Association between pig fetal measurements and gonadal development during intrauterine life

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It is known that the gonadal development in fetal life is directly related to the reproductive performance of the animal in its adult life. Since some histologic testicle parameters can predict the reproductive efficiency of animals, trying to relate these parameters to fetal measurements becomes interesting to predicting the reproductive capacity of males still in uterine life. Thus, the objective was to evaluate histomorphometric characteristics of male swine testicles in different gestational ages and correlate these characteristics with fetal length and weight, and head and thorax circumference of the fetuses. Twenty one male fetuses from ten DB-90 pregnant gilts (Danbred) in three different gestational ages (50, 80 and 106 days of gestation) were used. After slaughter of gilts, the weighing of fetus and measuring of fetal length, and head and thoracic circumference was performed. For the histomorphometric analyses of the testicle, those were dissected, weighed and fixed at 5% glutaraldehyde solution and analyzes were made by the software ImageJ. The gonadal parameters were subjected to ANOVA, statistically comparing the different gestational ages using the SNK test at 5% probability. Histomorphometric variables were correlated with fetal measurements by Pearson´s correlation test. The weight of the fetal testicles and the number of Sertoli cells increased ($P<0.05$) throughout gestation, especially between 80 and 106 days. The weight of the gonads, the cordonal length and the number of Sertoli cells were positively correlated ($P<0.01$) with the weight and length of the fetus and with the perimeters of head and thorax. On the other hand, there was a negative correlation ($P <0.02$) between the gonadossomatic index, percentage of tubular housing and nuclear volume of Sertoli cells with fetal weights and measures. Therefore, it is concluded that cell proliferation in the fetal testis is more pronounced in the final third of pregnancy and the fetal development is directly related to fetal testicular development.

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Characterization of hematopoietic stem cell from yolk sac of canine embryos

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In dog, the yolk sac persists until birth and it corresponds to a structure connect the ventral region of the embryo shaped bag. Thus, the canine yolk sac is a likely source of hematopoietic stem cells (HSC) which is a source of development of blood cells in mammals; erythrocytes, which express transcription factors to differentiation of the cell into hematopoietic cell. The present study aimed to characterize hematopoietic stem cells of yolk sac from canine embryos to contribute and provide data for future cell therapy. For analyzes, it was used canine embryo with a gestational age of approximately 35 days. It was made macroscopic analysis, cell culture, cell viability, colony assay (CFU) and flow cytometric. The cells were cultivated during 27 days in Stem-pro® 34 SFM with supplements, antifungals and antibiotics. The yolk sac canine showed a central portion yolk sac central call (SVc), which branches to the extremities (SVe), and this is close to the umbilical cord. Oliveira et al., (2015), also show this report in bovine embryos. As the cell culture, cultured cells supernatants were presented within 5 to 10 days in culture with circular or floating morphology. In the period from 15 to 25 days it was formed cell clusters like grapes. Cryopreservation of these cells was viable reaching a rate of 22.55% on average after-freezing cell death. Thus, at CFU it was found colonies with more than 200 cells and this is similar with the results from Oliveira et al., (Journal of tissue engineering and regenerative medicine, 2015, DOI: 10.1002/term.2016) in hematopoietic cells of bovine embryos, and different from findings in hematopoietic cells of sheep by Pessolato (Tese de Doutorado, Universidade de São Paulo (USP), Faculdade de Medicina Veterinária e Zootecnia, São Paulo, SP, 2011) which it was formed colonies after 1 day plated in methylcellulose. In flow cytometry, it was obtained high expression of the CD117 marker (88%) when compared with CD45 (20%) and CD34 (19%). These findings corroborate with Tárnok et al., (Cytometry A. 77(1):6-10, 2010. doi: 10.1002/cyto.a.20844.) which evaluated the expression of the same markers in hematopoietic cells from murine yolk sac. They observed that high levels of CD117 and low levels of CD45 suggest that these cells are able to form colonies with greater efficiency. The expression of these three markers showed that these cells have hematopoietic capacity. The analyzed results confirmed that the cells of the yolk sac of canine embryos have hematopoietic characteristics as we observed in cultured cell cluster formation expression of hematopoietic markers (CD34, CD45, and CD117) by flow cytometric analysis. It was noticed that these cells are able to form colonies and can be cryopreserved without losing viability.

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Could addition of resveratrol improve fertility rate of boar insemination doses?

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The exposure of spermatozoa to reactive oxygen species (ROS), produced mostly by cellular metabolism, induces a loss of motility, which is directly correlated with the level of oxidative stress endured by the spermatozoa. Oxidative stress affects several sperm function, resulting, amongst others, in impaired motility and sperm-oocyte binding, which could lead to lower fertility rate (Aitken e Baker, 2006. Mol Cell Endocrinol. 250:66-69). In this regard, resveratrol (RVT), a polyphenol compost, which plays an important role as antioxidant, due to its capacity to inhibit ROS formation (Stojanovic et al., 2001. Archives of Biochemistry and Biophysics, 391:79-89), has been used to improve sperm quality of different species with promising results (Bucak et al., 2015. Andrologia, 47: 545-552). Thus, the current study was carried out to evaluate whether the addition of RVT would exert a positive effect in fertility rate of boar sperm cooled at 17˚C for 72 h. For this purpose, females (n = 30) were fixed-timed inseminated after hormonal protocol with altrenogest (20 mg/day during 18 days), eCG (600 UI, IM) after 72 hours of altrenogest withdraw porcine LH (2.5 mg, IM) was administered then, 36 hours later, intra-uterine insemination was performed with pooled semen from two boars. After initial semen analysis, raw semen was extended in BTS medium (50 x 10^6 spermatozoa/mL in 50 mL) and RVT was added to form the following treatments: T0 (BTS + RVT 0 mM) without RVT, T1 (BTS + RVT 0.01 mM. Samples were cooled for 72 h before inseminations. Females were slaughtered for embryo collection five days after insemination in order to evaluate fertility rate of viable embryos (number of viable embryos/sum of oocytes and total embryos). Females inseminated with seminal doses containing RVT exhibited lower rates of viable embryos than those inseminated with seminal doses in the absence of RVT (59.96 ± 46.35^a; 12.15 ± 23.91^b, to T0 and T1 respectively). Thus, the presence of 0.01 mM of RVT in insemination doses has impaired fertility rate represented by viable embryos. Successful in sperm fertilization needs a capacitation step, which includes some necessary oxidative events. In this study, we possibly caused an imbalance in oxidant-antioxidant factors in favor to antioxidant, inhibiting the physiological oxidative events, and consequently impairing fertility rate. In conclusion, our results showed that the addition of RVT is not recommended, in concentration higher than 0.01 mM. However, additional studies of concentrations lower than 0.01 mM of RVT in boar insemination doses are needed to clarify these effects.

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Doppler ultrasonography of the umbilical artery and middle cerebral artery during pregnancy of dogs French Bulldog breed underwent caesarean delivery

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Gestational ultrasonographic evaluation is a critical tool for predictions of delivery time, as well as changes in maternal-fetal physiology, which can occur in late pregnancy. The French bulldog breed is well known by showing difficulties during natural parturition, so the determination of fetal maturity is essential in these animals for cesarean delivery decision. Twenty-six dogs French bulldog breed with average weight of 20 ± 2,80kg and average age between 3 and 5 years were evaluated by doppler ultrasonography for vascular flow patterns measurement of umbilical artery (UA) and middle cerebral artery (MCA). Vascular flow values were correlated with the respective average heart rate. The equipment used was a SonoSite ultrasound M-Turbo (USA, Washington), coupled to sectoral (5-8 mHz) and linear probes (12-15mHz). The calculations of gestational ages were performed automatically by measuring the biparietal diameter and abdominal diameter (tables inserted in the data platform by the authors). The vascular flow measurements were made by adjusting the pulse repetition frequencies (PRF) to the vessels studied as well as maintaining an insonation angle smaller than 20. The systolic flow velocities (SFV) and resistivity index (RI) were presented as follows: (a) after 28 days of pregnancy – HR= 245±3.45 bpm, UA, SFV= 37±1.24cm / s, RI= 0.87±0.06. (b) 40 days of pregnancy – HR= 235±2.73 bpm, UA, SFV = 42±1.13cm / s, RI= 0.82±0.04; MCA, SFV = 31±1.15cm / s, RI= 0.72±0.05. (c) after 56 days of pregnancy – HR= 215±1.60 bpm, AU, SFV= 53±1.21cm/s, RI= 0.89±0.04; MCA, SFV= 27±1.2 cm/s, RI= 0.64±0.08. The monitoring of the UA and MCA flows promoted valuable information on cesarean delivery decision. The hemodynamic effects promoted by increasing the RI of the UA (which tends to decrease in situations of normal and natural delivery) and the the reduction of SFV and the RI of the MCA associated with the relaxation of this artery assisted in the decision of cesarean delivery. The relaxation of the MCA was positively correlated HR reduction (r =0.86). All study animals were underwent caesarean delivery and there were no complications or death puppies, during the procedure and post-partum. The study identified vascular changes that may contribute to safe intervention by caesarean surgery in dogs french bulldog breed.

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Doppler ultrasounography of the aorta, common carotid artery and fetal renal artery during dogs pregnancy of French Bulldog breed bitches underwent caesarean delivery

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The prediction of fetal development complications in dogs is relevant to eventual post-partum management decisions or to establish caesarean intervention protocols. French Bulldog bitches often develop complications that hinder natural delivery. In such circumstances the establishment of fetal viability is fundamental. Twenty-six French bulldog bitches with average weight of 20 ± 2,80kg and average age between 3 and 5 years, were evaluated by doppler ultrasound for measurement of the vascular flow patterns of fetal aorta (AOfet), fetal common carotid artery (CCAfet) and right and left fetal renal artery ($RAfet_D$ and $ARfet_E$). Vascular flow values were correlated with the average heart rate of the animals studied. The equipment used for this study was a SonoSite ultrasound M-Turbo (USA, Washington), coupled to sectoral (5-8 mHz) and linear probes (12-15mHz). The calculations of gestational ages were performed automatically by measuring the biparietal diameter and abdominal diameter (tables inserted in the data platform by the authors). The vascular flow measurements were made by adjusting the pulse repetition frequencies (PRF) of each vessel studied as well as maintaining an insonation angle smaller than 20. The systolic flow velocities (SFV), resistivity index (RI) and heart rate (HR) were presented as follows: (a) after 28 days of pregnancy – HR= 245±3.45 bpm, AOfet, VSF=31±1.14cm/s, RI= 0.94±0.06; CCAfet, VSF=39±1.16cm/s, RI= 0.81±0.01. (b) 40 days of pregnancy – HR= 235±2.73 bpm, AOfet, VSF=48±1.2cm/s, RI= 0.92±0.09; CCAfet, VSF=42±1.14cm/s, RI= 0.72±0.03, $ARfet_D$, VSF=28±2.16cm/s, IR= 0.70±0.05; $RAfet_E$, VSF=29±2.10cm/s, RI= 0.71±0.02. (c) after 56 days of pregnancy – HR= 215±1.60 bpm, AOfet, VSF=57±1.19cm/s, RI= 0.81±0.01; CCAfet, VSF=45±2.11cm/s, RI= 0.61±0.02; $RAfet_D$, VSF=37±1.21cm/s, RI= 0.72±0.07; $RAfet_E$, VSF=39±1.11cm/s, IR= 0.73±0.05. There was no difference between the means of right and left renal arteries ($p>0.05$). The monitoring of AOfet, CCAfet and RAfet flows allowed us to suggest the normal vascular flow values during gestation of French Bulldog bitches underwent caesarean delivery. There was no alteration in the puppies viability and there were no complications or death during the procedure neither in post-partum. Thus, we suggest these hemodynamic values as a starting point for investigation of fetal hemodynamic complications during parturition, such as fetal stress in French Bulldog breed underwent caesarean delivery.

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Early development of the chorioallantoic membrane and cotyledons in the water buffalo (*Bubalus bubalis*)

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The villous synepitheliochorial placenta of ruminants is formed by close interaction between fetal and maternal tissues and complex cell differentiation mechanism. Whereas the bovine is extensively studied, for the buffalo as close relative early stages of placental differentiation are unknown. We investigated the early development of the chorioallantoic membrane (CM) and cotyledones in water buffalos. In total, 33 individuals with gestational ages from 15 to 58 days were studied by means of gross anatomy, histology, immunohistochemistry and electron microscopy. Data so far show that until 30 days the CM was smooth and thin. At 15-19 days, the CM had cubic epithelial cells associated with a very thin layer of collagen fibers towards the mesenchyme and a few small vessels. At 21-24 days, this layer increased in volume, as well as the number of vessels. Small folds as precursor of the villi occurred associated with dispersed binucleate trophoblast giant cells (TGCs). Abundant short villi were present at 26-32 days, characterized by a stratified cellular layer of small round cells and numerous TGCs. At 32-36 days, regions with dark color represented the origin of the cotyledons. They were villous at 45 days; especially in the middle of the CM. At 51-58 days, the cotyledons had increased in number and size and were distributed in rows along the caruncles of the CM. They possessed prismatic epithelium in the basal axis and numerous well-vascularized villi and projections. As occurred for bovine years ago, due to the role of CM for the successful of gestation, our results may contribute to the understanding and improvement of reproductive technologies applied to the *in vitro* buffalo embryo production, in order to compensate problems related to the low efficiency of superovulation and embryo transfer in this species.

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Effect of co-culture of cumulus cell and vitamin C in development of parthenogenetic embryos porcine

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This study was designed in order to assess the effects of cumulus cell coculture and PZM-3 medium supplemented with vitamin C (ascorbic acid) during the development of parthenogenetic embryos. The first experiment oocytes were used that were recovered from slaughterhouse ovaries and placed in maturation medium NCSU-23 for a period of 24h with hormones and 24h without hormones at 39°C with 5% CO2, 90% humidity, at the end He was evaluated those that were in metaphase II stage. They were subsequently activated using DC 150 V / mm by 100 µseg, placing them in micro drops of 30 for 5h in NCSU-23 medium to which the citocalaisna added B and cyclohexamide leaving for a period of 6 to 7 days. The second experiment, 1 µl of suspension of cumulus cells were used which were obtained during oocyte maturation, seeding them in the culture plates with the NCSU-23 for 24 h, after formation of the monolayer, was renewed NCSU-23 medium one time to onset of co-culture. The third experiment, in order to assess the amount of vitamin C (ascorbic acid) in the middle PZM-3, a preliminary study was conducted using concentrations of 0, 250 and 500 µM, with the optimum concentration result of 250 µM, which was compared to the co-culture. As 60% results in PZM-3 and 58% co-culture division≥ 2 cells was obtained. However the proportion of development to the blastocyst stage was significantly higher ($P < 0.01$) in the co-culture group (26.6% vs. 13.4%) compared to PZM-3. Also a count of the number of cells in the blastocyst stage was found more significant differences ($P <0.01$) in the coculture group 31.8% vs. 29.1% with PZM-3 medium supplemented with was ascorbic acid. In conclusion ascorbic acid at concentrations in the middle 250µM PZM-3 exerts an antioxidant action during cultivation, increasing embryo quality, as evidenced by the number of cells obtained blastocysts. Co-culturing cumulus cell used may be a suitable culture system could be used for the production of pig partenogenotes.

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Effects of caffeine daily consumption during two gestational phases on placental and fetal development in mice

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Several beverages containing caffeine as main component are regularly consumed worldwide to reduce fatigue. However, because of its ability to cross the placental barrier, its consumption should be restricted during pregnancy, especially in the last trimester, when a reduction in its degradation is noticed in humans and rodents. The aim of this study is to investigate the effects of different doses of caffeine in female Swiss mice during mid (d 11.5) and late (d 17.5) pregnancy on placental and fetal biometry. Female Swiss mice were divided into four groups (n=10), which received different caffeine doses (0-CC; 60-T60; 120–T120; 240–T240 mg/kg/day) for one week. Daily vaginal smears were collected to identify the estrous cycle phase. Females in estrus were placed together with one male overnight, and gestational day 0 (GD0) was designated as the day in which evidence of mating was observed. Pregnant females received the same caffeine doses after mating until day 11.5 or 17.5 GD, when they were euthanized. Data on estrus frequency, pregnancy rate, body and placental weight, as well as brain and liver weights of each fetus were recorded. The T240 group showed the lowest conception rate (16.7%). Even though T120 and T240 groups showed a numerically higher frequency of arrested diestrus, caffeine did not affect estrous cycle length ($P > 0.05$). Caffeine effects on fetal weights were evident at GD 17.5, as such parameter was lower in T120 and T240 groups ($P \leq 0.01$). In addition, fetuses of all treated groups were smaller than the CC group ones ($P < 0.01$) in GD 17.5. Placental weight was lower in T120 and T240 groups ($P< 0.01$) at GD 17.5, but not at GD 11.5 ($P > 0.05$). No differences were observed in brain or liver weights ($P > 0.05$) among experimental groups regardless gestational age. These results suggest that daily moderate and high consumption of caffeine during mice pregnancy may have negative effects on placental and fetal development, however such effects only become evident at late pregnancy (GD 17.5), when placental formation is complete. This project was approved by CEUA/UFMG (395/2015).

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Effects of oxygen tension in exosomal miRNAs isolated from culture medium of *in vitro* produced bovine embryos

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Exosomes released by bovine embryos potentially play important roles mediating communication between the embryo and the oviduct as well as with the uterus in physiological situation, and between embryos during *in vitro* development. This study aimed to determine the pattern of secretion and the main pathways regulated by exosomal miRNAs released by embryos to the culture medium during the first three days of culture in two different atmosphere tensions of oxygen: high (20% O_2) and low (5% O_2). Our hypothesis is that culture atmosphere can promote changes in exosomal miRNAs levels involved in mediating communication between the embryo and the environment. Groups of 25 embryos were cultivated *in vitro* in 90 μL drops submitted to high (20%) or low (5%) oxygen tensions. In the third day of culture 50 μL of medium was collected from each drop. Extracellular vesicles were isolated from culture media by centrifugations and ExoQuick-TC reagent. In order to perform the experiments we utilized three biological replicates per treatment. Samples were analyzed by NanoSight software to evaluate the size and concentration of the extracellular vesicles. Additionally, we performed RT-PCR to investigate the levels of 378 miRNAs and 3 housekeeping small RNAs. Results were compared using *Student's T-test* and p-value ≤0.05 was considered significant. Predicted targets of miRNAs were determined using mirBase and DIANA tools. Cleavage rates at Day 3 were similar between groups (80.87%±7.58 and 81.26%±4.45 for low and high tension, respectively). The average mode of exosome size (124.6±3.21 nm and 117.8±2.86 nm for low and high tension, respectively) was also similar between groups. Higher concentration of extracellular vesicles was identified in the culture media derived from low oxygen tension compared to high tension (8,518,000±7.22 and 5,340,000±2.83 particle/drop/day for low and high tension, respectively). This could be explained by hypoxic condition of the low-tension culture, which can induce exosomes secretion. From a total of 315 detected miRNAs, 17 were exclusively detected in low O_2 group while 15 were exclusively detected in high O_2 counterparts. From the 283 miRNAs expressed in both groups, 11 were upregulated and 10 were downregulated in low O_2 group compared to high O_2 group. Functional enrichment analysis of miRNAs upregulated in low tension (bta-miR-425-5p, bta-miR-664b, bta-miR-346, bta-miR-195, bta-miR-491, bta-miR-125a, bta-miR-374b, bta-miR-215, bta-miR-29e, bta-miR-149-3p, bta-miR-125b) indicate pathways associated with proteoglycans in cancer, p53 signaling, hippo signaling and adherents junction, while the downregulated ones (bta-miR-433, bta-miR-129-5p, bta-miR-429, bta-miR-380-3p, bta-miR-139, bta-miR-486, bta-miR-411a, bta-miR-129, bta-miR-574, bta-miR-181d) indicate adherens junction, colorectal cancer, glioma and prostate cancer pathways. Both up and dowregulated miRNAs were involved in regulation of actin cytoskeleton and cell differentiation. In addition, the miRNAs bta-miR-125a and bta-miR-125a, both upregulated in low tension, have the P53 signaling pathway as predicted regulated (by targeting TP53). This pathway regulates the expression of numerous genes associated with the cellular response to stress and DNA repair. In conclusion, different culture atmospheres lead to different concentrations of extracellular vesicles in the media probably due to different levels of exosomal secretion. Also, the variation of oxygen tension was associated with differences on exosomal miRNAs content. The exact roles and the bioactivity of these exosomal miRNAs are still elusive, however functional enrichment analysis indicate that they are potentially associated with the regulation of essential pathways to successful embryo development and embryo-maternal communication.

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Effects of reduced glutathione on sperm mitochondrial activity in boar sperm during the *in vitro* capacitation

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The capacitation is the one of the most important process that undergoes mammalian sperm after ejaculation. It has been defined as a combination of sequential and parallel events occurring in sperm structures that confers to cell the ability to reach the acrosome reaction and carry out the fertilization. With the advent of assisted reproductive technologies, through the *in vitro* studies, is being possible to verify the biological mechanisms involved in this process, that lead to the establishment of fully fertilizing ability. The reduced glutathione (GSH) is the most abundant thiol in cells and it is considered of vital importance. Among other functions, it is important for the maintenance of intracellular redox balance, and consequently, to modulate the sperm capacitation through of its influence on the mitochondrial activity. Thus, the aim was to evaluate the effects of GSH on sperm mitochondrial activity in boar sperm during the achievement of a standard *in vitro* capacitation (IVC). Four treatments (control and three different GSH concentrations: 1, 2 and 5 mM) for each assay (n=6) were analyzed after the incubation for 4h at 38.5ºC (in a 5% CO_2 atmosphere) of sperm in an IVC activation medium (CM) containing $NAHCO_3$ and bovine serum albumin (BSA). The mitochondrial membrane potential (MMP) was analyzed by applying the incubated method with the flurochrome JC-1 and the samples were processed through the flux cytometer. The incubation induced a significant ($P<0.05$) increase in the percentage of cells with high MMP, which ranged from 23.1 ± 3.7% at 0h of incubation to 54.6 ± 4.3% after 4h of incubation. These results were concomitant with capacitation-like changes of parameters like membrane fluidity, sperm motility and tyrosine phosphorylation levels. The addition of GSH prevented this increase at all of the tested GSH concentrations, although the maximal effects were observed at GSH concentrations of 5mM. Our results seem to indicate that IVC in boar sperm is related with a significant increase in MMP which can be prevented by the addition of GSH to the medium. These phenomena could play a role in the achievement of the capacitation status.

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Evaluation and prediction of the scrotal circumference in beef bulls

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Scrotal circumference (SC) is a more accurate predictor of age at onset of puberty than either age, or weight, regardless of breed. This trait is easily measured, highly heritable and repeatable, and correlated with sperm quality, body weight, and reproductive performance of the bull and the female offspring. Data of scrotal circumference (cm), body weight (kg), and age (days) were collected from 169,094 beef bulls from three breeds: Nellore (N), 112,149 animals; Angus (AA), 6,509; Brangus (BR), 41,205; Hereford (HH), 3,534; and Braford (BH), 5,297. Six different models were compared and included the effects of body weight (BW) and age (A) inside the each breed: Asymptotic Model, $y = a + b * \exp(cx) + \varepsilon$; Exponential Model, $y = a * \exp(bx) + \varepsilon$; Linear Model, $y = a * \exp(bx) + \varepsilon$; Log-linear Model, $y = a * \exp(bx) + \varepsilon$; Gompertz Model, $y = a * \exp(bx) + \varepsilon$; and Michaelies-Menten Model, $y = a * \exp(bx) + \varepsilon$. The choice of the models with better adjustment for each breed was defined by the following criteria: lowest value to Akaike's information criteria (AIC), highest value to R^2 (coefficient of determination), lowest root mean square deviation (RMSD), and model significance ($P \leq 0.05$). Bulls from HH (33.7±3.1 cm) and AA (33.5±3.0 cm) showed the highest unadjusted value of SC, followed by BH (32.2±3.6 cm), BA (30.6±4.1 cm), and N (26.9±3.6 cm). The constants in linear regression between BW and A for average daily gain (kg/day) were 0.817, 0.793, 0.700, 0.660, and 0.596 for HH, BH, AA, BA, and N, respectively. The effects of BW on SC in covariance analysis showed a non-linear pattern in the Michaelies-Menten Model ($P<0.001$), although the comparisons of models by the AIC criteria were similar in British breeds (HH and AA), crossbreed (BH and BA), and in both breed groups compared to N. The increase in BW and A, the average daily SC growth of curvilinear decreasing, and the values were 0.025, 0.035, and 0.042 cm/kg and 0.017, 0.026 e 0.034 cm/day for HH/AA, BH/BA, and N, respectively. There was change on testicular growth with the increasing of calves' age, with a distinctive sigmoid pattern: slow growth before 25 weeks followed by a fast growth during the prepubertal stage and a slow growth in the adult stage after 144 months. The best linear model that demonstrated the absolute growth curve of SC associated with BW was the non-linear behaviour (Michaelies-Menten) with three inclination patterns according to the breed groups ($P<0.01$). Bulls with 560-600 days of age or kg/BW showed higher adjusted SC values for all breeds, because in this A or BW the testicular mass growth was decreasing in British cattle breeds while increasing in Indian cattle breeds. In these evaluation parameters, bulls showed an average adjusted SC that represented 90-92% of SC, which highlighted the genetic progress from BH, BR, and N breeds. The puberty evaluation (510-540 days) seems to be the most appropriate moment to measure the SC of bulls, allowing to identify males that present accelerate testicular growth, that can be detected in animals with fast sexual precocity, and it has been observed that SC curve was stabilized between 550 and 599 days. The analysis of SC growth with this model can be important, since the parameters of the model can be used to select animals that present appropriate growth rate of SC; hence, increase the fertility and precocity of animals by the genetic selection. Non-linear models for evaluation and SC adjustments in young bulls showed better precision than linear models. However, independent of the model adopted, the characteristics of BW and A are essential to describe the testicular growth, since this information help the biological interpretation of growth and are easily comparable in different beef cattle production scenarios. On the other hand, the SC growth curve showed to better estimate the testicular size at the age of maturity, which should be obtained between 400 and 500 days for British cattle breeds and 50 and 100 days later for crossbreeds animals.

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Evaluation of mRNA expression of Bax and Bcl2 genes in goat semen cryopreserved in medium containing catechin or epigallocatechingallate

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In the cryopreservation process, particularly during freezing and thawing, the sperm cells are often damaged, which reduces the spermatozoa viability. Therefore, various studies have been made to find compounds acting to avoid cell damage. The (+) - catechin and especially the (-) - epigallocatechingallate (EGCG) are among the most currently phytochemical compounds studied due to their antioxidant activity. Catechins inhibit enzymes that induce oxidative stress and play a protective effect on the membranes and mitochondria and block apoptosis. In this regard, the present study aimed to evaluate the mRNA expression of Bax (pro-apoptotic) and Bcl2 (anti-apoptotic) genes in cryopreserved caprine semen in extender containing (+) - catechin or (-) - epigallocatechingallate (EGCG). Samples of semen in natura from six males were evaluated in phase contrast microscopy formotility (0-100%) and vigor (0-5). After evaluation, the samples were grouped into pools, and then divided into aliquots that were diluted in skimmed milk based extender [10 g skim milk powder, 194 mg D - (+) - glucose, 100 ml Milli-Q 7% glycerol, pH 6.8] (control group) or the same extender containing 75 uM of (+) - catechin hydrate or 75 uM EGCG. The semen was diluted to a final concentration of 200 x 10^6 sperm/mL, packaged in straws (0.25 mL) and frozen in automatic system. After thawing, semen aliquots were evaluated for sperm kinetics, plasma membrane integrity and acrosome, morphology and oxidative stress at 0 and 1 h incubation. Immediately after thawing, these parameters were evaluated and it was observed that both EGCG and (+) - catechinhave a transient inhibitor effect on goats sperm kinetic. After this preliminary evaluation, six pools from each experimental group were used for total RNA extraction using Trizol technique. The samples were subjected to qPCR technique to establish the level of mRNA expression of apoptosis related genes, Bcl-2 and Bax. Amplification results were normalized using constitutive gene GAPDH. Statistical analysis was performed by ANOVA and Tukey test. The results show a high BAX expression in cryopreserved catechin group ($P < 0.05$) compared to both the control group and EGCG group. In normal conditions, there is a balance between anti-apoptotic (eg: Bcl-2) and pro-apoptotic (eg: Bax)proteins. Stimulus such as DNA damage, leading to increased expression of pro-apoptotic proteins, producingan unbalance and inducing apoptosis. In this study, the increase in Bax expression in semen cryopreserved withcatechin, suggests that this antioxidant was not protecting the cryopreserved semen, allowingthe apoptosis more than EGCG treatment. However, more deep studies are necessary to clarify the pathway of this antioxidants in the cryopreserved goat semen.

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Fetal sex determination by transretal ultrassographic evaluation of fetal gonads in jennies

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In Brazil, the equine population is currently estimated about 7,751,532 animals, with 5,508 million horses, 974,532 donkeys and 1,269 million hinnies. This horse population is the fourth largest in the world, which has held steady in recent years. In 1989, the first description of fetal sex determination in horses by identifying the genital tubercle in the first third of pregnancy was performed. About 11 years later, it was reported in the same specie sexing by identifying gonads and external genitalia, performed when the fetus reaches the middle third of pregnancy. However, on donkeys only two works were published about fetal sexing technique by evaluating the fetal gonads. Thus, the aim of this study was perform fetal sexing by transrectal ultrassonographic evaluating the fetal gonads in jennies. Therefore, we used 10 jennies Pega breed weighing between 230 and 400kg, aged 6 and 13, located in the Vassouras city, Rio de Janeiro, Brazil (LAT 22º39'58.80 "S and LONG 43º67'14.73."). It used the Ibex Pro vet ultrasound equipped with linear probe of 7.5 MHz for transrectal B mode exam, between 107 and 156 days of gestation. It was possible to identify fetal sex in 10 jennies and in only one of them we need two tests for identification of fetal sex. We obtained 70% (7/10) ultrasound images similar to testicular parenchyma and 30% (3/10) ultrasound images similar to ovarian parenchyma. These results were confirmed at birth, reaching 100% (10/10) right evaluation using the technique. Crisci et al. (2014) also reported 100% (5/5) accuracy using transabdominal technique, allowing sex determination between 150-265 days. Mancuso et al. (2007) considered that the optimal time to perform fetal sex determination by the fetal gonads evaluation was around 100-150 days, similar with the results of this study. So, with these results we can considerer that the transrectal technique can be used successfully in jennies to identify the fetal sex between 107 and 156 days of pregnancy.

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In vitro culture medium supplemented with L-carnitine increases production and viability of vitrified/warmed *Bos Indicus* X *Bos Taurus* embryos

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In vitro produced embryos (IVP) are more sensitive to cryopreservation by several factors inherent gamete quality and manipulation during the whole process. Because their higher lipid concentrations, *Bos indicus* IVP embryos are more sensitive than Bos taurus. This study was designed to evaluate the effects of exogenous L-carnitine (LC), an enhancer of lipid metabolism, on the in vitro development and freezing survival of bovine embryos. Viable oocytes obtained from abattoir-derived Nellore cows ovaries were in vitro matured (IVM) for 24 hours at 38.5°C at atmosphere of 5% CO_2 and fertilized with conventional semen (Holstein, $1x10^6$/mL) to produce a *Bos Indicus* X *Bos Taurus* embryo. After 18 hours presumed zygotes were cultured for seven days under the same IVM conditions. Blastocysts (BL) and expanded blastocysts (Bx) Grade I and II were vitrified, thawed and cultured in the IVC medium. The experimental groups were: Culture medium with LC (T1) culture+re-culture media with LC (T2), maturation+ culture+re-culture with LC (T3) and control (C). Embryo production rates (D7) and re-expansion rate (RR) after 2 and 12 hours were evaluated and data were submitted to the Chi square test. Total number of cells (TC) and number of apoptotic cells (NAC) count was performed by TUNEL and data were evaluated using ANOVA. Cleavage rate did not differ between groups. Day 7 embryo production rate was higher T1 (48.85%) and T2 (44.1%) than Control (34.89%) ($P < 0.05$), as well as the number of embryos grade I (24.92%, 27.63%, and 17.45% respectively). Re-expansion rate of the embryos re-cultured during 2h was higher in T3 (90.48%, $P<0.05$), but no differences were verified between groups after 12 hours in the culture medium. TC did not differ between treatments [$P>0.05$; C (175.6±7.1), T1 (179.4±7.0), T2 (172.1±7.0), T3 (178.7±7.0)]. NAC decreased in T2 and T3 (3.8±0.53 and 3.4±0.53 respectively) when compared to T1 (7.9±0.53) and control (6.0±0.54) ($P<0.05$). In conclusion, the supplementation of L-carnitine during in vitro embryo culture (before and after vitrification) improves the development and recovery after warming of Bos Indicus X Bos Taurus embryos.

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Melatonin during bovine oocyte maturation and embryonic development improves the quality of *in vitro* produced embryos

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Recent studies have been shown that melatonin has an important role in oocyte maturation and embryo development, mainly for its antioxidant action, however, these studies evaluated the effect of melatonin just in one stage, or *in vitro* oocyte maturation (IVM) or embryo culture (IVC). Therefore, the aim of this study was to evaluate the effect of melatonin on *in vitro* embryo production (IVP) when added in the medium of IVM and IVC. For IVP, ovaries coming from abattoir were punctured and the *cumulus*-oocyte complexes (COCs) were selected randomly according to Experimental Groups: 126 COCs in Control (IVM and IVC without adding melatonin); 121 COCs in Melatonin 1 (IVM and IVC with 10^{12}M melatonin) and 121 COCs in Melatonin 2 (IVM with 10^{12}M and IVC with 10^{9}m of melatonin). For IVM culture medium (M199, Gibco, Carlsbad, CA, USA) was supplemented with 10% FCS, 0.5µg/ml FSH, 50 µg/ml LH, 22µg/ml pyruvate, 50µM betamercaptoethanol and 50 µg/ml gentamicin, for a period of 18 h. *In vitro* fertilization was used frozen semen from a single bull (*Bos taurus taurus*) at a concentration of $2X10^{6}$ sperm/ml after separation by discontinuous Percoll density gradient. The IVF was performed in the synthetic oviductal fluid (SOF) medium according to Holm et al. (Theriogenology, 52: 683-700, 1999) without glucose and supplemented with 6 mg/mL BSA, 10µg/ml heparin, 2µM penicillamine, 1µM hipotaurina, 0,25µM epinephrine and 50 µg/ml gentamicin, for a period of 32 hours. The embryo cultures were grown in co-culture system with *cumulus-oophorus*-cell monolayer in SOF medium supplemented with 10% FBS, 6 mg/ml BSA, 50µM betamercaptoethanol and 50 µg/mL gentamicin. All IVP was performed in 35 mm polystyrene petri dishes, drops 100µL of culture medium under sterile mineral oil, in a culture oven with 5% CO_2, 20% O_2 and 75% N_2, under humid atmosphere and temperature of 38.5 °C. The results of cleavage, blastocyst formation and expression of genes octamer-binding transcription factor 4 (*OCT-4*), interferon-tau (*IFN-t*) and heat shock proteins (*HSP70*) were analyzed with ANOVA (post test turkey, program BioEstat 5.0).The significant difference between treatments was set at $P < 0.05$. There was no difference ($P > 0.05$) in cleavage rates and blastocyst formation, respectively, among the Control Groups (84.92 and 46.03%), Melatonin 1 (84.30 and 40.50%) and Melatonin 2 (86.78 and 49.59%). At the molecular level, the gene expression of *OCT-4* and *IFN-t* genes were significantly ($P < 0.05$) higher and lower, respectively, in Melatonin 1 compared to Control and Melatonin 2. Melatonin 2 had low *HSP70* expression compared to Control and Melatonin 1. Thus, supplementation with melatonin, IVM and IVC, influence the expression of important genes that are used as indicative of embryo quality, but not altered the cleavage rate and blastocyst formation showing its importance on *in vitro* production of bovine embryos.

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MicroRNA levels during maternal-zygotic transition in bovine embryos produced in vitro

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The transition from maternal to embryonic genome activation (EGA) is an important step during the early embryonic development. During this period, the maternal messenger RNAs (mRNAs) are replaced by embryonic mRNAs. Regulation of signaling pathways involved in cell differentiation, proliferation and metabolism are crucial to embryo development. Recent studies showed that microRNAs (miRNAs), short non-coding RNAs, can regulate gene expression through post transcriptional mechanisms. Therefore miRNAs can play a critical role regulating transcripts such as the maternal mRNA during the maternal-to-embryonic transition as well as after the major EGA. The aim of this study was to determine the dynamic of microRNAs (miRNAs) levels during early bovine embryonic development in vitro. For this, bovine embryos were produced by in vitro fertilization (IVF) and samples were collected, on day 2 and on day 3 after the IVF, for the analysis of 380 bovine miRNAs. Initially, cumulus oocyte complexes (COCs) were aspirated from 3-6 mm ovarian follicles (Nellore) obtained from local slaughterhouses. COCs were selected and groups of approximately 25 COCs were matured in vitro during 24 hours. The matured oocytes were fertilized (with semen from a single bull) and remained in fertilization medium 18 hours to 20 hours and then they were replaced in culture medium. The in vitro culture (IVC) was made at 38.5 °C, 5% CO_2 and 5% O_2. The day of IVF was considered day 0 for sample collection. Samples were grouped in 3 polls of 10 embryos collected on day 2 (D2), containing 4 cells, and 3 pools of 10 embryos collected on day 3 (D3), containing embryos at 8-16 cells. Embryos were collected from 6 different embryo production routines and homogeneously distributed to generate the pools. The Total RNA extracted by combined QIAzol reagent (Qiagen) and miRNeasy kit (Qiagen) was isolated and the quality determined by Nanodrop analysis. Reverse transcription was performed with 100ng of total RNA utilizing the miScript PCR System kit (Qiagen). The Real-Time PCR analysis of the miRNAs was performed with a custom miRNA profiler plate. Data were normalized by the geometric mean of 2 endogenous small RNAs (RNU43 snoRNA and bta-miR-99b). The relative levels were transformed by $1/\Delta Ct$ and analyzed by Student's T-Test. A total of 249 miRNAs were detected during the early embryonic development, and of these, 13 were exclusively expressed on day 2 while 54 on day 3, suggesting a relation with the major EGA on day 3. A total of 10 miRNAs were differentially expressed between D2 and D3 embryos. Bioinformatics analysis identified a total of 60 possibly regulated pathways such as Hippo signaling (71 genes), TGF-beta (42 genes), Wnt signaling (62 genes), Adheren junctions (49 genes), RNA degradation (39 genes), mRNA surveillance (43 genes), Fatty acid elongation (11 genes) and Fatty acid metabolism (25 genes). Analysis also revealed that 4 miRNAs (bta-miR-200b, bta-miR-129, bta-miR-423-3p and bta-miR-143) were highly abundant on D2, modulating 14 important pathways such as PI3K-Akt signaling pathway (70 genes), Insulin signaling pathway (31 genes) and signaling pathways regulating pluripotency of stem cells (31 genes). Similarly, 6 miRNAs (bta-miR-363, bta-miR-106a, bta-miR-211, bta-miR-139, bta-miR-9-5p and bta-miR-15b) were highly abundant on D3, controlling 17 pathways such as fatty acid degradation (13 genes) and RNA polymerase (15 genes). Thus our analysis demonstrated that miRNAs levels are dynamic during early embryo development suggesting that they play an important role modulating cell differentiation and metabolism. The results of this project will allow a better understanding of pathways regulated by miRNAs during the early embryonic development, with great impact on the assisted reproduction industry in cattle and humans.

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Nellore and crossbred dairy cows have similar inflammatory responses to lipopolysaccharide in *ex vivo* endometrium and are not influenced by ovarian steroids

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Uterine infections are common in half of postpartum dairy cows and can lead to infertility, but in beef cows these diseases are infrequent and less significant than in dairy cows. Also, the risk of bacterial infection of the endometrium causing uterine diseases in cattle is increased in the progesterone-dominated luteal phase of the ovarian cycle, whilst estrogens or estrous are therapeutic or protect against disease. However, recent publication did not found effect of the stage of the oestrus cycle, exogenous ovarian steroids or inhibition of their nuclear receptors on key cytokine and chemokine responses to *E. coli* or LPS in endometrial tissues of *Bos taurus* mixed-breeds. This study tested the hypothesis that Nellore *ex vivo* endometrium when challenged with LPS has better inflammatory responses than crossbred dairy cows (*Bos Taurus x Bos indicus*) and that these responses are influenced by ovarian steroids. To evaluate these conditions, uteri with no gross evidence of genital disease or microbial infection were collected from 32 postpubertal Nellore beef (*n=15*) and crossbred dairy (*n=17*) cows, and blood for progesterone (P4) and oestradiol (E2) analysis was collected. *Ex vivo* organ cultures (EVOCs) were collected from the endometrium using sterile 8-mm diameter biopsy punches; immediately placed in 24-well plates containing 2 ml culture medium/well; Endometrial EVOCs were treated with the control medium or a medium containing 1 µg/ml LPS for 24 h, and both culture supernatants accumulated IL-1β and IL-6, as expected, but there was no difference between inflammatory responses intensity among breeds. Furthermore, these inflammatory responses were not affected by the serum P4 concentration when samples were divided into three groups, according P4 dosage (P4<1 ng/ml; 1<P4<5 ng/ml; P4>5 ng/ml). Also, it was not found good correlation between inflammatory responses with IL6 and P4 (*r=0,370*) or E2 (*r=0,104*) serum concentrations and with IL1B and P4 (*r=-0,222*) or E2 (*r=0,065*) serum concentrations. In conclusion, we have demonstrated a similar inflammatory response to LPS between *ex vivo* organ cultures of Nellore and crossbred (*Bos Taurus x Bos indicus*) dairy cow's endometrium and no effect of the ovarian steroids.

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Optimization of a decellularization protocol for the production of scaffolds from murine placentas

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Placentas as organs of reproduction that are well vascularized and that possessed an extensive extracellular matrix to preserve the inner structure are promising for building biological scaffolds. To establish an effective decellularization protocol for murine placentas we used 40 mice placentas from C57BL/6 of 17.5-20 days of gestation and 20 placentas of Wistar rats of 14.5 days, tested for 4 protocols and controls were performed by histology and immunohistochemistry for collagen I, III and IV, fibronectin and laminin. The protocols included washing with PBS (Phosphate-Buffered Saline) 1X + EDTA (Ethylenediaminetetraacetic acid) + ATB (Antibiotics Penicillin/Streptomycin) 0.5%: (P1) 0.25% SDS (Sodium Dodecyl Sulfate) for 72 hours; (P2) 0.3% SDS for 72 hours; (P3) 0:25%, 0.5%, 1.0% SDS each for 24 hours; (P4) 0.001% 0.01% and 1.0% SDS for 24 hours each. Finally, incubation with Triton X-100 1% followed for 48 hours in all groups. For mice, protocol 1 resulted as the best, whereas decellularization in rats was successful by protocol 2. Controls by histology revealed the absence of nuclei and cells in these samples. Immunohistochemistry showed the presence of collagens, fibronectin and laminin in the extracellular matrices. Especially in mice, they were mainly found in the labyrinth zone in contrast to the junctional zone towards the decidua. Ongoing research concentrates on the biological meaning of this finding. In conclusion, we found adequate protocols for the decellularization of mice and rat placentas in order to build biological scaffolds for further applications.

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Partial ablation of endometrial glands in dogs after exposure to progestin during the neonatal period

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Bitches with uteri devoid of endometrial glands should be sterile, and consequently could contribute to the population control of dogs. Considering that an inadequate exposure of the female reproductive system to steroids can lead to the formation of the uterine gland knock-out (UGKO) phenotype in some species, the aim of this study was to evaluate the effect of serial applications of medroxyprogesterone acetate (MPA) from birth until the age of 6 months on the development of endometrial glands in bitches. For this purpose, 16 female mongrel dogs from different litters were distributed into either an MPA group (n = 8), animals treated with medroxyprogesterone acetate (MPA,10 mg/kg/sc), from day 1 after birth until the age of 6 months, at 3-week intervals, or a control group (n = 8), composed of animals that only received a 0.9% NaCl solution in place of MPA. At 6 months of age, ovariohysterectomy was performed and uterine horn samples were collected for histological and immunohistochemical examinations. The variables evaluated included the total thickness of the uterine wall (μm), endometrium (μm), and myometrium (μm); diameter of the endometrial glands (μm); number of endometrial glands/mm^2; and the epithelial height (μm). Five fields per histological sample were evaluated for the calculation of the total thickness of the uterine lining, endometrium, and myometrium, and the epithelial height. The glandular diameter was calculated by measuring 10 glands per histological sample. The measurements were performed using the ImageJ software version 1.43u (Wayne Rasband, National Institutes of Health, USA). The expression of the ER-α, ER- β and PR on the surface epithelium and endometrial stroma was evaluated. A total of 200 cells with stained nuclei were counted in each slide. The histological data were evaluated by descriptive analysis, and the morphometric data were analysed by the Tukey's test using the System for Statistical Analyses (SAS) software version 9.1 (2007). The immunohistochemical data were analysed using the GraphPad Prism 5.0 software (Prism Software, Irvine, CA, USA). Data normality was demonstrated using the Kolmogorov-Smirnoff test. Statistical differences were evaluated using the Student's t-test, and the analyses were considered significant if the P-value was ≤ 0.05.The bitches from the MPA-treated group presented a 35% decrease in the number of endometrial glands, a larger diameter of the endometrial glands, a greater epithelial height, as well as a greater thickness of the uterine wall, endometrium, and myometrium. However, no significant differences were observed between the two groups in the expression of ER-α, ER-β, and PR on the surface epithelium and endometrial stroma. Therefore, serial injections of MPA from birth until the age of 6 months caused a decrease in the development of endometrial glands by approximately 35% in bitches. However, the ER α and β and PR were expressed at normal levels on the uterine surface epithelium and endometrial stroma.

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Pulmonary embryo-fetal development in domestic cat (*Felis silvestris catus*). Preliminary results

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Lung development begins in early prenatal period and extends to the post-natal period and is divided into four periods: pseudoglandular, canalicular, saccular and alveolar. The lung becomes mature and functional only after birth. Amniotic fluid that fills the lungs prevents gas exchange during fetal life, but at birth, with the onset of ventilation, the liquid is absorbed by the epithelial cells. In the alveolar phase the surfactant is produced by the pneumocytes type II, it is an essential substance for the survival of the animal and its impaired production may be related to respiratory distress and high neonatal mortality. Based on this knowledge, the present study aims to evaluate the chronological development of the respiratory system in domestic cats (*Felis silvestris catus*), and identify the stages of specialization of the parenchymal lung, correlating them with fetal maturity. For this purpose, 10 fetuses with gestational ages between 33 to 48 days were collected from pregnant females submitted to ovariohysterectomy. Fetal age was estimated by measuring crown-rump length (CR) standard for cats. And based on fetal age, the following groups were formed: G1 fetuses between 30 and 37 days of gestation (n = 2); G2: fetuses between 38 and 42 days of gestation (n = 4); G3: fetuses between 43 and 50 days of pregnancy (n = 4). Samples of 0.5 cm^3 from the left lung lobe were washed with 0.9% saline solution, fixed in 4% paraformaldehyde solution buffered to pH 7.4 and 0.1M. Fetuses with CR below 5 cm were fixed entirely and after 48h were dissected. The samples were included in paraffin, sectioned at 5 um histological sections of the slides, stained with hematoxylin and eosin and evaluated by light microscopy. As a result, in a single section of evaluation, two stages of lung maturation could coexist due to a centrifugal lung development, in which the central portion of the organ developed earlier than the peripheral portion. The pseudoglandular phase was not observed until this moment. A reason of this may be because the gestational interval established at this project was 30 up to 50 days. Thus, the inicial differentiation of lung parenchyma might begin erlier in gestation. Canalicular phase was identified in the fetuses from 33 days to 38 days of gestation (G1). The tubular structures were more extended and branched. The amount of mesenchymal tissue reduced gradually, blood vessels began to emerge and proliferate and the epithelium became thinner. The saccular phase has been described between 43 and 48 days of gestation, corresponding to the experimental G3. At this stage, the tubular structures had great expansion with many saccular structures, and resembled an adult lung. There was an increased area of exposure to air. The mesenchymal tissue was greatly reduced and formed septa that divided sacs, and thus, there was an approximation of type II pneumocytes and blood capillaries, allowing gas exchange in air environment. Thus, with the results obtained so far, it is possible to observe a significant difference in lung development between cats and dogs. Regarding lung maturation, we observed that the canalicular stage in cats begins with at least fifteen days in advance compared to its appearance in dogs, a pattern also observed in the development of saccular phase. This result can justify the fact that problems related to respiratory distress by neonatal immaturity immediately after birth are more common in dogs to cats. Future studies are being developed to identify the degree of lung development at other moments of pregnancy, including birth prodrome. In addition, the morphometry results of the area from sacs and alveoli will be critical to the comprehension of the respiratory adaptation immediately at birth.

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Relationship between histological characteristics of fetal ovarian and pig fetuses measures

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The gonadal efficiency of the female adulthood is directly related to her development during uterine life, since the number of follicles and oocytes that can be recruited at puberty is determined before birth. Thus, the objective of the study was to evaluate the histomorphometric characteristics of fetal ovaries at different gestational ages and correlate them with the fetal length and head circumference and thoracic perimeters of fetuses. Twenty three female fetuses from eight DB-90 pregnant gilts (Danbred) in three different gestational ages (50, 80 and 106 days of gestation) were used. After slaughter of gilts, fetuses were separated, weighed and were measured the fetal length and head and thoracic circumferences. The fetal ovaries were dissected, weighed and fixed in Bouin's for the preparation of histological sections which were stained with hematoxylin-eosin. Histomorphometric analyzes were performed by software ImageJ. The gonadal parameters were subjected to ANOVA, and the different gestational ages were by statistically comparing the SNK test at 5% probability. After this, histomorphometric variables were correlated with fetal measurements by Pearson´s correlation test. The total number of follicles was higher ($P < 0.05$) at 80 and 106 days of gestation, whereas the number of oogonia decreasing ($P < 0.05$) during pregnancy. The weight of the fetus and fetal measures were positively correlated ($P < 0.01$) between the total diameter of the primordial follicle, the oocyte and the diameter oocyte nucleus of these follicles with fetal weight measures. There were also positive correlations ($P < 0.01$) between the diameter and volume of growing follicles as well as cell and nuclear diameter of oocytes with their weight and fetal measurements. Negative correlations ($P < 0.03$) between the gonadossomatic index, total number of oogonia and percentage of nucleus of oogonia with weight and fetal measurements were observed. It is concluded that the weight and fetal size are related to ovarian development in fetal life, which can an indicative of a reproductive potential in the adult.

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Resveratrol supplementation enhance oocyte nuclear maturation *in vitro*

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Supplementation of resveratrol (3,4',5-trihydroxystilbene) (RESV), a phytoalexin naturally occurring, has provided a beneficial impact on in vitro maturation of oocytes and pig embryonic development (Kwak et al., 2012). More recently observed beneficial effects also in the microenvironment oocyte by increasing glutathione peroxidase, decreased ROS levels, leading to stimulation of embryonic development and regulation of gene expression (Mukrerjee et al., 2014). The aim of this study was to determine whether the inclusion of resveratrol on *in vitro* maturation of oocytes could improve the maturity indexes. In this regard, cumulus-oocyte complexes (COCs) were aspired from slaughterhouse ovaries at Frigosaj (Feira de Santana-BA, 12º 16' 00" S and 38º 58' 00" W) and subjected to IVM (*in vitro* maturation). Fifty COCs (homogeneous cytoplasm and a minimum of three layers of compact cumulus cells were selected for further analysis) were matured per well of four-well plates, under mineral oil, containing 1 ml of maturation medium: TCM 199 with Earle's salts and 2.2 g/L sodium bicarbonate and L-glutamine were supplemented with 0.5% fatty acid-free BSA, 0.2 mM sodium pyruvate, 83.4 µg/ml amikacin and hormones (15 ng/ml of FSH, 50 µg/ml of hCG and 50 ng/µl EGF), following RESV supplementation with 0; 0.25; 0.5 e 0.75µM. Resveratrol was diluted in DMSO (dimethyl sulfoxide). IVM was performed for 24 hs at 38.8°C under 5% CO_2 in air and high humidity. Approximately 100 oocytes were used per treatment in three replications. The maturation rate was evaluated by expansion of COCs cells, according to Gomez et al. (2012): 0 = no expansion of COCs cells; 1 = minimal observable response; 2 = expansion of the outer layers of the COCs; 3 = expansion of all layers except the corona radiata; 4 = COC expansion of all layers. Nuclear maturation was evaluated after 24 hours of IVM under light microscopy with phase contrast (1000X). To this, 20% of the oocytes from each treatment were denuded mechanically and fixed with methanol-acetic acid (3: 1) and stained with 1% orcein in 40% glacial acetic acid solution, mounted in slide and cover slip and observed at microscope. Nuclear configuration analyzed were: germinal vesicle (GV), metaphase II (MII) metaphase I (MI) without chromosomal configuration (SCC). Data were analyzed by logistic regression using SAEG. Considering the significance level of $P < 0.05$. After 24 hours of MIV bovine COCs showed 100% expansion, but the level of 2, 3 and 4, which varied according to the treatment were not different ($P<0.05$). The degrees of expansion 3 and 4 are considered best in relation to others and possibly reflect the oocyte competence. For nuclear maturation as assessed by chromatin configuration 74% of total oocytes showed MII stage, and they differ for the control compare to different RESV concentrations evaluated ($P > 0.05$) (0 µM: 60.3±2.1; 0.25 µM: 73.3±1.3; 0.5 µM: 74.1±2.5 e 0.75µM: 75.1±1.3).The supplementation of resveratrol can improve maturation and development competence of bovine oocytes and may reflect in best embryo production further.

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Spermatogonial proliferation after testis injuries in man

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Several etiologies are related to human azoospermia, known as the absence of sperm in the ejaculate, being some of them originated from malformation or diseases, at a non-testicular level. The spermatogonial or proliferative phase of spermatogenesis, which contain the stem cells so called spermatogonia, is responsible for maintaining the number of sperm produced from spermatogonial mitosis. An effective way to predict sperm production would be through the assessment of spermatogonial proliferation. Although it is known that important changes take place within the testicular parenchyma in azoospermic subjects, the way such alterations affect spermatogonial proliferation is still not determined. Thus, the present study compared whether there are spermatogonial proliferation patterns among different groups of obstructive azoospermia, represented by individuals with agenesis of the vas deferens (AD) and orchiepididymitis (OE) and non-obstructive azoospermia, represented by individuals with varicocele (VA) and orchitis due to mumps (OM). Testicular biopsies from 24 patients [AD (9); OE (5); VA (7) and OM (4)] were fixed with 4% paraformaldehyde in phosphate buffer 0.05, pH 7.3 and embedded in paraplast for immunohistochemical analysis. Proliferating cells were positively labeled with anti-MCM7 antibody. The seminiferous tubules perimeter was measured using the Image-Pro Plus software (Media Cybernetics). Thereafter, the number of dividing (+ labeled) and non-dividing (- labeled) spermatogonia were counted in the same seminiferous tubules (ST) sections. The spermatogonial proliferation index was calculated as the number of dividing spermatogonia by millimeter of seminiferous tubules. The total number of spermatogonia was significantly lower in the non-obstructive groups, showing that there is a higher cell loss in the spermatogenic process than in the obstructive ones. The same behavior was found for the spermatogonial proliferation index, which indicates a higher probability to develop infertility problems. Therefore, the deleterious effects due to the increase of testicular temperature (VA) and of viral infections (OM) were the most severe, due to the reduced spermatogonial number, interfering with their proliferation capacity. (ETIC 032/04).

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Standardization of decellularization of canine placentas as biological *scaffolds* for regenerative medicine

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Placentas as temporary organs of reproduction offer interesting possibilities of application for regenerative medicine. A recent, but promising aspect is the use of placentas as biological scaffolds after decellularization processes that could be subsequently repopulated with various groups of stem cells in order to rebuild organs or tissues for transplantation. A good scaffold needs adequate blood supply options and preserved inner structure by an extracellular matrix to promote cell adhesion, migration and proliferation. Since the endotheliochorial placenta of canines possesses both fetal and maternal vessels in close association it seems to be of interest. For the first time, we intended to use dog placentas for such purposes. Primarily, we established an adequate protocol for the decellularization process, resulting in the addition of 5Mm Ethylenediaminetetraacetic acid (EDTA) for washing the organ and using a 0.1% solution of Sodium dodecyl sulfate (SDS) only, because stronger solutions destroyed the inner structure. Controls by histology indicated that cells were nevertheless completely removed. Immunohistochemistry was performed for collagen I, III and IV, fibronectin and laminin. Data showed that collagen III and IV were mainly associated with the cells, whereas type I was mainly found in the extracellular matrix. Our next steps will include Picogreen analysis to quantify the remaining DNA, molecular analysis and standardization of repopulation processes of the scaffolds. However, data so far confirmed that canine placentas indeed resulted as good choices for building biological scaffolds.

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Strategies to improve reproductive efficiency in recipient mares

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Pharmacological treatment and hormonal protocols utilized during the diestrus with the objective of improving pregnancy rates in recipient mares have been the main focus of studies in several research groups. This study was conducted to evaluate the effects of pharmacological interventions in regards to gestational rate. Experiment 1 consisted of the evaluation of administration of oxytocin and enrofloxacin IM to recipient mares on the day of ovulation. One hundred and sixty Mangalarga Marchador mares were divided into groups, according to cytological evaluation. Mares with negative cytology were divided into 2 groups, where Group 1 (n = 40) received oxytocin (20 UI IM) and enrofloxacin (2g IM) on ovulation day and Group 2 (n = 40) received no treatment. Mares with positive cytology were also divided into 2 groups, where Group 3 (n = 40) received oxytocin (20 UI IM) and enrofloxacin (2g IM) on ovulation day and Group 4 (n = 40) received no treatment. Experiment 2 evaluated the efficacy of slow release progesterone (P_4 LA) administered on ovulation or embryo transfer (ET) days in recipient mares with reproductive history of Poor or low pregnancy historic in ET programs. Eighty Mangalarga Marchador recipient mares participating in reproductive programs with a history of two previous unsuccessful ETs were selected and divided into two groups: Group P_4 LA (n=40) received 1500mg of P_4 LA (IM) after ovulation detection while the Control Group (n = 40) received 5mL of saline solution IM after ovulation detection. All mares were used as embryo recipients and in Experiment 1 Group 1 achieved 82.5% initial gestation rate, Group 2 70%, Group 3 57.5%, and Group 4 35.0%. Logistic regression model demonstrated a significant difference ($p < 0.05$) between groups 1 and 3, but not between 1 and 2 or 2 versus 3. In Experiment 2 initial gestational rate was 62.5% for Group P_4 LA while 35% for the Control Group. This difference was statistically significant ($p < 0.05$). Results from the present study indicate that treatment with 1500mg of intramuscular P_4 LA on ovulation day improves initial gestational rate in recipient mares with previous history of ET failure.

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Temporal and Cell-Specific Expression of Arginase, ODC1, ADC and AGMAT mRNAs and proteins in uterine endometria, placentomes and conceptuses during pregnancy in ewes

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Arginine is a conditionally essential amino acid that is important in pregnancy for mammals due to it being a substrate for synthesis of important molecules such as nitric oxide and polyamines. Arginine can be used to synthesize polyamines by a classical pathway in which arginine is converted by arginase (ARG1 and ARG2) to ornithine which is then converted to polyamines by ornithine decarboxylase (ODC1). Recent studies revealed an important alternative pathway for metabolism of arginine in which it is decarboxylated to form agmatine by arginine decarboxylase (ADC) and agmatine can then be converted into putrescine by agmatinase (AGMAT). In this regard, this study evaluated the expression on ARG1, ODC1, ADC and AGMAT mRNAs and proteins, and their cell-specific localization in endometria and conceptuses from Days 13, 14, 15 and 16 of the peri-implantation period of pregnancy and placentomes from Day 40, 60, 80, 100, 120 or 140 of gestation. On Days 13, 14, 15 and 16 of pregnancy the uterine horns were flushed with 20 ml of sterile PBS to collect conceptuses. Conceptuses were frozen in liquid nitrogen, stored at -80ºC, and subjected to RNA extraction to evaluate gene expression by qPCR. Sections of conceptuses and sections from the mid-portion of the uterine horn ipsilateral to the CL were fixed in fresh 4% paraformaldehyde and embedded in Paraplast-Plus (Sigma). For late pregnant ewes, several sections of placentomal tissues from the gravid uterine horn were fixed in fresh 4% paraformaldehyde for 24 h and then embedded in Paraplast Plus (Oxford Labware, St. Louis, MO). The data were analyzed as a completely randomized design with the fixed effects of day (df = 3) using MIXED procedure of SAS v9.4 (SAS Inst. Inc., Cary, NC). The effect of days was decomposed into three orthogonal polynomial contrasts (linear, quadratic, and cubic). Studentized residuals were plotted against predicted values to verify model assumptions. Outliers were defined as those values with a Studentized residual outside the ± 2.5 range of values. The relative abundance of *ARG1, ODC1* and *AGMAT* mRNAs in conceptuses differed among days (P=0.0165, P=0.0066, and P<0.001; respectively). Relative abundances of *ARG1* and *AGMAT* mRNAs decreased linearly as days of pregnancy increased (P =0.002 and P = 0.001, respectively); there was no significant difference between Days 13 and 14, but Day 13 differed from Days 15 and 16. The relative abundance of *ODC1* mRNA decreased from Day 13 to Day 16 (P=0.02) and the greatest expression was observed on Day 13 which differed significantly from Days 14, 15 and 16 among which days there was no difference. Immunohistochemical analyses detected all proteins on all days evaluated in the following tissues: conceptuses, uterine luminal (LE), superficial glandular (sGE) and glandular (GE) epithelia, and placentomes. For conceptus trophectoderm, ARG1, ODC1 and ADC protein abundances increased from Day 14 to Days 15 and 16, while the relative abundance of AGMAT protein was similar across all days. Levels of ARG1, ADC, and AGMAT appeared more abundant in uterine LE, while ODC1 was detectable at a similar level in both uterine LE and GE. The detectable levels of ADC protein increased in uterine epithelia over time, whereas ARG1, ODC1, and AGMAT levels appeared similar across all days examined. ARG1 and ADC were more readily detectable in placentomes from Days 60, 120 and 140 compared to inter-placentomal tissues. ODC1 was most abundant on Days 60 and 120, and AGMAT on Days 80 and 120 of pregnancy. Results of this study indicated the relevance of the alternative ADC:AGMAT pathway in different tissues for metabolism of arginine during both the peri-implantation period and later stages of pregnancy in sheep.

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The dynamics of different patterns of markers in the development of canine germ cells

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Primordial germ cells (PGCs) are known as the only cells capable of generating a new individual, the gametes will originate from them and will transmit the genetic material to future generations (CINALLI; RANGAN; LEHMANN, 2008). The specification of PGCs, as well as formation, proliferation and migration towards the gonadal ridges becomes different for each species. Usually, the mammalian germ line is determined by genetic factors and specific cell markers have essential functions to guide the direction of PGCs. The complete understanding of the pathways and "triggers" for initial migration and differentiation of these cells may be important to identify and correct possible flaws in these processes, which will be important in future for the development and reproductive performance to the specimen. It is known that the study material of human embryos are scarce and difficult to obtain, so it is necessary to use other animal models, such as the Canids, which also resemble humans. More than half of the hereditary canine diseases analogously affects the humans, showing similar genetic and cellular kinetics(KIRKNESS, 2013; PARKER; OSTRANDER, 2005; WHITWORTH; OVCHINNIKOV; WOLVETANG, 2012). Then, it has become interesting to expand the studies on canines' PGCs in order to show the importance of different models that might resemble humans. Therefore, this proposal aims to identify the canine's primordial germ cells (PGCs) in canine embryos in the first (15 to 30 days pregnancy), second (30 to 45 days pregnancy) and late (45 to 60 days pregnancy) pregnancy. Canine embryo gonads the first pregnancy (n=5), canine fetal gonads (n=9) were obtained during dogs neutering campaigns (Pirassununga-SP) and conducted in accordance with the Committee of Ethics of the Faculty of Animal Sciences and Food Engineering, University of Sao Paulo, Brazil (13.1.1729.74.2). Immunophenotypic characterization were performed in the gonadal ridge with the pluripotency marker and initial germ cell OCT4 (POU5F1), the marker VASA germ cell (DDX4), receptor c-Kit (CD117), and germ cell-specific RNA binding protein, DAZL. We observed that during the first period of pregnancy OCT4 were detected in the gonadal ridge. However, we were able to detect some OCT4 positive germ cells migrating along the way to the gonads, especially in mesentery and around of aorta. We have identified few positive VASA PGCs present in the gonads. The markers C-kit and DAZL were undetectable. On the second and third period, the marker of DAZL increased. OCT4, VASA and c-kit were low undetected on the 2nd and 3rd period of pregnancy. The conclusion is that patterns of expression to all the markers are temporally and spatially distinct of the others mammalian species, especially rodents. The markers OCT4 and DAZL match with results that were found in humans (ANDERSON et al., 2007). However, VASA and c-Kit are down regulation during development of canine germ cells.

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The Effect of Feeding in the production of embryos in culture media without antioxidants, with 50 uM/ml cysteamine and 5 ug/ml *Lippia origanoides*

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The production of genetically superior animals in a shorter period of time has allowed the use of large-scale production of biotechnology *in vitro* embryo. To have the production quality of embryos, the use of suitable means during embryo development is critical. Thus, the addition of antioxidants such as *Lippia origanoides* improves the environment and increases the production and quality blastocysts. The aim of this study was to evaluate the final production of embryos with and without performing the addition of new medium during cultivation (Feeding in D3) supplemented or not with antioxidants. Bovine ovaries were obtained from abattoirs and transported in saline 0.9% NaCl 38.0ºC to the laboratory. The follicular aspiration was performed with a needle (40x12G) coupled to a 5 ml disposable syringe, and follicular fluid was deposited in 50mL tubes for sedimentation of oocytes. After 10 minutes, the *pellet* was removed from the tube and washed in TCM-HEPES 199. We selected only those oocytes degrees 1 and 2, according to the analysis of complex *cumulus oophorus* cells. Oocytes were washed in TCM bicarbonate supplemented with 10% fetal bovine serum + 22 ug/ml sodium pyruvate + 50 ug/ml gentamicin sulfate + 5ug/ml LH + 1 µg /ml of FSH + 10 µg/ml of estradiol + 2.5 mg/ml of *Lippia origanoides* antioxidant and subsequently incubated in greenhouse cultivation in a humidified atmosphere with 5% CO_2, 38.5ºC for 22 hours.Then, the oocytes were fertilized with among $1x10^6$ sperm *in vitro* fertilization and incubated in a humidified atmosphere with 5% CO_2, 38,5ºC for 20 hours. After this period, the oocytes were denuded and directed to three treatments containing SOF-m (modified) without adding antioxidant (T1) medium supplemented with 50 uM/ml cysteamine (T2) and medium supplemented with 5.0 ug / ml of *Lippia origanoides* oil antioxidant (T3). Routines were divided into those who received half again the third day after fertilization (Feeding D3) and those where there was no such replacement. Normality of continuous variables were analyzed by the Shapiro-Wilk test. Statistical analysis was performed using the Student t test, with significance level of $P < 0.05$. Cleavage rates were 77.2; 81.1 and 70.7% for T1, T2 and T3, respectively, who underwent Feeding the D3. As for the treatments that did not perform the D3 Feeding the cleavage rates of T1, T2 and T3 were 73.8; 61.7 and 81.4%, respectively. The blastocysts production rate was 37.1; 27.8 and 31.6 for T1, T2 and T3, respectively with Feeding on D3. Without Feeding on D3, the production rate was 44.4; 38.7 and 49.3% for T1, T2 and T3, respectively. Treatment 2 was only one difference ($P < 0.05$) in cleavage and blastocyst rates as the realization of Feeding on D3. Preliminary results showed that supplementation with Cysteamine at a concentration of 50 uM / ml in culture medium without Feeding the D3 positively influenced both the rate of cleavage as blastocysts. In addition, treatment with *Lippia origanoides* oil at a concentration of 5.0 ug / ml, along the non-operation of Feeding on D3 showed a tendency to increase in cleavage rate and blastocyst.

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The trans-10, cis-12 conjugated linoleic acid reduces apoptosis and increases the number of cells in the inner cell mass of bovine in vitro produced embryos

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In vitro produced embryos (IVP) have high concentration of cytoplasmic lipids, interfering with survival after transfer and resistance to cryopreservation (1). One strategy to achieve better quality IVP embryos would be the addition of conjugated linoleic acid *trans-10, cis-12* (CLA) in the culture medium (2). CLA reduces lipogenesis in cells, improved embryo quality and possibly decreasing its sensitivity to cryopreservation (2, 3). Was evaluated the effect of CLA addition to *in vitro* culture medium in the blastocyst production and embryo quality. Three culture media were used: control (n = 1052 oocytes): SOF medium plus BSA and FBS, without the addition of CLA; FBS + CLA (n = 1054 oocytes): SOF medium plus BSA, FBS and CLA; CLA (n = 1061 oocytes): SOF medium plus BSA and CLA without adding FBS. On the eighth day of culture, blastocyst from the three treatments were stained, 22 blastocysts Control group, 14 group FBS + CLA and 16 of the CLA group. All embryonic blastomers marked by DAPI were visualized at 460nm blue filter fluorescence microscope. Embryonic cells undergoing apoptosis were stained in green and identified at 520 ± 20nm filter in an epifluorescence microscope. The counts of total embryonic cells and apoptotic cells were performed using the software Image J 1.41. Was found cleavage rates of 74.05%, 67.65% and 75.40% for control groups, FBS + CLA and CLA respectively (P> 0.05). Embryo production rate in relation to the cleaved embryos was 39.15% for the control, 26.93% for SFB + CLA and 20,50% for CLA (P> 0.05). The number of cells contained in the inner cell mass was similar for all three treatments, but the control showed a higher number of cells in the trophoblast (70.6) compared to other groups, found 41.3 cells to SFB + CLA and 47.2 cells for the CLA group (P <0.05). Additionally, the average number of apoptotic cells was also higher than the control (79.6) than in FBS + CLA (50.3) and CLA (58.5) (P <0.05). The addition of CLA to the culture medium increases the embryo quality, reducing apoptosis and increasing the proportion of cells in the inner cell mass in relation to trophoblast cells in bovine embryos produced in vitro.

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Use of dexamethasone as antenatal therapy affects placentome microcirculation in goats? - Preliminary data

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Antenatal therapy with corticosteroids have been widely established as standard protocol in women at high risk to premature birth, with the purpose to reduce respiratory distress syndrome in newborns (Bonanno et al., 2008. Am J Obst. 5:448-456). Small ruminants are often used as an experimental model for these studies with the aim of optimizing the dose and time of administration of these drugs (Morel et al., 2012. Theriogenology, 78:1763-1773). The aim of this study was to evaluate and compare the Doppler indices of arterial branches of placentome in pregnant goats submitted to different dexamethasone base protocols. Three protocols were tested: GI (n=4) goats received a single injection of 20mg/i.m at 139 day of gestation; GII (n=5) were given every 24h 2mg/i.m between 133 and 136 days, 4mg at 137 and 138 days and 20mg at 139 day; and GIII (n=5) were given 16mg/i.m every 12h at 139 and 140 days. All animals were submitted elective cesarean section to 141 days of pregnancy to obtain premature kids. Hemodynamic changes of two placentome were assessed by spectral Doppler ultrasonography (Mylabvet70®, ESAOTE, Genova, Italy). There was no difference between the different treatments for flow velocity integral (6.78±1.38, $p>0.67$), pulsatility index (1.61.69±0.68, $p>0.21$), resistive index (0.60±0.17, $p>0.40$); peak velocity (25.14±3.41, $p>0.55$), end diastolic velocity (11.26±1.93, $p>0.86$) and mean velocity (15.40±1.69, $p>0.36$). In conclusion, the placentome microcirculation is not affected by the different doses or by corticosteroid administration time.

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Gonadal steroid hormones levels in a bitch with true bilateral hermaphroditism

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True hermaphroditism is one type of intersexuality that may involve swine, goats, cattle, horses and dogs. It's characterized for the presence of gonads from both sexes in the same individual. True hermaphroditism can be bilateral (both gonads are ovotestes), unilateral (one gonad is an ovotestes and the other is an ovary or a testis) and lateral (one gonad is an ovary and the other a testis). Hermaphroditism is a not well-understood desordered sexual development in dogs. Thus the aim of this summary is to demonstrate testosterone, progesterone and estrogen levels recovered from a bicth with true bilateral hermaphroditism, diagnosed by histopathological examination of the gonads after ovariohysterectomy surgery, due to the few case reports published involving hormonal levels in dogs with this intersexuality. Serum was obtained from the animal before surgery and was submitted to hormone measurements. Progesterone and testosterone were evaluated by radioimnunoassay using kits from Beckman Coulter and Gamma Counter Perkin Elmer and estrogen was evaluated using Cayman plates by ELISA method with Biotek ELx808. The values of progesterone, testosterone and estrogen levels were: 2.26 ng/mL, 0.05 ng/mL and 9,70 pg/mL, respectively. The proportion of ovarian tissue found in the ovotestes after histopathological examination was higher in relation to seminiferous tubules, which may explain the low level of serum testosterone. Low concentration of serum testosterone may contributted to the parcial virilization of external genitalia, since the animal only had clitoral hypertrophy. Estrogen and progesterone levels found were compatible with the final stage of proestrus, which shows that ovarian follicles found in the bilateral ovotestes were functional contributing to the occurrence of all stages of the estrous cycle before surgery. More studies are needed to elucidate the real cause of intersex in dogs. Quantitation of serum hormones may also shed light on the cause of canine ovetestes.

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Treatments with eCG stimulate courtship behaviour in rams during the breeding and the non-breeding seasons

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Equine Chorionic Gonadotrophin (eCG or PMSG) is a hormone with FSH/LH effect, that has been widely used in ewes and cows to induce ovulation, but has been scarcely used in males. ECG increases rams' ability to stimulate ovulation anoestrous ewes (the "ram effect"), but the pathways by which this is achieved are not known. Thus, the aim was to determine if administration of eCG improves rams' sexual behaviour in Saint Croix rams in both, the breeding and the non-breeding seasons. Two doses of 1000 IU of eCG were administered separated 4 days to 10 rams during the breeding and 5 rams during the non-breeding seasons on Days 0 and 4 of the study, remaining the same number of animals as untreated controls. On Days -7. -5, -3, 2 and 6 blood samples were collected for testosterone measurement and sexual behaviour of each ram toward a non-oestrous ewe was recorded. Each ram was tested with a different ewe secured at a portable station fixed in the middle of a 2 x 5 m pen, equipped with plastic walls to isolate them from visual outside distractions and sight from other rams. The number of anogenital sniffs (AGS), lateral approaches (LA), mount attempts, mounts and mounts with ejaculation were recorded by blinded observers during 20 min. The interval from the onset of the test to the first ejaculation was also recorded. The total number of courtship behaviors (AGS+LA), of mounts (mounts without ejaculations + mounts with ejaculation), and the ejaculation/total mounts ratio, were calculated. During the breeding season, testosterone concentrations were greater in treated than untreated rams (P=0.008), and there was a significant interaction with time (P<0.0001). This interaction was explained by greater concentrations in treated rams on days 2 (P<0.0001) and 6 (P=0.0008). The number of AGS and LA, as well as total courtship behaviours were greater in treated than untreated rams [AGS: log (x+1): 0.87 vs 0.72, SEM: 0.05, P=0.002; LA: 43.9 vs 27.1, SEM=5.0, P=0.028; total courtship: 52.2 vs 33.3, SEM=5.0, P=0.015 for treated and untreated rams, respectively] (Figure 1B, 1C and 1D, respectively). Mount attempts (0.87 vs 0.87, SEM=0.2), mounts without ejaculation (16.8 vs 15.0, SEM=2.0), and total sum of mounts (18.4 vs 16.3, SEM=1.9) were similar in treated and untreated rams. Treated rams tended to mate more than untreated rams (1.68 vs 1.02, SEM=0.24, P=0.07), and had a greater mate/total mount ratio (0.15 vs 0.09, SEM=0.02, P=0.026). There was no effect of eCG in the time elapsed for the first ejaculation (127.1 vs 147.5 s, SEM=32.1). During the non-breeding season, testosterone concentrations were greater in treated than untreated rams (P=0.0009), and there was a significant interaction between treatment and time (P<0.003), explained by greater concentrations in treated rams on days 2 (P=.0001) and 6 (P=0.007). The number of LA, as well as total courtship behaviours were greater in treated than untreated rams (LA: 51.1 vs 33.6, SEM=4.6, P=0.029; total courtship: 59.2 vs 41.7, SEM=4.8, P=0.034 for treated and untreated rams respectively] (Figure 2B and 2C, respectively). The number of AGS (8.1 vs 8.1, SEM=0.8), mount attempts (6.8 vs 4.4, SEM=1.2), mounts without ejaculation (4.6 vs 5.8, SEM=1.0), and total sum of mounts (7.5 vs 8.7, SEM=1.1), mates (2.8 vs 2.9, SEM=0.4), mate/total mount ratio (0.4 vs 0.4, SEM=0.1), and the time to the first ejaculation (124.9 s vs 99.9 s, SEM=31.1) were similar between treatments. Administration of eCG determined a great increase in testosterone concentrations associated with an increase of sexual behaviours, mainly courtship events. During the breeding season there were also slight positive effects on mating behaviour.

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Use of Kisspeptin-10 to induce ovulation in prepubertal gilts. Preliminary results

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The use of hCG associated with a previously injection of eCG is the standard treatment recommended to induce estrus and ovulation in gilts and sows. Recently, it was shown that the neuropeptide kisspeptin is thinly associated with the pituitary LH release in different mammalian species. Thus, this study aimed to determine whether kisspeptin-10 associated with eCG could be an efficient method to induce ovulation in prepubertal gilts. Twenty commercial hybrid gilts of 5 months of age, with an average weight of 87 ± 8.4 kg were randomly distributed into four groups of five animals each. Group 1 (control) received an injection im of saline solution, while groups 2, 3 and 4 received an injection im of 750 IU eCG (Novormon® MSD Animal Health, São Paulo, Brazil). After 72 hours, the group 2 received an injection im of 0.5 mg of kisspeptin-10 (Kisspeptin-10, Genscript, USA), the group 3 received an injection im of 500 IU of hCG (Chorulon®, MSD Animal Health, São Paulo, Brazil) and groups 1 and 4 were injected with saline solution. The animals were slaughtered seven days later and ovulation was confirmed by the presence of corpora lutea on the ovaries. Data were analyzed by Anova (SAS 9.2 TS). All females in groups 2 and 3 ovulated, whereas animals of group 1 and 4 did not ovulate. Mean ovulation in groups 2 and 3 was 18.5 ± 4.5 and 17.5 ± 3.5 respectively, (P>0.05). There was no influence of treatment on the weight or diameter of the ovaries (P>0.05). These results suggest that kisspeptin-10 presents biological activity comparable to hCG to induce ovulation in prepubertal gilts. Additional studies with a larger number of repetitions are in progress to validate the results of this study, including the incorporation of variables related to fertility of the induced ovulation.

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AMH in buffalo's ovaries from fetuses: plasma concentration and immunolocalization

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The anti- Mullerian Hormone (AMH) is responsible for the regression of the Mullerian duct in the male fetus and it is produced by the Sertoli cells (Jost, 1953), however specific receptors AMH are present in fetuses from both sexes (Rey et al, 2003). In the ovary, granulosa cells are embryologically homologous to Sertoli cells and also produce AMH (Vigier et al, 1984 Munsterbrg & Lovell-Badge, 1991). There are no studies on AMH in buffalo fetuses, therefore the objective of this study was to analyze AMH expression by evaluating the plasma concentration of maternal and fetal AMH, make the immunolocalization of AMH in fetal ovaries and thus determine the population of ovarian primordial follicles in buffalo fetuses. For this we used mother's blood samples (n = 14) and fetus (n = 16), ovaries from fetuses (n = 16) aged between 3 and 8 months (15-98cm CRL) (Abdel- Raoulf et al, 1974) collected at a slaughterhouse. Blood samples were processed to obtain plasma, and the ovaries were processed for conventional histology and stained with H.E for quantification, according follicular Gougeon and chainy (1987). Plasm concentration of AMH was determined using the ELISA kit (MOFA 21700/1000). The immunohistochemistry was performed using anti AMH AMH SC - 28912 antibody (1:50, Santa Cruz) according to the manufacturer 's instructions. The plasma concentration of AMH in mothers was 50.82 pg/mL (± 16:22) , 71.53 pg/mL (± 49.54) , 45.81 pg/mL (± 9.14), 100.18 pg/mL (± 25.6), ranging from 30 to 130 pg/ml at 3-4 , 5, 7 and 8m of gestation, respectively. At 6 months of pregnancy only maternal blood sample were collected and they showed a concentration of 33.09 pg /ml AMH, no statistically significant difference ($p \leq 0.05$) was detected between gestational ages. The plasma concentration in fetuses was 234.48 pg/mL (±79.88), 366.27 pg/mL (± 383.94), 43.51 pg/mL (± 14.74) , 352.36 pg/mL (±513.36) and 81.13 pg/mL (± 43.12), at 3-4 , 5 , 6, 7 and 8m of gestation , respectively , ranging from 33e 944 pg / ml , with no statistically significant difference ($p \leq 0.05$) between gestational ages.The immunolocalization of AMH was carried out in fetal ovaries from 5 to 8 months and showed intense staining in the cytoplasm of oocytes from primordial follicles at 5 and 6 months (35-62cm CRL) and weak staining at 7 and 8 months (CRL 63-98cm). Granulosa cells showed low signal intensity while theca cells and ovarian stroma showed no signals. The average number of primordial follicles was 28,020.00 (± 26,736.09), 32,479.33 (±13,743.11), 11,581.33 (±10,811.42) and 9,996.66 (±4331.27) in 5, 6 , 7 and 8 months, respectively , showing no statistically significant differences ($p \leq 0.05$) between the ages, ranging from 2,635-62,283 primordial follicles. Primary follicles were observed at 5 months, 6 months and secondary/antral at 7 months. Our results shows for the first time the presence of AMH in buffalo fetal blood and ovaries, suggesting no relationship between the concentration fetal AMH with the population of primordial follicles. About AMH concentration in pregnancy, the results suggest that AMH production in the mother is independent of AMH production in the fetus. The variations observed between AMH levels in maternal and fetal plasma and between immunolocalization in different fetal ages and follicular population is large and difficult to interpret the role of AMH in ovarian physiology so is necessary further studies particularly related to the participation of AMH in ovarian development.

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Characteristics of the estrous cycle of jaguars *(Panthera onca)* revealed by estrogen concentrations assessed noninvasively Via enzyme immunoassay

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The jaguar (*Panthera onca*) is one of most charismatic species of the native fauna of Brazil, and is the largest cat of the Americas; yet, compared to other large felids, little has been studied about this species' reproduction. Valuable information on the reproductive function of wild felids can be collected noninvasively by assessing concentrations of reproductive hormones in fecal samples; therefore, the objective of this study was to test an enzyme immunoassay protocol to assess ovarian function activity noninvasively in jaguars to study the estrous cycle in the species. Four adult female jaguars housed in Brazilian zoological institutions were used in this study. Fecal samples were collected during two to four consecutive months. Hormone extraction was performed by adding a total of 5 mL 90% ethanol to 0.2 g of lyophilized feces and homogenizing the mixture overnight. Fecal estrogen concentrations were assessed using the polyclonal antibody anti-estrone-glucuronide R522-2. This method was considered efficient in detecting changes in estrogen concentrations after serial dilutions through parallelism. Individual baseline concentrations of fecal estrogens (Females #1-4), observed during interestrus, were as follows: 149 (± 51 SD); 50 (± 17); 97 (± 32); and 81 (± 30) ng per gram of lyophilized feces, respectively. Overall, mean baseline concentration of fecal estrogens was 94 (± 41) ng/g. Individual peak values of fecal estrogens, which were considered indicative of estrus, ranged from 334 to 1,812 ng/g, with an overall mean of 752 (± 429) ng/g. The length of the estrous cycle (n = 7 estrous cycles) was determined by the interval between two consecutive peaks, having a mean value of 41 (± 11) days (range, 22-62 days). A parallel study using the same samples to measure progestin concentrations did not detect increases that would be indicative of spontaneous ovulation in these four females during the period of sample collection. In conclusion, the present protocol was efficient at detecting fluctuations in estrogen production in jaguars, and the mean duration of the estrous cycle was in agreement with the few previous descriptions of estrous cyclicity in the species assessed through different methodologies. (CEUA approval protocol: #053/13).

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Diameter follicular and ovulation rate in Girolando heifers treated with different fixed time artificial insemination protocol

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The Brazilian dairy industry has gone through a time where search increasingly intensifying and improving production efficiency for maintenance in the activity. For this it is essential to search for better reproductive rates, which can be achieved with the help of reproductive biotechnologies, among which we can highlight the artificial insemination in fixed time (FTAI). The FTAI have substantial advantages in reproductive management of dairy cattle, including allowing the improvement of the reproductive performance of high milk yield. The objective of the present study was to evaluate the follicular diameter on the day of artificial insemination (DAI), the diameter of the ovulatory follicle (OF), the estrus expression and ovulation rate in heifers receiving two different TAI protocols. Girolando heifers (3/4 to 7/8 Holstein) were subjected to two TAI protocols: Protocol 1 (P1) (n = 30): Administration of GnRH (Gonaxal®)10.5µg intramuscular (IM) on day of insert of intravaginal progesterone device (P4) (Primer®) (D0); administration of 150µg IM of prostaglandin F2α (PGF2a) (Croniben®), and removal of the P4 device on the fifth day (D5), PGF2α IM was administered on the sixth day (D6), AI and administration 10.5µg IM of GnRH on the eighth day protocol (D8). Protocol 2 (P2) (n = 30) administration of 2.0 mg IM of estradiol benzoate (EB) (Bioestrogen®) and insert of intravaginal P4 device (D0), administration of 150µg IM of PGF2α on day seven (D7), withdrawal P4 device and application 1mg IM Estradiol Cypionate (E.C) (Croni-cip®) on the ninth day (D9), AI on the eleventh day of the protocol (D11). Transrectal ultrasonography were made (Mindray®, DP2200vet model, the linear transducer coupled 5,0MHz) at the DAI extended 12 in 12 hours until moment before to ovulation (the ovulatory follicle - OF) in each animal. Animals that had some moment the absence of the largest follicle between evaluations were to have ovulated deemed. Follicular diameters on DAI, and OF were analyzed for normality by the Shapiro-Wilk test. Confirmed the normality of the data, the T test was used to analyze the effects of treatment on this variables (averages). Data for estrus expression and ovulation rate were analyzed by Chi-square test, and was considered significant when $p<0.05$. Results are expressed as means ± the standart error from de mean. The diameter of the largest follicle did not differ between P1 and P2 on DAI, it is 13.33 ± 0.45 mm and 12.18 ± 0.86 mm, respectively ($p>0.05$). The OF also did not differ and showed values of 13.47 ± 0.47 mm and 13.67 ± 0,86 mm, for P1 and P2, respectively ($p> 0.05$). The estrous expressions rate was lower in the animals P1 (50.0%) than in animals P2 (93.1%) ($p<0.05$). However, the ovulation rate show tendency ($p = 0.06$) to be higher in animals P1 (83.3%) than in the animals of P2 (62.1%). In conclusion, no treatment effect was detected on follicular diameters at AI day and ovulatory follicle, and estrus expression rate, but the ovulation rate tend to be higher in heifers treated with protocol 1. (CEUA/UFU/Protocol#056/2016).

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Effect of *Justicia insularis* on *in vitro* culture of sheep ovarian tissue

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This study aimed to evaluate the implication of *Justicia insularis (Jus.)* on the morphology, activation and levels of Reactive Oxygen Species (ROS) of ovine preantral follicles (primordial and developing follicles) included in ovarian tissue *in vitro* cultured for 1 or 7 days. Ovarian fragments were immediately fixed (non-cultured control) or *in vitro* cultured in α-MEM$^+$ (negative control), α-MEM$^+$ supplemented with FSH 50 ng/mL (positive control), or in α-MEM$^+$ supplemented with lyophilized plant extract *Jus.* (0.3; 1.25 and 5 mg/mL) for 1 or 7 days, at 39°C, 5% CO2. The ovarian fragments were processed, coded and analyzed by a blinded observer by classical histology and the follicles were classified individually as histologically normal or degenerated based on their morphology. Furthermore, the follicular activation was calculated based on the percentages of healthy primordial and growing follicles before (fresh control) and after culture in each medium. The percentages of morphologically normal preantral follicles in non-cultured ovarian tissue (control) was 84.6% and after 7 days of culture, there was a significant decrease ($P<0.05$) on these percentages in all treatments except *Jus.* 0.3 mg/mL when compared with non-cultured control. Lower ($P<0.05$) percentage of activation 25,4% and 34,5% with *Jus.* 1.25 and *Jus.* 5 mg/mL respectively was observed when compared to the fresh control. Furthermore, *Jus.* 0.3 mg/mL showed positive antioxidant effects on day 1 and maintained the ROS level unchanged from day 1 to day 6. It can be concluded that 0.3 mg/mL of *Jus.* have an important role in the maintenance of ovine preantral follicle viability and promote low follicular activation. This medicinal plant seems to be a good scavenger of ROS. (CEUA/UECE/# 6004720/2015).

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Effects of exogenous melatonin on resumption of ovarian function in undernourished ewes at Argentina

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Reproductive seasonality and nutritional condition are the main factors that influence reproductive performance in sheep, particularly in the central region of Argentina, where the availability of food is highly seasonal. Undernutrition can compromise follicle-oocyte competence, luteal function and embryo development. On the other hand, melatonin treatment is an effective method for inducing estrous cycles, increasing ovulation and improving embryo viability during anestrus. Therefore, we hypothesized that melatonin can override the detrimental effects of undernutrition to improve reproductive performance in ewes. In this regard, the effect of exogenous melatonin on ovarian function in undernourished ewes during anestrous season in Argentina was investigated. In October, 24 adult ewes (weighed 51.2±2.1 kg, body condition scored (scale 1-5) 2.65±0.16, mean±SEM) housed in an open barn at the Río Cuarto University facilities (30º07´S, 64º14´W), were assigned into two groups: treated (MEL) or not with a subcutaneous implant of melatonin (Melovine®, CEVA). They were kept under natural photoperiod. After 40 days, both groups were synchronized with intravaginal pessaries and eCG (400 IU) and fed to provide 1.5 (Control, C) or 0.5 (Low, L) times daily maintenance requirements. Therefore, ewes were divided into four groups (n=6): C-MEL, C+MEL, L-MEL and L+MEL. Estrus detection was performed twice daily and at estrus (Day=0) ewes were mated. From Day 15, for 5 days, a second estrus detection and mated was performed. Ovulatory follicles from Day -5 to ovulation were monitored daily by transrectal ultrasonography with a real time, B mode and rigid 7.5 MHz linear-array transducer. The day of ovulation was detected by collapse of a large follicle. Pregnancy diagnosis was determined at Day 28 after estrus. Statistical comparisons of proportional values were based on the Chi-squared Test. The level for statistical significance was set to $P < 0.05$. After 21 days, groups L-MEL and L+MEL had an average weight loss of 5 kg ($P<0.05$). Neither nutrition and melatonin nor their interaction had a significant effect on ovulation rate or maximum diameter of ovulatory follicle between groups. However, melatonin increased ovulation rate in undernourished ewes (L-MEL: 33%; L+MEL: 67%; $P<0.01$). No effect of melatonin treatment on pregnancy rates was observed (C-MEL: 50%, C+MEL: 67%, L-MEL: 33% and L+MEL: 33%). Only non-pregnant ewes from melatonin treatment were capable to had a natural ovulation after the induced estrus ($P<0.01$). In fact, after the second period of estrus detection and mated, pregnancy rates was significantly higher in melatonin groups than the others (C: 50%, C+MEL: 83%, L: 50%, L+MEL: 67%; $P<0.01$). In conclusion, this study shows that melatonin improved resumption of ovary activity in anestrus, particularly in undernourished ewes at Argentina. A better understanding of modulating effects of melatonin on the ovarian function is essential to develop management systems looking for to enhance reproductive performance.

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Endometrial thickness of bitches in different periods of diestrus

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The phases of the canine estrous cycle are divided into anestrus, proestrus, estrus and diestrus and the bitch is considered nonseasonal monoestric. The dog has a very characteristic diestrus phase that is longer than other animals, lasting 55-75 days. The diestrus phase is characterized in bitch for increasing of P4 in the first middle and E2 fluctuations in the second middle of the phase with a peak of P4 between days 15 to 25 post ovulation (P.O.) and peak of E2 around day 40. During the estrous cycle, the endometrium of bitch undergoes morphological and biochemical changes that are associated with the proliferation, apoptosis and cell differentiation. The objective of this study was to determine the thickness of the endometrium in diestrus phase of bitches on specific days 10, 20, 30, 40, 50 and 60 P.O. Right and left uterine horns of 26 clinically healthy dogs of different ages and with no defined breed were analyzed histologically. The experiment was approved by the Ethics Committee on the use Animals (CEUA), number 1863. The dogs underwent ovariohysterectomy (OSH) to 10 (n = 10), 20 (n = 8), 30 (n = 8), 40 (n= 8), 50 (n = 8) and 60 days (n = 10) P.O. Hematoxylin-eosin staining was performed on slides and subsequently the slides were photographed by a camera attached to the optical microscope objective of 4x. The evaluation were made in 20 endometrial thickness per slide and it was measured by using the software MOTIC Imageplus 2.0®. All parameters were assessed for normality assumption Shapiro-Wilk, considering normal data when P> 0.01. The samples were subjected to ANOVA followed by Tukey test (P <0.05). The mean thickness of the endometrium from the uterine horns was: 10 days = 1017.98 μm; 20 days = 1237.8 μm; 30 days = 1137.54 μm; 40 days = 1179.19 μm; 50 days = 1048.52 μm; and 60 days = 1222.01 μm. No differences were found statistics in endometrial thickness at different times of the diestrus, 10, 20, 30, 40, 50 and 60 days after ovulation. The conclusion is that endometrial thickness of bitches did not differ between the post-ovulation days.

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Estradiol changes the immunohistochemical profile of the endometrial proteins PKCγ, AKR1B1 and estradiol α receptor in cattle

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In cattle estradiol (E_2) has an important role in the endometrial PGF2α release associated with luteolysis, however, the molecular mechanisms involved in such process are poorly understood. The PGF2α synthesis is the result of a series of intracellular events that include the participation of kinase C gamma protein (PKCγ), aldo-keto reductase family 1 member B1 (AKR1B1), estradiol receptor α (ERα) and progesterone receptor (PGR). The main objective was to investigate using immunohistochemistry the PKCγ, AKR1B1, ERα and PGR immunostaining in endometrial tissue of Nelore cows treated or not with 3 mg of 17β-estradiol intravenously on day 15 of the estrous cycle 0, 4, and 7 hours after injection. Nelore (N = 52), pluriparous, cyclic and non-lactating cows received 2 mg of estradiol benzoate (Sincrodiol Ourofino®) and an intravaginal progesterone device (1g; Sincrogest Ourofino®) during 8 days. The cows received 0.5 mg of sodium cloprostenol (Sincrocio; Ourofino®) via IM, 48 hours before the device's removal and a second application the day of removal. On day 15 of the estrous cycle (D0; estrus) the following treatments were administered: placebo (P; 5 ml of ethanol 50%; IV), estradiol (E; 5mL of 50% ethanol containing 3 mg of 17β estradiol; IV) or control (not treated). Time 0 was the moment of the treatment application. Cows were subjected to a transcervical endometrial biopsy, and according to the time of biopsy were divided into the following groups: time 0 in the control group (C; n = 10), time 4 hours (E4, n = 11 and P4, n = 10), and 7 hours (E7, n = 10 and P7; n = 11). The tissue was fixed in 4% buffered-formalin for 24 hours and then stored in 70% alcohol until paraffin embedding. Endometrial sections were evaluated by immunohistochemistry and immunostaining was evaluated in the luminal epithelium (LE), glandular epithelium (GE) and stroma (S). The statistical differences were determined by t test and considered when $P < 0.05$. The results of PKCγ protein showed higher immunostaining in the LE of E4 and E7 groups compared to P4 and P7 and increased labeling in GE of E7 compared to P7, but increased labeling in GE of P4 compared to E4 . The AKR1B1 protein showed higher immunostaining in the LE of E4 compared to P4. The ERα shows a higher immunostaining in the GE of P4 and P7 groups compared to E4 and E7 and higher immunostaining in LE of P4 when compared to E4 group. The PGR shows a higher immunostaining in the LE of E4 and E7 compared to P4 and P7, and a higher immunostaining in the GE of E7 compared to P7. It is concluded that E_2 increases immunostaining of the PKCγ, AKR1B1 and PGR and reduces the immunostaining of ERα in endometrial tissue. Thereby E_2 modifying the concentration of these endometrial receptors and proteins that are involved on PGF2α synthesis.

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Evaluation of ovine pre-antral follicles after *in situ* culture in medium supplemented with EGF and GDF -9

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Ovarian sheep contains thousands of immature oocytes predominantly included in the pre-antral follicles. These follicles are considered a potential source of fertilizable gametes, what is of great interest to ensure the *in vitro* growth and allow the acquisition of competence of oocytes from these follicles. However, during the female reproductive life, few primordial follicles reach the pre ovulatory follicle stadium, after starting their *in vivo* growth, because during this process about 99.9 % of follicles undergo atresia. The follicular growth and maturation are controlled by a perfect interaction between endocrine, autocrine and paracrine factors. Many studies demonstrated that growth factors such as EGF, FGF, IGF, TGF- ß, GDF -9 and KL are of great importance during this process, which may act in different ways on all stages of folliculogenesis. Therefore, this study aimed to verify the population of pre-antral follicles enclosed in sheep ovarian tissue after *in situ* culture in medium supplemented with different concentrations of epidermal growth factor (EGF) and growth differentiation factor-9 (GDF-9). Ovaries were obtained from abattoirs (n = 60) and fragmented. A fragment of each pair of ovaries was used for the histology (fresh control), and the others were cultured *in vitro* for 8 days at 39 °C and 5% CO_2. The base medium was α-Minimum Essential Medium added of supplements (α-MEM+), and the fragments were cultured in pure α-MEM+ (culture control) or added with 10, 25, 50, 75 or 100 ng/mL of EGF or GDF-9 (treatment groups). After culture, all fragments were processed for conventional histology and stained with hematoxylin-eosin technique (HE). The number of pre-antral follicles was evaluated in the slides of fresh control, culture control and treatment groups in triplicate. It was counted 30 follicles in each section, which were classified in primordial (PL), primary (PO) and secondary (SE) follicles. Data were subjected to ANOVA ($P < 0.05$). The results showed that medium containing 25 and 50 ng/mL of EGF or GDF-9 showed an increase ($p < 0.05$) in the follicular activation (SE follicles) when compared to fresh control. In addition, treatment with 50 ng/ml of EGF and 25 ng/mL of GDF-9 showed the better results ($p < 0.05$) compared to culture control, after 8 days culture. Given the above, it was concluded that α-MEM+ medium supplemented with reduced concentrations of EGF or GDF -9 is enough to promote the follicular activation of ovine primordial follicles in the in vitro culture. Other studies will be performed in order to test the efficiency of these two growth factors used together in the medium.

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Evaluation of vitrified goat ovarian tissue after autotransplantation

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Transplantation of cryopreserved ovarian tissue before chemotherapy is a promising assisted reproductive technology (TRA) to preserve fertility for prepubertal girls or adult women with cancer. Therefore, this technology can also be applied to preserve the reproductive function in animal's farm. In this way. The current study was designed to compare fresh and vitrified-warmed goat ovarian tissue after autotransplantation. Eleven adult goats (n =11) were completely ovariectomized after premedication and anesthesia. After ovariectomy, each ovary pair was fragmented ($5x5x1mm^3$) and distributed as follow: seven fresh ovarian fragments were directly autotransplanted in the left side of the peritoneum and three fresh fragments fixed in paraformaldehyde 4% for classical histology and named as *Fresh Control*. Simultaneously, ten fragments were vitrified for one week. After warming, the fragments were autotransplanted to the right side of the peritoneum after a second surgery. The remaining vitrified/warmed fragments (3 fragments) were then fixed in paraformaldehyde 4% for classical histology and named as *Vitrified Control*. After 90 days of autotransplantation, three antral follicles (two in fresh graft and one in vitrified-warmed graft) were observed on the surface of the grafts. Moreover, one oocyte was punctured from an antral follicle of a fresh fragment. The percentage of normal preantral follicles reduced ($P < 0.05$) in vitrified control in comparison with fresh control. However, similar percentage ($P > 0.05$) of normal follicles was found between fresh control, fresh transplant, and vitrified transplant groups. Moreover, the percentage of developing follicles was similar between fresh graft and vitrified graft. Fresh graft and vitrified graft had a similar odds ratio ($P = 0.17$) to presence of normal preantral follicles. Thus, these results show that transplantation of vitrified goat ovarian tissue is able to promote development of preantral follicles until antral stage. (CEUA-UECE/protocol#2917497/2015).

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Folliculogenesis effects on IGF1R and NFKBIB in mare ovaries

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During the folliculogenesis in all mammalian species, there is a complex signaling mechanism resulting from a cascade of events required for the formation of a dominant follicle. Insulin-like growth factor 1 is known as one of the important factors for follicular development in the mare. The gene expression of insulin-like growth factor 1 receptor (IGF1R) during folliculogenesis may be related directly or indirectly with NF-kappa-B inhibitor beta (NFKBIB). The aim of this study was to verify the gene expression of IGF1R and NFKBIB in equine ovaries during the folliculogenesis. Ovaries of 12 mixed-breed mares with unknown reproductive history were collected in a slaughterhouse located in São Gabriel, (31°S) Brazil. Only the ovaries of cyclic mares were selected. Based on follicle diameter, ovaries were divided into two groups: Development group (DEV) and Dominant group (DOM). DEV group was comprised of 6 animals, whose follicles were <28 mm in diameter (development follicles) and the DOM group was comprised of 6 animals, whose follicles were ≥28 mm in diameter (dominant follicles), this group was divided in two sub-groups: ovary with the dominant follicle (DOM-D) and contra-lateral ovary (DOM-C). Each ovary was longitudinally sectioned and two fragments were taken from the hemi-ovary for quantitative real-time polymerase chain reaction (qPCR). One of the fragments was removed from the ovulation fossa, and the other was taken from the central portion of the ovary (stroma). In hemi-ovaries with follicles, the central fragment was removed together with a portion of the largest follicle. Fragments were conserved in stabilization and storage reagent (RNAlater®, Life Technologies). For qPCR, equine IGF1R and NFKBIB-specific primers were used. Amplification was performed at 95°C for 10 minutes, followed by 40 denaturation cycles at 95°C for 15 seconds, annealing at 59 and 54°C for 30 seconds, and elongation at 60°C for 30 seconds respectively. Relative quantification was performed and the mRNA levels of the target genes were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels. The comparative threshold cycle (CT) ($2^{-\Delta\Delta CT}$) method was used for calculating relative mRNA expression. No significant differences to IGF1R were observed in DEV group between fossa and stroma or between both ovaries. There was an IGF1R tendency to be more expressed in DOM-D group than DOM-C group (P=0,064). In samples from the ovarian stroma was verified that DOM-D group showed a high gene expression than DOM-C group (P=0.021) for IGF1R. For NFKBIB the DEV group showed more gene expression than DOM group in stroma region (P=0,010). In DEV group, the gene expression of NFKBIB was high in stroma than in fossa region (P=0,029). In summary, NFKBIB showed significant difference during the development phase, suggesting that a complex signaling mechanism is important but this is not directly related to the expression of IGF1R. Gene expression to IGF1R showed difference between stroma DOM-D and DOM-C suggesting that this receptor is important to development of dominant follicle.

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Folliculogenesis in the Argentine brown bat *Eptesicus furinalis* (Vespertilionidae: Chiroptera)

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Bats belong to the second largest mammalian Order and exhibit a great variety of reproductive strategies. Although tropical regions have the greater variety of bats, the majority studies of reproductive aspects in bats are done just with temperate species of bats. *Eptesicus furinalis* is a neotropical bat from the Vespertilionidae family that shows a great distribution, and the males are known to have a process of testicular regression, but no studies were previously done with the females. Our aims were to analyze the ovarian morphology and the folliculogenesis process in this species, to increase the knowledge about the reproductive diversity of tropical bats. Sexually mature females of *E. furinalis* were collected in northwestern São Paulo State – Brazil (20°49'11'' S, 49°22'45''W), and the ovaries were carefully removed and prepared for histological analysis. The diameter (µm) of each ovary, follicles (without theca layers and just the sections that showed the oocyte nucleus), and corpus luteum were measured - the diameter was calculated by the formula: (Dµm) = √ab, where 'a' is the measure of the ovary/follicles/corpus luteum diameter, and 'b' is the diameter in 90^{o} angle. The ovary (~878,8 µm) is round to oval, it is divided into cortex and medulla, shows an abundance of stromal tissue, and there was no difference in size between right and left ovaries. Five follicle stages were observed in the ovaries: 1. primordial (~15,6 µm) - surrounded by a single layer of flattened granulosa cells, 2. primary (~62,4 µm) - the single layer of granulosa cells becomes now cubic cells; 3. secondary (~101,3 µm) - two or more layers of granulosa cells surrounding the oocyte; 4. tertiary (~180,1 µm) - the granulosa cells start to secret the antrum fluid, which accumulate between the cells of the granulosa layer, forming small cavities; 5. antral follicle (~214,1 µm) - the cavities start to increase and merge into each other, until it became a major cavity, known as antrum. We observed more than one dominant follicle in the ovaries and two corpora lutea in the same ovary of some females. Besides, atresia was present in all follicle stages, except in the primordial follicles, and we recognized two types of follicular atresia: Type I – degeneration and fragmentation of the oocyte before starts atresia in the granulosa layers; type II – degeneration of the granulosa cells before starts atresia in the oocyte. The process of folliculogenesis and the morphology of the ovaries in *Eptesicus furinalis* are similar to the mammal general pattern. Furthermore, *E. furinalis* presents the ovary morphology similar to other species from the same family: bilateral symmetry of both ovaries and abundance of the interstitial tissue, they present more than one dominant follicle and can ovulates more than one ovum. Polytocous species of bats are generally related with bilaterally symmetry of the female reproductive organs, i.e., both ovaries presenting the same size. Although the folliculogenesis process is well preserved between mammals, bats show different specializations between different taxa, and here we described, for the first time, the folliculogenesis process in this neotropical species.

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Gene expression of IGF-1 in the ovaries of female Wistar rats submitted to growth hormone and exercises

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Some people who seek a better physical performance often use the growth hormone (GH) indiscriminately. Studies have provided better knowledge about the effects of GH in reproduction. The GH acts directly or indirectly on the folliculogenesis and corpus luteum formation. However, until now, has not been investigated the frequent use of GH combined with physical exercises in the expression of genes that respond to GH in the gonads, as the insulin-like growth factor (IGF-1). The objective of this study was to verify the gene expression of IGF-1 in ovaries of female rats that were subjected to the use of GH associated or not to exercises, as an experimental model of human. For this aim, 40 rats were divided into 4 groups: CT (control group), GH (group with administration of 0.2 IU/kg of GH), Ex (group which was submitted to physical exercises for 4 sets of 10 vertical jumps in water with a weight of 50% of the animal's weight) and ExGH (group which was submitted to physical exercises and was administered GH). Vaginal cytology was performed daily and after 30 days all rats were anesthetized and induced to death by exsanguination in diestrus phase of estrous cycle. The RNA extraction of the ovaries was performed, followed by reverse transcription and quantitative analysis of gene expression of IGF-1. The analysis of IGF-1 was by real time PCR, using RPS-18 and HPRT-1 endogenous genes as a normalizing of reaction. The statistical analysis of the data of gene expression was performed by ANOVA and the comparisons of means by Tukey test ($P<0.05$). The mean values and standard errors of the groups were CT=0.93±0.19; GH=1.44±0.17; Ex=0.95±0.24; and ExGH=1.08±0.17. No difference was found between the groups in gene expression of IGF-1 ($P>0.05$). Despite the IGF-1 is synthesized by the liver, and the majority of organic cells, when stimulated by GH and physical exercise to stimulate an increase in the release of GH (GOMES et al, R Bras Ci Mov, 11:57-62, 2003), in the present study no difference was found in the gene expression of IGF-1 in the ovaries between the groups.
We conclude that the GH and physical exercise under the conditions of this experiment do not alter the gene expression of IGF-1 in ovaries of female rats.

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Immunolocalization of anti-Mullerian hormone in bovine fetus ovary

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The anti-Mullerian hormone (AMH) or Mullerian Inhibiting Substance (MIS), produced by the Sertoli cells induces the regression of Mullerian ducts in male embryos (Jost, 1953), however its receptors are present in both sexes (Rey et al, 2003). Granulosa cells from ovarian follicle are homologous to Sertoli cells and also produce AMH (Vigier et al, 1984). Münsterberg & Lovell-Badge (1991) observed AMH restricted to granulosa cells on the sixth day after birth. In antral follicles only cells near to the oocyte and a few granulosa cells surrounding the antrum produced AMH (Lee et al, 1996). In humans, the AMH blocks meiosis in fetal ovary and it is observed in granulosa cells of preantral follicles on fetal ovaries only from the 36th week (Rajpert-De Meyts et al, 1999). In ovaries of ewes AMH was detected in granulosa cells pre and post birth (Bézard et al., 1987) and females without AMH show early follicular depletion (Durlinger et al, (1999). Since there are no published studies on AMH in bovine fetuses this work aimed to immunolocalize AMH in ovaries of bovine fetuses between 24-79cm CRL (4 to 7 months) collected from a slaughter cows. Ovaries were fixed in 10% of paraformaldehyde, processed for conventional histology, cut with a rotative microtome, deparaffinized, and subjected to immunohistochemistry protocol using the anti- AMH 1:50 (SC 28912, Santa Cruz Biotechnology) according to the manufacturer's instructions. There was light staining in granulosa cells but not in the theca cells. However, intense staining was observed in the cytoplasm of the oocyte of primordial follicles, primary, secondary and antral follicles, at all ages analyzed, suggesting that despite being produced by granulosa cells, AMH concentrates in the oocyte cytoplasm. According Modi et al (2006), differences in AMH expression patterns between species make the understanding of AMH role in ovarian physiology difficult and taking into account that there are no studies in the literature on the presence of AMH in fetal bovine ovaries more studies are necessary for better understanding of the role of this protein in ovarian development of fetuses.

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In vivo effects of GnRH on expression of interleukin 1 (IL-1) system members in bovine preovulatory follicles and the effects of IL-1β on gene expression of cultured cumulus cells

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An increasing number of evidences shows that the intraovarian interleukin 1 (IL-1) system can be an important regulator of the preovulatory period. These evidences emerged because IL-1 is an established mediator of inflammation, and ovulation is assimilated to a cyclic inflammatory-like process. In preovulatory period, gonadotropins (LH/FSH) activate several genes in ovarian cells that regulate critical events to release a fertilizable oocyte, such as cumulus cells expansion. The purpose of this study was (1) to quantify the levels of mRNA for IL-1β, IL-1RI and IL-1RA in granulosa cells from bovine preovulatory follicles after *in vivo* GnRH treatment (2) to evaluate the influence of gonadotropins on expression of the IL-1β, IL-1RI and IL-1RA mRNA on cultured cumulus cells and (3) to investigate the *in vitro* ability of IL-1β to regulate its own expression in cumulus cells. In order to obtaining the follicular cells, cows treated with GnRH were ovariectomized at different times post-GnRH treatment (0, 3, 6, 12 and 24 h) by colpotomy. Immediately after ovariectomy, granulosa cells from preovulatory follicles were isolated. After collecting, granulosa cells from individual follicles were stored in liquid nitrogen until mRNA extraction. Granulosa cells were collected and submitted to total RNA extraction protocol with TRIzol®. The resulting RNA underwent a reverse transcriptase reaction to produce cDNA. Contamination of granulosa cells with theca cells was tested by quantitative polymerase chain reaction (qPCR) to detect (CYP17A1) mRNA, and samples which express this gene were discarded. The expression of IL-1β, IL-1RI and IL-1RA mRNA in granulosa cells was evaluated by qPCR. To evaluate the *in vitro* effects of LH/FSH on expression of IL-1β and its functional receptors in cumulus cells, COCs were recovered and selected under a stereomicroscope. Grade 1 and 2 COCs were randomly distributed into 100µl of maturation medium and cultured in an incubator for 24 hours. After culture, cumulus cells from COCs were removed by vortexing, pooled and washed in PBS twice by centrifugation. Isolated cumulus cells were stored in cryotubes in liquid nitrogen until RNA extraction. Total RNA extraction and quantification of mRNA for IL-1β, IL-1RI and IL-1RA was performed in the same conditions as described previously. *In vivo* studies demonstrated that IL-1RA mRNA levels were increased ($P<0.05$) after 24 h of GnRH treatment. *In vitro*, the presence of gonadotropins increased the levels of mRNAs for IL-1RI and IL-1RA in cumulus cells after 24 h of culture. IL-1β (10 ng/mL) treatment induced its own mRNA expression and promotes the mRNA expression of IL-1RA after COC culture. In conclusion, IL-1RA mRNA is upregulated in granulosa cells at later stages of the ovulatory process in cattle. In cumulus cells cultured *in vitro*, the expression of IL-1RI and IL-1RA mRNA is induced by gonadotropins. IL-1β has a self-regulatory effect in cumulus cells of COC after *in vitro* culture.

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Morphological Changes in Vagina and Ovary Throughout Estrous Cycle in *Galea spixii* (Rodentia: Caviidae Wagler, 1831)

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The rodents Spix's yellow-toothed (SYT) cavies (*Galea spixii*, Wagler, 1831) belong to the family Caviidae and are found in Brazilian northeast where they are used as source of protein for inhabitants of that region. For this reason, the species is bred in captivity for the preservation and to use as experimental model for research on reproductive biology. During the estrous cycle, the vaginal epithelium is influenced by different demands of sexual steroid hormones produced by ovarian follicles at different stages of development and this fact is related to the different cell types found in samples of vaginal exfoliative cytology. Present study aimed to determine if the characterization of the estrous cycle in females of SYT cavies by vaginal exfoliative cytology were corresponding with microstructural variations in vaginal epithelium revealed by proliferating cell nuclear antigen (PCNA) and to the follicular development in the ovaries at different phases of the estrous cycle. After determination of the estrous cycle phases by vaginal exfoliative cytology, females were euthanized at metestrus, diestrus, proestrus and estrus phases (n=3 for each estrous cycle phase). Histological study of the vaginal epithelium and ovary were performed and besides, immunohistochemistry for proliferating cell nuclear antigen (PCNA) was performed using samples of vaginal tissue at each estrous cycle phase. Present data shows that the vaginal exfoliative cytology may be associated with the follicular development in the ovaries and the proliferation and desquamation of the vaginal epithelium in SYT cavies. At estrus, superficial cornified cells in vaginal exfoliative cytology and vaginal epithelium besides early post-ovulatory follicles in ovaries were found. At metestrus, the vaginal epithelium undergone desquamation and lost the superficial cornified cells; basal and intermediate cells appeared in the vaginal exfoliative cytology and post ovulatory follicle formed early corpus luteum. At diestrus, corpus luteum was developed and the vaginal epithelium showed basal and intermediate cells by vaginal exfoliative cytology. At proestrus, due to the growth and maturation of ovarian follicles, the vaginal epithelium suffered intense proliferation. Vaginal exfoliative cytology showed large intermediate cells and nucleated and enucleated superficial cornified cells. In the ovary, mature follicles were present. PCNA was present in vaginal tissue at diestrus, proestrus and estrus but not at metestrus. In summary, present data shows association between the findings by vaginal exfoliative cytology, changes in microstructure of vaginal epithelium; proliferating cell nuclear antigen (PCNA) expression; and, follicular development in the ovaries.

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Color Doppler ultrasonography for evaluation of corpus luteum in mares induced to ovulate

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Considerable efforts have been made to improve the application of assisted reproduction techniques in equine species, however there are still few studies in vascularization of the corpus luteum (CL) and its relation to progesterone in mares induced with deslorelin acetate. The aim of this study was evaluate the influence of deslorelin in CL vascularization and progesterone production. Mares with natural ovulation (GC) and induced with deslorelin acetate (follicles ≥35 mm) (GT) were used. The CLs vascularization was analyzed using color Doppler ultrasonography (6.5 MHz) on days 4, 8 and 12 days after ovulation and images were recorded in video. The analysis of the videos was considering the percentage of luteal parenchymal area with color signals. The concentration of progesterone was analyzed by ELISA, in the same time points. The results were presented as mean ± SEM of percentage of vascularization and progesterone concentration (ng/mL). The average vascularization was in GC: 76.25 ± 8.98; 70.00 ± 8.16 and 57.50 ± 13.31 % and in GT: 85.00 ± 5.40, 90.00 ± 0,00 and 58.75 ± 9.24 % respectively for the days 4, 8 and 12 after ovulation. Progesterone concentrations were in GC: 6.21 ± 0.61; 5.82 ± 1.44 and 4.63 ± 0.79 ng/mL and GT: 6.10 ± 1.18; 4.67 ± 1.06 and 4.49 ± 1.37 ng/ml, respectively for the days 4, 8 and 12 after ovulation. The patterns of vascularization were higher in D4 and D8 compared to D12 of GT ($P<0.05$). However, there were no differences between groups or days in GC. The concentration of progesterone didn't show significant differences between groups or studied days. It was concluded that ovulation induction with deslorelin acetate, were effective for the induction of ovulation in mares which does not alter the physiological responses of the corpus luteum in respect of its progesterone production, however affected luteal parenchyma vasculature. More in-depth studies are necessary to clarify the implications of vascularization changes in the luteal function.

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Protein profile in corpus luteum during pregnancy in cows

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The corpus luteum (CL) is a transitory endocrine gland necessary for the onset and maintenance of pregnancy in mammals. Progesterone (P_4) is secreted by the CL and is critical for embryo survival and development. The objetive of this study was to characterized the CL proteome of pregnant and non-pregnant cows using two dimensional gel electrophoresis (2DE). Ovaries were collected from pregnant (n=7) and non-pregnant cows (n=4) at a near abattoir. For the pregnant cows, the fetus had to be visualized to be included in the study. Gestational ages were estimated (between 30 and 160 days). At the laboratory, CLs were carefully dissected and pools were made. For protein extraction, 5 mg of luteal tissues were combined with 250 uL lysis buffer (TRIS-HCL, SDS, B-Mercaptoetdanol) and vortex was applied for 1min. Insoluble material was precipitated by centrifugation for 45 min (5000 g at 4°C). The supernatant was collected and proteins were precipitated with acetone during two hours at -20°C. After two hours of precipitation, another centrifugation was perform as described above and the protein pellet formed was dried overnight at -4°C. Posteriorly, the pellet was resuspended in sample buffer (Urea and Tiurea) and protein concentration was determined by Bradford assay. Three hundred micrograms of protein were subjected to isoelectric focusing in 13 cm strips (pH 3-10). The second dimension was conducted by SDS-PAGE 12.5%. Gels were scanned with an ImageScanner II (GE Lifesciences, USA) and analyzed using PDQuest® version 8.0.1 (Bio-Rad Laboratories, USA). In the gels, for the pregnant CL had 433 spots and those of non-pregnant CL, 259 spots. There were 174 spots only present for the pregnant CL. Most proteins (69%) were found between 10 and 60 kDa (33.0 ± 14.2 mean ± SD) and between 5.1 and 10 pH (7.0 ± 1.4 mean ± SD) (77%). One hundred and fifteen spots were up-regulated and 142 spots were down-regulated in CL of pregnant cows, compared to non-pregnant (3-fold difference). Two spots were significantly expressed (150-fold) in the pregnant CL, with 76 and 78 kDa and 5.7 and 5.8 pH. Our results show that there are significant changes in protein CL expression during the pregnancy. These proteins are most likely involved with mechanisms in production of P_4 by the corpus luteum.

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Alpha-L-fucosidase of epididymal fluid is active on bull spermatozoa

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The mammalian epididymis plays an important role in the acquisition of motility and fertilizing ability of spermatozoa. During passage through the epididymis, spermatozoa undergo biochemical and morphological changes, resulting in a gamete competent for fertilization. These changes include modifications of glycoconjugates on the cell surface as the result of sequential interactions with proteins secreted by the epithelium along the epididymal duct. Among the several proteins secreted by the epididymal epithelium, glycosidases have been found in the epididymal lumen of domestic animals, such as bulls. Alpha-L-fucosidase (FUC) appears to be important for the fertilizing ability of the spermatozoa, since, high levels of this enzyme have been found in the epididymal fluid of bulls with high fertilizing capacity. Regarding this, we proposed to study the secreted FUC of bull cauda epididymis and correlate with fucosylated status of spermatozoa. We incubated spermatozoa from caput or cauda with FITC-conjugated UEA-I lectin and analyzed the fucosylation by fluorescence miscroscopy and flow cytometry. It was observed a strong post-acrosomal detection in spermatozoa from the caput, which is much lesser in cauda spermatozoa. This change was significant as judged by flow cytometry quantification. Moreover, we evaluated the pattern of protein fucosylation, employing biotin-conjugated UEA-I after blotting onto nitrocellulose membranes. Consistent with the previous results, we observed a decrease of fucosylation in several proteins of cauda spermatozoa, although an increase was observed in other proteins. This decreased fucosylation may be related to an increased activity of FUC in the lumen of cauda epididymis. By western blot, using anti-FUC, we found that the enzyme is expressed in the tissue of caput, corpus and cauda, although only is secreted in the cauda epididymis. These results were confirmed evaluating the enzyme activity by spectrofluorometry. In order to determine if FUC from epididymal lumen is responsible of fucose removal in the sperm surface, we performed an assay in which a crude cauda fluid was incubated with caput spermatozoa. After incubation we observed (by flow cytometry) a significant decrease of fucose detection which was reversed with synthetic specific inhibitors for FUC. In conclusion, we provide direct evidence that FUC from epididymal fluid participates in removing fucose from spermatozoa, as a step of sperm maturation in bull epididymis.

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Association between Abiraterone acetate and PI3K inhibitor (BEZ235) reduced tumor growth in dorsolateral prostate of Fischer 344 rats

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The anti-androgen Abiraterone acetate (Abi), an inhibitor of CYP17 enzyme, has been used to treat prostate cancer (PCa), however its use can trigger drug resistance with activation of additional pathways such as PI3K/Akt. To overcome the resistance caused by monotherapies, the association with PI3K/Akt inhibitor (BEZ235) appears as an alternative strategy to control PCa progression. Thus, the aim of this study was to evaluate the effect of single and combined Abi and BEZ on PCa. Male adult F344 rats were inoculated with MNU (15mg/kg) in the dorsolateral prostate (DLP) capsule and received testosterone-cypionate twice a week for 220 days, subcutaneously. After tumor-induction animals were randomly divided into four groups and received the following drugs by gavage for 10 days: I- Tumor-induced control (vehicle); A (Abi-14mg/kg); B (BEZ-45mg/kg) and AB (Abi+BEZ). After the treatment, serum testosterone and DHT levels were measured, DLP was removed, weighed and submitted to quantitative and statistical analysis of lesions incidence and multiplicity, epithelial proliferative index (Ki67) and Western blot for AKT-related proteins (IL-6, TNF-α, IL-10, PI3K, pAKT, p-BAD, XIAP). DLP weight and testosterone serum levels were significantly lower in the AB group compared to the control, while DHT serum levels decreased in all treated groups compared to the control, with the lowest levels in AB group. The effective reduction of testosterone and DHT levels in the AB group shows Abi action on androgen blockade. After tumor induction, DLP develops relevant number of proliferative lesions in higher frequency than those found in ventral prostate (data not shown). Treatment with drugs decreased multiplicity of inflammatory-related disorders such as reactive hyperplasia and intraluminal inflammation, while stromal inflammation was significantly reduced in AB compared to control. Oppositely, atypical hyperplasia increased in A and B compared to control and AB. Incidence and multiplicity of premalignant and malignant lesions decreased significantly in treated groups and a greater reduction was observed in AB. Additionally, drugs significantly reduced epithelial proliferative index. Supporting histopathological data, IL-6 expression decreased in all treated groups (I=2.99±1.08; A=1.43±0.38; B=0.92±0.24; AB=1.51±0.22) while TNF-α expression decreased in A and AB (I=3.8±0.5; A=2.22±0.2; B=3.37±0.01; AB=2.84±0.19), suggesting reduction of inflammation. Oppositely, IL-10, an anti-inflammatory cytokine, reduced its expression in AB group (I=3.46±0.78; A=3.7±0.0.08; B=2.67±01.02; AB=0.7±0.57) and a possible explanation is that in this group the lesions are scarce and less extensive. In relation to the effect of drugs on AKT pathway, was observed a decreased in PI3K expression in all treated groups (I=1.47±0.35; A=0.42±0.16; B=0.22±0.13; AB=0.37±0.16). Consequently, pAKT expression were reduced in the same groups (I=2.84±0.59; A=1.06±0.78; B=1.31±0.72; AB=0.79±0.45) and these events can contribute to decrease cell proliferation. As a result of AKT pathway blockade, the expression of apoptosis-related proteins such as p-BAD (I=1.83±0.07; A=0.34±0.29; B=0.18±0.13; AB=0.34±0.16) and XIAP (I=1.42±0.64; A=0.36±0.11; B=0.57±0.47; AB=0.38±0.11) decreased in treated groups, which possibly increased apoptosis and contributed to reduce tumor growth. Together, histopathological and biometric data, decrease in epithelial proliferation and in expression of proteins related to cell proliferation, survival, inhibition of apoptosis and inflammation suggest that association between Abiraterone and BEZ can reduce tumor growth effectively. Further investigation is necessary to clarify the mechanisms by which drugs act synergistically and exert anti-inflammatory effects.

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Biometric evaluation organs of the male reproductive apparatus and sperm motility of Swiss mice treated with hexanic and hydroalcoholic extract of leaves of *Davilla elliptica*

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The *Davilla elliptica* (Leguminosae - Mimosoideae), popularly known as "dry-testis", is one of the 330 cataloged plants of the Brazilian Cerrado, which are used in traditional medicine. Presents as remarkable feature high polyphenol content, and is widely used for its digestive properties, sedative, laxative and diuretic. However, despite popular name, studies considering the effects of gametogenesis in plants are not found. The aim of this study was to evaluate the effect of hexanic and hydroalcoholic extracts of *D. elliptica* leaves, on biometrics of male reproductive organs and sperm motility of Swiss adult mice. Thus, 35 male mice, 82 days old, were divided into seven experimental groups (n = 5) G1: distilled water (0.6 mL / day); G2: 2% Tween 20 in 0.6 ml of distilled water (vehicle solution); G3: hexanic extract (HE) 100mg / kg +; G4: HE 200 mg / kg +; G5: hydroalcoholic (HA) 100 mg / kg; G6 HA 200 mg / kg and G7 HA 400mg / kg. The hexanic extracts were diluted in Tween 20 (vehicle). The treatments were administered by gavage with duration of 42 consecutive days. Twenty-four hours after the last dose, the animals were anesthetized and euthanized and the male reproductive tract was removed and weighed. Testis, prostate gland and seminal vesicles were fixed in Karnovsky in cacodylate buffer for 24 hours and transferred to alcohol 70%. The left epididymis had their tails cut in order to obtain a rich liquid sperm. The sperm were collected from the epididymal tail, the sperm motility were examined under the microscope at a magnification of 400x. Sperm cells were classified as either motile or immotile and total motility was expressed as percentage. This experiment was approved for CEUA/UFV with number of process 40/201. Results were analyzed by ANOVA followed by post-hoc Student-Newman Keuls test ($p \leq 0,05$). There was a reduction in body weight of the animals of the G7 group (36.04g) in relation to the G2 group (44.22g). However, the values to GSI and testicular weight of the treated groups did not differ from control (G1) and vehicle (G2). The G3 (0.041g) showed epididymal weight less than the G1 (0.057 g). Prostatic masses of the G3 (0.12 g), G5 (0.10 g), G6 (0.11 g) and G7 (0.11 g) were smaller than the mass of G2 (0, 17 g) and the seminal vesicle values were not different between groups. Concerning sperm motility, all showed reduction treatment groups compared with the control group G1 (78.67%) and G2 (55.50%), G3 (25.74%), G4 (33.75%), G5 (36.00%), G6 (46.75%) and G7 (24.33%). Regarding to the vehicle (G2), all other groups showed reduced motility, less the group G6. Quantification motile sperm is one of the parameters used to evaluate male reproductive potential and can, through these tests, provide sperm quality. Thus, it is possible to infer that the animals treated with extracts, hexanic and hydroalcoholic, of *D. elliptica* leaves can showed semen quality inferior to that of the control.

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Biometric evaluation organs of the male reproductive tract and sperm motility of Swiss mice treated with infusion and fraction enriched flavonoid of leaves of *Davilla elliptica*

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The use of plants as drug is an ancient practice commonly used in folk medicine. However, most plants used as medicines have not had their therapeutic potential, or possible toxic effects, effectively tested and proven. The *Davilla elliptica*, popularly known as "trash bin" and "dry-testis", is used in traditional medicine for its digestive properties that are distinguished by their high flavonoid content. However, it is not known the effect of *D. elliptica* on reproduction, as well as flavonoids present in that plant. The aim of this study was to evaluate the effect of infusion of the leaves of *Davilla elliptica*, and its fraction enriched in flavonoids (EF), on the biometrics of male reproductive organs and sperm motility of Swiss adult mice. Forty male mice, of reproductive age, were randomly divided into eight experimental groups (n = 5): G1- distilled water (0.6 mL / day); G2- dimethylsulfoxide (DMSO) in 0.6% 4 ml of distilled water (vehicle); G3- infusion of 100 mg / kg; G4- infusion of 200 mg / kg; G5- infusion of 400 mg / kg; G6- FE 100 mg / kg +; G7- FE 200 mg / kg +; and G8- FE 400 mg / kg +. The enriched fractions were diluted in DMSO. Treatment was administered by gavage for 42 consecutive days. After 24 hours of the last dose the animals were euthanized being removed and weighed testes, epididymis, prostate and seminal vesicles. The tail of the epididymis was sectioned to obtain a fluid rich in spermatozoa. The fluid was diluted in 200µl of Tris-citrate-fructose. The total motility was assessed by automatic sperm analysis (Sperm Class Analyzer - automatic sperm analysis computer MICROPTIC © S.L., Barcelona, Spain). Results were analyzed by ANOVA followed by post-hoc Student-Newman Keuls test ($p \leq 0.05$). This experiment was approved for CEUA/UFV with number of process 40/2015. There was no change in body weight of the animals compared to the control group. However, in comparison with the vehicle, G4, G5 and G8, showed significant reduction in this parameter of 8.93, 8.53 and 15.73%, respectively. There was a reduction of 20% of epididymal weight on G4, G5 G8, compared to the control. The motility of sperm was significantly reduced in the treated groups, also in the vehicle group, which had reduced motility in 27.55%. However, the animals in groups G3, G4 and G5 had reduced motility 57.63, 45.02 54.24%, respectively. The groups treated with the enriched fraction (G6, G7, G8) reduced motility in 49.57, 62.82 and 60.59%, respectively. As for testicular weight, GSI, prostate weight and seminal vesicles weight, there was no significant difference between groups. As sperm motility is directly related to the ability of fertilization, decreased motility indicates a potential error in sperm maturation process which can lead to the inability to fertilize an oocyte. It was observed in this study decreased motility in all treated groups, particularly highlighting the infusion in groups (G3 and G5) also in the groups receiving enriched fraction (G7 and G8). Morphological analysis should be performed to elucidate the effect of the infusion and the fraction enriched in flavonoids (EF) of the leaves of *D. elliptica* on the organs of the male reproductive system.

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Can Single Layer Centrifugation with Androcoll-Equine improve freezability in poor freezing stallions?

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Despite much research to improve cryosurvival of stallion sperm, it is not always possible to obtain acceptable post-thaw motility i.e. ≥ 30%, from all ejaculates. In some studies, colloidal centrifugation has been shown to improve cryosurvival and to increase the number of stallion ejaculates that can be frozen with acceptable post-thaw semen parameters. The use of Single Layer Centrifugation (SLC) and the inclusion of a prolonged equilibration step prior to freezing were tested on sperm samples from stallions known to be poor freezers. An ejaculate was collected from six "poor-freezing" stallions using an artificial vagina; Gent extender (Minitüb GmbH; Tiefenbach, Germany) was added, adjusting to a concentration of $100x10^6$ sperm/mL. The samples produced were treated as follows: control samples were either centrifuged immediately, or after 8h storage at 5°C or 15°C; in each case the sperm pellet was resuspended in EquiPlus Freeze (EF; Minitüb GmbH; Tiefenbach, Germany) and frozen. For the SLC samples, the SLC was either carried out immediately after semen collection or after storage of the samples for 8h at 5°C or 15°C, followed by freezing. All sperm pellets were resuspended in EF at a concentration of $200x10^6$/mL for freezing in 0.5mL straws. After thawing, the percentage of sperm with progressive motility was assessed by AndroVision (Minitüb GmbH; Tiefenbach, Germany) and treatment means were compared using Kruskal-Wallis and Tukey tests ($P < 0.05$). None of the control samples gave acceptable post-thaw motility regardless of whether they were processed immediately (mean 8.41%± 2.1) or after storage (5^oC, 4.63%± 2.1; 15^oC 3.87% ± 1.9). However, all SLC samples processed immediately had a post-thaw motility ≥ 30% with a significant different average of 46.02%± 5.3 in comparison with all other means. None of the samples that were stored before SLC had acceptable post thaw motility (5^oC, 11.55%± 2.9; 15^oC 15.16%± 1.5). In summary, for some poor freezing stallions, processing by SLC and freezing immediately results in samples with acceptable post-thaw motility.

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Chrysis, an Aromatase inhibitor, alter the prostate microenvironment of Mongolia Gerbils

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The prostate physiology is regulated by steroid hormones, mainly androgens and estrogens. Androgens induce differentiation, development and secretory activity, and, estrogens modulate androgenic effects, regulating the growth and normal prostate physiology.The majority of estrogens present in the organism is derived from testosterone (T) aromatization by action of the enzyme aromatase (Aro), in adipose tissue, liver and also in the prostate. This study aimed to elucidate the effects of hormonal imbalance caused by inhibition of Aro in prostate microenvironment of Mongolia gerbils. Male gerbils aged 120 days old were treated weekly by gavage with chrysin (Cr) (50mg/kg/day in 0.1 ml of corn oil), an inhibitor of the Aro. The control group (Cn) was formed by intact animals. Five animals per group were euthanized on week 1 of treatment and other five animals per group on week 4. The ventral lobe of the prostate glands were removed and fixed in paraformaldehyde 4% and processed for histological analyses. The slides were stained with Hematoxilina-Eosina, Gomori trichrome, Gomori reticulin and P.A.S. (Ácido Periódico de Schiff) and used for general and morphometric analysis. In addition, it was performed immunohistochemistry for marking α-actin. The testosterone levels were assessed by ELISA assay. The data were compared statistically by analysis of variance (two-way-Anova), and subsequently by the Bonferroni post tests ($P \leq 0.05$). This study showed that the use of the inhibitor resulted in alterations in the glandular structure, as the secretory activity decreases, the presence of inflammatory cell infiltrate in the prostatic compartments, accumulation of collagen fibers in the subepithelial layer and a glandular greater vascularization, in both treatment periods. The serum of testosterone levels decreased only in the group treated with the inhibitor for 4 weeks. Epithelial cells have become atrophied in the groups treated for 1 and 4 weeks and the thickness of the stromal compartment reduced only in the group treated for 1 week. The percentage of immunostaining for α-actin decreased only in the group treated for 4 weeks. The results obtained with Aro inhibition highlight the importance of this enzyme in the prostate hormonal regulation, moreover, it can be concluded that 4 weeks of treatment triggered more expressive. (CEUA/ Ibilce-UNESP/Protocol: 130/2016)

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Comb, cloaca and feet scores and testis morphometry in male broiler breeders at two different ages

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In broiler breeder roosters, the weight of the testes is positively associated with daily sperm production and fertility. In birds, the testes are located in the coelomic cavity, not accessible to reproductive evaluation as mammalian testes. The reproductive evaluation of roosters is based on phenotypic traits. Any changes on testes will reflect on fertility levels. The aim of this paper is to evaluate the potential association of phenotypic traits, such as body weight (BW), comb scores (COS), cloaca scores (CLS) and feet scores (FS), with testicular morphometric parameters in adult Cobb® Mx roosters. The experimental design was fully randomized in a 2x3 factorial arrangement: two ages (25 weeks and 45 weeks) and three bodyweight categories (light, medium or heavy) with 10 replicates each; the experimental unit was a single animal. After being weighed and divided into body weight categories, all roosters had the comb and cloaca morphology, as well as the presence of lesions in the feet, inspected by a single trained evaluator. Comb, cloaca and feet scores followed the methodology described by Rezende et al. (2014). The testes were then removed, individually weighed and prepared for histology. Normal data and homogeneous variances were analyzed by a one-way analysis of variance (ANOVA) and the means were compared by the Tukey test (coefficient of variation lower than 10%) or Duncan test (coefficient of variation greater than 10%) with a significance level of 5% ($P \leq 0.05$). The Pearson-Spearman correlation was used to assess the correlation between the different traits. The low BW roosters had lower testes weight (TW), smaller seminiferous tubule diameter (STD) and lower comb and cloaca scores than the medium and high BW roosters ($P \leq 0.05$). Heavy roosters had the highest COS and greater seminiferous epithelium heights (SEH). There was no statistical difference in FS and volume ratio of seminiferous tubule (ST) and interstitial tissue (IT) among the BW groups. As to the age effect, 45-week roosters had lower TW and ST than 25-week roosters. Positive, moderate and significant correlation was found between testes weight and phenotypic traits (BW, COS and CLS) in 45-week roosters. Positive, moderate and significant correlation was found between body weight and histological features (STD and SHE) for both ages. In conclusion, the testes weight regressed with age, and this was more intense in Cobb® Mx roosters with lower BW. The BW positively influenced testes weight and histological features. COS and CLS may be used with moderate potential prediction to identify roosters with low testes weight at 45 weeks of age (CEUA/UFMG/Protocol#79/2015).

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Consequences of the treatment with corn oil on the prostate histology and sperm parameters of Mongolian gerbils (*Meriones unguiculatus*)

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A wide range of substances has been used as vehicle in researches with endocrine disrupting chemicals. The main point in the choice of the most adequate vehicle is that it should not influence the results obtained for the compound under investigation. The corn oil, a vehicle widely used in these researches, has phytoestrogens and a high content of linoleic acid in its composition. Due to the fact of some researchers consider this category of fatty acid able to induce inflammatory disorders, it is relevant to evaluate if the corn oil has biological effects and can interfere with the normal development of reproductive organs. Therefore, this study analyzed the effects of treatment with corn oil during gestation on prostate histology and sperm parameters of Mongolian gerbils at adulthood. Male gerbils (n=10 animals/group) were randomly divided into Control (C) and Oil-treated (O – animals treated with 0.1mL of corn oil from 8th to 23rd gestational day, via maternal gavage) groups. Animals (16w old) were weighted, euthanized, the ventral prostatic lobe was fixed in methacarn and processed for paraffin embedding, and testis and epididymis were processed for evaluation of sperm parameters. Testosterone (T) and 17β-Estradiol (E_2) analyses were performed by ELISA. Serial prostate sections were stained with Hematoxilin-Eosin (HE) and Gömöri's reticulin, submitted to immunohistochemistry for PCNA and alpha-smooth muscle actin, and the incidence of lesions was evaluated in 25 microscopic sections/group. Stereological analysis of HE-stained histological sections was performed. There was a tendency to reduce the body weight in the O group (C: 70.6±3.3, O: 67.6±3.6g; p=0.0901). The treatment with corn oil during gestation decreased the prostatic weight at adulthood (C: 17.1±4.5; O: 13.3±3.9mg; p=0.0277). Stereological analysis indicated that this reduction was due to a decrease of 32.3% and 24.7%, respectively, in the volume of epithelial and luminal compartments. There was no alteration in the cell proliferation index in the prostate after the treatment with corn oil during the fetal period, but the analysis of lesions incidence indicated the occurrence of reactive hyperplasia in 20% (5/25) of all microscopic sections. The treatment with corn oil during the fetal life did not alter the testis weight (C: 543.2±47.6, O: 519.0±26.3mg; p=0.1616), but there was a tendency to diminish the epididymis weight (C: 203.1±17.5, O: 188.5±12.8mg; p=0.0621). There was no alteration in the daily sperm production; nevertheless, the sperm reserve and sperm transit time in the epididymis diminished in O group. Treatment with corn oil also affected the sperm motility; sperms with progressive movement decreased while those with non-progressive movement enhanced. There was no alteration in the T levels between the groups, but the E_2 levels increased 38.6% in O animals. These data indicate that the corn oil treatment during gestation affects the prostatic gland histology, sperm parameters and the levels of circulating estrogen in Mongolian gerbils at adulthood. Taken together, these results are worrying, because they show that the corn oil interfered with the normal development of the reproductive organs, and this fact can result in misinterpretation of the real results of the compound under investigation. (CEUA protocol 93/2014).

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Doppler ultrasound of the normal testis in peccary (Tayassu tajacu, Linnaeus, 1758): B-mode, color and spectral doppler imaging

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Peccary is an intensely hunted animal in the northeast of the country. Efforts have been established for a better understanding of their reproductive biology. In this context, imaging methods can help identify morphologic and hemodynamic characteristics of the reproductive system of these animals. Eight males peccaries, between 4 to 6 years, were used. The animals were submitted to testicular ultrasound examination (bilateral) B-mode, color Doppler and spectral Doppler. Images of Doppler and color flow Doppler spectral (pulsed) were obtained using an ultrasound machine SonoSite M-turbo coupled to a linear probe multifrequency (12-15 MHz). The testes were accessed in longitudinal and transverse sections. Testicular and epididymis biometric parameters were measured. Using color Doppler we evaluated the distribution pattern of testicular vascular bilaterally. Spectral Doppler was used aligning the sample volume in the pampiniform plexus checking the flow velocity, and resistivity index. The testes showed homogeneous echotexture, echogenicity average (moderately echogenic and uniform standard throughout the parenchyma). The *rete testis* was viewed as a hyperechoic structure in longitudinal topography (centrally), from the proximal to the distal end. The parameters are expressed as follows: 1) Right testicular length=4.15±0.21cm; 2) Right testicular diameter=2.3±0.31cm; 3) rete testis diameter (right testis)=0.15±0.02cm; 4) Right epididymis head length=1.73±0.31cm; 5) Right epididymis head Diameter= 0.81±0.12cm; 6) Right testicular artery velocity=36.3±1.13cm/s; 7) Right testicular resistivity index 0.67±0.01; 8) Left testicular length=5.07±0.13cm; 9) Left testicular diameter=2.27±0.1cm; 10) rete testis diameter (left testis)= 0.13±0.04cm; 11) Left epididymis head length=2,07±0.08cm; 12) Left epididymis head Diameter= 1,08±0.09cm; 13) Left testicular artery velocity=34.2±1.10cm/s; 14) Left testicular resistivity index 0.68±0.06. The B-mode sonographic pattern was similar to that seen in humans and domestic males, like the dog and the cat, demonstrating reduced vascular flow centrally, and highest volume at the periphery of the testes. The spectral waveform pattern showed a well-defined peak systolic with a slope deceleration similar to that described in domestic animals. No citations were found to peccary testis doppler ultrasound. The resistivity index showed remained stable, characterized by a moderate systolic peak and lower diastolic peak. These findings were also written in humans and domestic animals. There was no statistical difference between the right and left testes compared bilaterally ($p>0.05$). Thus, these preliminary results allow us to suggest that the testicular flow presented with moderate average velocity, which we attribute probably tortuous vascular plexus associated with thermogenic function. Further studies are being developed and have allowed us to better define these flow characteristics, to associate them with the reproductive profile of reported animal.

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Effects caused by Finasteride, an 5α-reductase inhibitor, in the Mongolian Gerbil prostate

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The prostate is a gland of the reproductive system regulated by androgens and estrogens. The 5α-reductase enzyme (5α-R) participates in the regulation of prostatic levels of these hormones. The 5α-R is responsible to convert testosterone (T) to dihydrotestosterone (DHT), the biologically active metabolite of T. This study aimed to elucidate the effects of hormonal imbalance caused by castration and the inhibition of 5α-R in the microenvironment prostate. The control group was formed with intact male gerbils aged 120 days old. Animals at 120 days of age were submitted to a bilateral orchiectomy, another portion of the animals received weekly gavage of Finasteride (10 mg/kg/day in 0.1 ml corn oil), an inhibitor of 5α -r. The animals were euthanized after 1 (n=5) and 4 (n=5) weeks of treatment and the ventral prostate was removed and processed for histological analyses. For general analysis, stereological and morphometric measurements were performed with colorations of Hematoxilina-Eosina, Gomori trichrome, and P.A.S. (Ácido Periódico de Schiff) and also was realized immunohistochemistry for α-actin. The testosterone amd estradiol levels were assessed by ELISA assay. The data were compared statistically by analysis of variance (two-way-Anova), and subsequently by the Bonferroni post tests ($p \leq 0.05$). This study showed that castration and enzyme inhibition led to a hormonal imbalance, wherein the T serum levels decreased in castrated group of both period and in the finasterida treated group for 4 weeks; furthermore it was also observed an increase in estradiol levels in the finasteride group treated for 4 weeks. The hormonal imbalance observed in the castrated and finasterida treated groups led to a glandular regression evidenced by a reduction of the secretory activity and the percentage of epithelial cells, that showed atrophied, and a stromal compartment with reduced thickness. In addition, a stromal remodeling was observed as muscle cells with irregular shape, a subepithelial layer with accumulation of collagen fibers, increased vascularization and a compact layer of smooth muscle. The percentage of α-actin reduced only in the group treated for 4 weeks. The effects observed after castration were more intense than those observed in groups treated with finasteride and 4 weeks of treatment was more effective for both treatments. (CEUA/ Ibilce-UNESP/Protocol: 130/2016).

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Evaluation of the stages of the seminiferous epithelium cycle in the testis of *Chinchila lanigera* according to the acrosomic system

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The *Chinchila lanigera* is a rodent endemic in Andes, South America and is considered critically endangered due to the large use of its fur in the market. Despite its economic importance, there are only few reports about its reproductive biology. So far, some peculiar characteristics have been described, for instance, the puberty in male chinchillas takes place at 3 months of age (mo) and the sexual maturity is reached at 17 mo, a very long period of time when compared to other mammals. In this context, the main objective was to determine the stages of the seminiferous epithelium cycle according to the acrosomic system in order to provide the basis for other studies in this species. Therefore, four adult chinchillas were utilized. Testes were perfused-fixed with glutaraldehyde and processed for histological analyses. Sections stained with PAS were used to better determine the size and position of the acrosome. The stages were characterized based on the presence, angle and size of the acrosome, shape and location of spermatid nuclei, presence of meiotic divisions, and overall seminiferous epithelium composition. In order to help the stages determination, the angles of the acrosome were measured from 10 photos per animal and 20 spermatids per photo in stages IV to VII using Image J 1.47t. Based on the acrosomic system, 11 stages of the seminiferous epithelium cycle were found for chinchilla. Stages I to VI had two generations of spermatids (round and elongate spermatids), while stages VII to XI had only one generation. In stage I, early round spermatids (step 1) were found with no acrosomal vesicle, some elongate spermatid bundles were located deeply within the epithelium. Young pachytene primary spermatocytes were situated between round spermatids and the basal lamina. Subsequently, in stages II/III it was possible to see, in the same cross section, round spermatids with one or more granules on their nuclear surfaces (step 2) or with one rounded acrosomal vesicle on it (step 3), making it difficult to designate as stages II or III, separately. The stage IV is characterized by the presence of step 4 round spermatids. The acrosomal vesicle starts to spread over the nuclear membrane, depicting an angle (~65°±0.04). Bundles of elongated spermatids had started to move toward the seminiferous tubule lumen. Also, pachytenes were found more distant from the basal lamina in this stage. The acrosome in round spermatids (step 5) kept developing over the nucleus in the stage V, and its angle mean was ~77°±0.05. Elongated spermatid bundles continued moving toward the lumen. Next, in stage VI, nuclei of elongated spermatids were located along the lumenal surface of the seminiferous epithelium as long as the spermiation occurs at this stage. Many residual bodies were found just below elongated spermatids. Also, the angle depicted by the acrosome over the round spermatid (step 6) nucleus was around 107°±0.05. After spermiation, only one spermatid generation was occurring in the epithelium. Hence, the angle formed over the round spermatids nuclei (step 7) by the acrosome vesicles represented ~130±0.05 degrees. In stage VIII, step 8 spermatids nuclei began the elongation process and started to be organized with the acrosome side facing the basal lamina. Two types of primary spermatocytes (leptotene and pachytene) were found at the bottom of the epithelium. Spermatids at the step 9 were present in the stage IX and they were more elongated compared with the same cells of the previous stage. Nuclear condensation has started at this stage. Leptotene and pachytene spermatocytes were observed at this stage as well. The nuclear condensation of elongated spermatids (step 10) was more evident than the precedent cells. Also, these cells were arranged in bundles, with the acrosomal side facing the basal lamina. Zygotene and diplotene corresponded to the spermatocytes present in the seminiferous epithelium at the stage X. In stage XI, it was possible to see meiosis I and meiosis II cells. Besides that, zygotene and secondary spermatocytes were also observed. Eventually, diplotene spermatocytes were still present at this phase of the seminiferous epithelium cycle. The spermatogonia were present along the 11 stages of the seminiferous epithelium cycle. However, ongoing studies are being performed in order to thoroughly characterize these cell types and their generations.

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Fertility assessment of sperm cell subpopulations in the bull *(Bos taurus indicus)*

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In bulls of the Nellore breed (*Bos taurus indicus*) it was observed four sperm cell subpopulations exhibiting asymmetrical distribution (Santos, et al. 2016. 18th International Congress on Animal Reproduction. Abstract book, Le Vinci in Tours, France, PW 913. p. 331-332). In this work we have investigated the relationship among these clusters and the fertility of semen samples, using *in vitro* fertilization rates. Nine straws were thawed in a water bath at 37° C for 30 seconds and fixed in buffered formal saline. Two hundred images of normal cells per animal were acquired by a CCD camera attached to a microscope and computer. The images were processed using tools of the software Image J, generating data on the morphometry of the sperm head. Multivariate analysis was performed using the Ward hierarchical clustering technique with Euclidean distance and determining the optimal number of groups by analysis of the level of distance and similarity behavior. Later, the k-means method was applied in the partition of each subpopulation. The Tukey test ($P < 0.05$) was used to the validation of groups, in order to check the difference among variables averages. Until now, semen samples of nine animals were used for *in vitro* fertilization of matured oocytes from slaughterhouse ovaries. Twenty hours after fertilization, the potential zygotes were fixed in 4% paraformaldehyde, stained with Hoechst 33342 (5µg / ml) and pronuclear formation rate was evaluated by epifluorescence microscopy in order to determine the fertilization rate. Three replicates per animal were performed. The correlation among the proportion of sperm in each subpopulation and pronuclear formation rate was calculated. Statistical analysis was performed using the softwares Minitab 17.1.0 and R version 3.3.1, MVar.pt package version 1.9.4. Four different subpopulations were determined for all the evaluated bulls which differ in relation to the head area, with SP1 composed by the smaller cells ($X = 966.00 \pm 66.80$ pix^2), SP2 ($X = 981.84 \pm 69.27$ pix^2) and SP3 ($X = 1007.99 \pm 82.91$ pix^2) by intermediate cells and SP4 composed by the larger head area sperm ($X = 1075.51 \pm 49.03$ pix^2). Six bulls out of nine animals evaluated showed a predominance of SP4 ($X = 37.0\% \pm 5.6$), two did not show this predominance ($X = 23.25\% \pm 10.25$) and one animal presented homogeneous clusters distribution and no significant difference in the head area. For correlation analysis, in addition to the animal which did not differ by head area, a second animal was excluded because it was considered an outlier showing a high pronuclear formation rate (68.52%). A significant and negative correlation ($P < 0.05$; $r = -0.91$) was found between the relative numbers of SP4 cells and pronuclear formation rate. These results showed that higher proportion of larger head area sperm cells in *Bos taurus indicus* ejaculates decreases semen *in vitro* fertility. It is possible that the presence and asymmetry of different morphometric sperm cell clusters in the bull could be determined by histones and the degree of nuclear protamination during spermiogenesis and epididymal transit, and also that cells with larger area have a lower degree of nuclear organization and lower fertility in relation to the other subpopulations. Currently a new approach is being conducted in order to investigate these possibilities. (CEUA/UFLA/Protocol#073/15).

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Gynecological examination in Pantaneiro mare

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Pantaneiro horses are very well adapted to Pantanal´s environmental conditions due to natural selection. They are of great use in beef cattle management which is the main economic activity of this region. The gynecological examination is an essential tool for endometrial evaluation in mares. With the endometrial biopsy, cytology and culture is possible to evaluate the structural integrity, inflammation degree and the internal environment of the uterus. This study aimed to characterize the epidemiology of the Pantanal mare´s status reproductive system pathologies to stablish the fertility prognosis and determine the reproductive condition. Gynecological examinations were carried out in 10 Pantaneiro mares aged 3-30 years old performing the culture, cytology and endometrial biopsy. For the uterine culture the mares were properly contained and cleaned and it was used a sterile swab on a stem protected by a metalic tube. After the collection, the sample was placed in the Stuart medium for transportation. Endometrial cytology was performed with a sterile gynecologic brush and after collection was made a smear on a slide and stained by Panótico® method. The uterine biopsy was performed using two different kinds of forceps. With the first forceps, the samples measured approximately 0.5 cm long and 0.2 cm wide, and with the second forceps the samples measured 2 cm long by 1 cm wide. Samples were withdrawn from the uterus body near the junction of the uterine horns, fixed in 10% formalin and processed in routine histology techniques. The classification of mares was made according to the proposal by Doig and Kenney (1986), which includes four categories (I, IIA, IIB and III). Biopsies collected with the first forceps were not successful, because the materials were insufficient for the histopathology of the endometrium. The findings in histopathology were discrete, the main changes consisted of discrete periglandular infiltrators and perivascular inflammatory cells, macrophages containing brownish pigment, small number of eosinophils in the compactum stratum, discreet presence of lymphocytes and plasma cells in the low epithelium, fibrosis areas, glandular dilatation, glandular lymphatic lacunae in island form and edema due to the phase of the estrous cycle. Only one animal showed alteration in the cytology exam, with a pronounced number of inflammatory cells composed of neutrophils and some macrophages. In the endometrial culture isolation was obtained the results of 7 animals, 6 of these with bacterial isolation (*Pseudomonas* sp., *Actinomyces* sp., *Escherichia coli*, *Corynebacterium* sp.), and one with fungus (*Candida* sp.). In examining endometrial cytology, the presence of more than 3 neutrophils per light field is considered to be inflammation, which was observed in a mare who also had rounded negative images of approximately 1µm diameter compatible with fungal structures, which was confirmed by isolation of *Candida* sp. Endometrial culture results should always be interpreted with cytological and histological findings or symptoms. In the endometrial culture two animals isolated Pseudomonas sp., witch may be considered venereal disease in horses, so it is suggested that more detailed examinations are performed in these two animals. The use of endometrial biopsy can provide some links for the endometrium, what other techniques could not provide. Infiltrates of plasma cells, that were under the epitelium, was found in only one mare, and, they are not observed in normal endometrium, the presence of this is indicative of a chronic process. The siderophores, which are macrophages containing hemosiderin, were found in the stratum spongiosum in one animal, its presence indicates chronic inflammation. In relation of periglandular fibrosis, only three mares showed this lesion. According to the degree of fibrosis, only one demonstrated moderate fibrosis and other with slight fibrosis, an animal was classified in category I, and the other mares in the category IIA. It is concluded that samples collected with the first forceps not obtained enough material for histopathological analysis, unlike the second forceps used, which removed optimum amount of material for analysis. There was 4 animals that showed no alterations in the performed tests and were considered in optimal conditions for reproduction. (CEUA/UFMS/Protocol#736/2015).

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Histomorphology of the glans penis in *Eumops glaucinus, Molossus rufus* and *Nyctinomops laticaudatus* (Chiroptera:Mammalia)

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The evolution of internal fertilization resulted in arise of a vast diversity of anatomical forms in the copulatory organs, especially in the penis. Indeed, external and internal penile morphology varies widely among mammal species. The importance of the penis and its variation in morphology to the success of the reproduction in mammals is undoubted. In bats, the morphology of the penis varies between species, and this may be related, among other factors, to the different habitats they live, which may have influenced, in an evolutionary way, the morphology of the copulatory organ. Between some variations in this structure are the presence of an *os penis* (*baculum*) found in the tip of the glans, that may provide an additional rigidity for the erect penis to facilitate intromission, or may assist in sperm transport and to stimulate the female, and the presence of additional erectile tissue known as the *accessory corpus cavernosus* (in the glans either in the prepuce); and in the number of *corpora cavernosa*. So, the aim of our work was to describe the morphology of the *baculum* and the histomorphology of the glans of three species of Chiroptera: *Eumops glaucinus*, *Molossus rufus* and *Nyctinomops laticaudatus*. The penises were removed and processed for light microscopy, stained with HE. For the morphological analyses we submitted 5 penises from each species to the process of diaphonization. The baculum was isolated and analyzed under stereoscopy. In *M. rufus* the glans penis is larger – 4.47mm long, cone-shaped and has spine-shaped epidermal projections. It tapers toward the apex, which is constituted by three lobes. In dorsal surface there is a deep furrow in the level of urinary meatus. The glans penis of *E. glaucinus* is smaller- 1.61mm long, and lacks epidermal projections. Its shaft shaped, oval at its base and widens at approximately two-thirds the distance from the prepucial junction. In the ventral surface there is a medial ridge, and ending the ridge there is a circle shaped furrow, in level of urinary meatus. In *N. laticaudatus* the glans has 2.73mm in length, is cone shaped, tapering toward the apex and ending in a rounded bacular mount. It has spine-shaped epidermal projections, but they are smaller and more spaced than in *M. rufus.* In the ventral surface there is in the subapical region a circular cleft which surrounds the entire ventral portion, the urinary meatus, which separates the ventral end portion of the glans in a small rounded tip, bacular mount.The baculum of *E. glaucinus* has approximately 0.62 mm in length, is curved in about two-thirds of its length when viewed laterally, and narrower in the apical portion than in the basis. Already *M. rufus'* baculum is lower, has about 0.34 mm in length, its shaft-shaped with rounded ends, narrowing in the the midline. *N. laticaudatus*'s baculum has about 0.64mm, has an arrow shape in dorsal view but in lateral view it is straight until 2/3 its length, widening in the remaining third. All species exhibited a vascular penis indeed each one has particular histological features.When observed the basis of the penis all have two corpora cavernosa, but when we analyzed at the midpoint we notice that the penis of *M. rufus* shows three corpora cavernosa while there is no variation in the number of this tissue in the other species. The three species have another erectile tissue, which is more diffuse, called accessory *corpora cavernosa*, which is more developed in *M. rufus* and *N. laticaudatus* than in *E. glaucinus*. In *M. rufus* and *N. laticaudatus* the epithelium is covered with a deep layer of keratin, which forms the epithelial projections. Already in *E. glaucinus* we were able to identify a pair of dorso-lateral nerves, that are absent in the other species. According to the exhibit data we can confirm that the analyzed taxa keep the basic histological structure of vascular penis in mammals, with variations both in morphology and in histological arrangement in the penises of the three species. (IBAMA Processo: 02027.001957/2006-02).

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Impact of chronic exposure to low doses of bisphenol A on the epithelial and stromal compartments of the ventral gerbil's prostate

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Recent research has shown that exposure to various chemicals in the environment can cause permanent morphophysiological changes in the prostate. These substances, know as endocrine disruptors, represent a variety of components that are found in significant quantities in water, air, soil, food and industrial products in general. These chemicals mimic steroid hormones and have the potential to cause damage to the endocrine system, in order to directly affect the metabolism of hormones in the body. Consequently, these substances may act directly on the organs of the reproductive tract, both of males and females in order to compete with endogenous steroids for the binding sites of their specific receptors. The aim of this study was to evaluate the potential of the chronic exposure to bisphenol A on promotion of neoplastic prostate lesions in ventral prostate lobe of gerbil (*Meriones unguiculatus*) and analyze the impact of bisphenol A on the epithelial compartment and on the elements of the stromal compartment. Adult gerbils (120 days/ n=7 per group) were divided into Control Group- animals that received filtered water; and BPA group – animals that received bisphenol A - 50μg/kg/day in drinking water. Fourteen animals were sacrificed after twenty-nine weeks of treatment and the ventral prostate lobe was removed and processed for light microscopy. The methodologies involved the monitoring of body weight of the animal and the consumption of bisphenol A. Morphological analyzes of ventral prostate were performed by cytochemical and immunohistochemical (α-actin, PCNA, CD163) methods (Ethics Committee Protocol: 122/2015 - CEUA-IBILCE/UNESP). Bisphenol A stimulated a higher number of histopathological lesions in prostate (Premalignant and Malignant lesions). Among the lesions, the most common was Prostatic Intraepithelial Neoplasia. These lesions showed papillary growth, commonly forming epithelial invaginations restricted to certain sites. Microinvasive lesions were less frequent and characterized by rupture of smooth muscle cells layer and basement membrane, allowing stromal invasion by the abnormal cells. In general, it was possible to observe a large number of macrophages (CD163 positive cells) associated with the areas affected by lesions. Stereological analysis of epithelial and stromal components showed that in BPA group, the relative volume of epithelium was greater than the control group. This suggests that BPA promoted instability in epithelial cells homeostasis, which was reflected in higher proliferative index (PCNA positive cells). Consequently, there was a decrease in the relative volume of the luminal compartment due to the frequent proliferative foci. In the stromal compartment were analyzed the fibrilar elements (Collagen I and III) and smooth muscle (α-actin positive cells) and was observed an increase in the volume occupied by all of them in the BPA group. Additionally, these elements have suffered restructuration in neoplastic sites. Thus, we conclude that BPA alters the epithelial and stromal microenvironment of ventral prostate and may be considered a promoter of neoplasms in the gland.

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Increased prevalence of clinical varicocele in Brazilian young adults

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Male infertility is a major public health problem that has been increasing in recent decades. Varicocele, an abnormal dilation of the veins of the pampiniform plexus with blood reflux, is the most treatable cause of male infertility. Few studies show that the prevalence rates of varicocele among adolescents and young adults ranges from 3 to 25%. Despite an abundance of studies on the effects of varicoceles and their prevalence within the adult population, there are comparatively few data examining the effects of varicocele on youth. Considering the lack of data about varicocele prevalence in Brazilian young men, our main objective was to evaluate the clinical varicocele prevalence in young men from Belo Horizonte metropolitan area. Men aged between 18 and 23 year-old from several universities of Belo Horizonte and its metropolitan region were invited to participate as volunteers through posters, flyers, electronic media, social networking, radio and television. The inclusion criteria were place of residence in the Belo Horizonte metropolitan area, and both the man and his mother being born and raised in Brazil. The participation rate among recruited men was 60%. The testicle examination of the 235 young men were performed under warm temperature in a private room, in orthostatic position, with and without the Valsalva manoeuver. Varicocele was classified in grades as follows. First grade: when the enlargement of the venous plexus of spermatic tone is evident only by palpation during the Valsalva manoeuver. Second grade: when the enlargement of the venous plexus of spermatic tone is evident only by palpation at upright position. Third grade: when the enlargement of the venous plexus of spermatic tone is visually evident. The criteria for varicocele diagnosis was unchanged during the study period. Almost half-analyzed young men (47.5%) were diagnosed with varicocele. From these, 53% and 14.8% had varicocele in the left and in the right testis, respectively. Another 32.2% showed bilateral varicocele. Left side unilateral varicocele was present in 25.3% of the analyzed young men and 15%, 6.6% and 3.7% were classified as grade I, grade II and grade III, respectively. Right side unilateral varicocele was present in 7% of the analyzed young men and 5%, 0.8% and 1.2% were classified as grade I, grade II and grade III, respectively. Moreover, 15.2% of the analyzed young men showed bilateral varicocele. To our knowledge, this is the first study in Brazil concerning young adult's rates of varicocele prevalence. A better understanding of the varicocele in relation to testicular function, semen analysis, and fertility outcomes would better guide practitioners in their counseling and management of patients with regard to intervention.

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Influence of exogenous melatonin on rat prostate response under experimental diabetes and testosterone supplementation

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Prostate damage induced by diabetes is due to perturbations in insulin signaling, increment of oxidative stress and androgenic scarcity. Because of its antioxidant activity, melatonin (MLT) is a potential agent to be used in prevention of prostate impairment due to diabetes. MLT also inhibits testosterone testicular synthesis and its action on androgen-dependent organs must be better evaluated. In this regard, we investigated the influence of therapeutic doses of MLT in morphology and proliferative activity on ventral prostate of healthy and diabetic rats. Testosterone treatment was used to discriminate the MLT inhibitory effect on androgen synthesis and to better clarify the MLT specific action on this gland. The experiment was approved by the Ethics Committee of Experimental Animals of Sao Paulo State University (Protocol number: 137/2016). Wistar rats (12 weeks old) were divided into 6 groups (n=15 per group): control (C), MLT treatment (M), diabetic (D), diabetic treated with MLT (DM), diabetic treated with testosterone (DT) and diabetic treated with MLT and testosterone. Diabetes was induced at 12^{th} week by streptozotocin injection (40mg/Kg body weight, ip) and the animals which showed glucose levels over 200mg/dL were considered diabetic. Animals were treated for one week with MLT (0.1mg/kg/day, gavage), testosterone (200ug/day/animal, sc) or both and were euthanized at 13^{th} week of age. Serum testosterone levels were measured by ELISA, prostate histological sections were stained with Hematoxylin and Eosin for stereological analysis and submitted to immunohistochemistry for proliferating cell nuclear antigen (PCNA). Our results indicated that MLT treatment did not influence glycemic levels of healthy animals but increased these levels ~70% at D and DM group. There was a drastic prostate atrophy in D ($p=0.002$) and DM ($p=0.007$) that did not occurred in DT and DMT groups. Stereological analysis indicated that M group displayed a reduction in absolute frequency of epithelial component and D group showed a decrease of epithelial relative weight (~43%) and lumen (~42%), possibly due to the decline of epithelial cell secretory capacity. Proliferative rates declined ~70% after one week of induced-diabetes. The MLT treatment to diabetic rats did not avoid epithelial and lumen atrophy however there was an increased ~32% the relative stromal weight. This effect could be explained by the fact that MLT preserved testosterone serum levels in DM group. As expected, testosterone treatment to diabetic rats attenuated acinar atrophy of prostate. DMT group displayed a reduction of acinar epithelial and stromal hyperplasia as well an increase of mitotic rate which surpassed DT group. In conclusion, the main effect of treatment of healthy rats with therapeutic doses of MLT for short-term was a discrete epithelial atrophy. Such doses ameliorated prostate atrophy induced by experimental diabetes but also induced stromal hyperplasia of the gland in this rodent. Besides, our results indicate a synergistic action of MLT and testosterone on proliferative activity of rat prostate under hyperglycemic conditions.

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Male reproductive system in *Coendou prehensilis* (Rodentia, Erethizontidae)

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The knowledge about reproductive aspects has great importance in the conservation of endangered species and captive breeding techniques can answer some behavioral issues. In rodents we can see a wide diversity of characteristics found in the reproductive system even in animals belonging to the same suborder as the *histricomorfs*, for example, which belongs the *Coendou prehensilis*. The present study aims to acquire more knowledge about this rodent species morphologically describing the male reproductive system and characterizing all the components present in this system both macro and microscopically, being helpful in the management and captive reproduction of *C.prehensilis*. Seven males were used for the reproductive system study, all organs were examined macroscopically and microscopically, five animals were destined for histological analysis and by scanning electronic microscopy. The specie's reproductive system is formed by a pair of testes and ductus deferens, a pair of vesicular, prostate and bulbourethral glands, both with fundamental importance in the semen production, but as in other *hystricomorphs*, the *C. prehensilis* doesn't have a pair of ampullary glands. The *C. prehensilis* male doesn't have an apparent sexual dimorphism, the ovoid testes are found in the abdominal and pelvic cavities, varying according to their age, not reaching the scrotum, keeping it empty. The ductus deferens has no expansion in its extension and just carries the sperm to the urethra, requiring its pseudostratified epithelium is more resistant and thick, externally presenting a thick layer of smooth muscle, necessary for ejaculation of sperm. All the three glands are shown in pairs and histologically with three layers, an outer coating, a medium muscle and an internal secretory epithelium, as in other *hystricomorphs*, but the inner layer of the glands had a very particular characteristic in this species and not found in other animals of the same class. The penis with "S" inverted format can be found in both cylindrical and conical form during its extension, full of keratinized spicules on its surface. Inside the penis, a large amount of smooth muscle around the spongy and cavernous bodies was found. The spongy body wrapped by a slight layer of connective tissue surrounds the urethra, which contains a transitional epithelium in its structure. The results analyzed confirmed that the reproductive system of male C. prehensilis has many features similar to other hystricomorphs rodents. Some few differences were found, such as the format of the glands, the epithelium and the amount of the penis spicules.

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Metabolome profile of seminal plasma associated with bull fertility

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Metabolites play essential roles in biological systems; however, detailed identities and significance of the seminal plasma metabolome in the bovine have not been reported. The objectives of this study were to develop a comprehensive metabolome profile of seminal plasma from Holstein sires, and to evaluate the potential relationships between seminal plasma metabolites and bull fertility. To achieve these goals, metabolomic profiles of seminal plasma from 16 Holstein bulls with different fertility (high fertility, n = 8 and low fertility, n = 8) scores were determined using gas chromatography-mass spectrometry (GC-MS). Moreover, multivariate and univariate statistical analyses of the data were performed by uploading data onto MetaboAnalyst 3.0. Pathways associated with the seminal plasma metabolome were identified using a bioinformatics tool (Metscape, version 3.1.2, which is a plug-in for Cytoscape, version 3.2.1). Based on GC-MS, 63 metabolites were identified in the seminal plasma of all bulls, including 21 amino acids, such as glutamic acid, alanine, isoleucine, leucine, and serine. Other 20 organic compounds, such as citric acid, lactic acid, urea, uric acid and myo-inositol were described as the second major group of metabolites. Twelve carbohydrates and carbohydrate conjugate metabolites were identified in the bull seminal plasma, such as fructose, mannitol, sorbitol, glycerol, and ribitol. In addition, we identified fatty acids and conjugates (malic acid, hexadecanoic acid, methylmaleic acid and stearic acid); steroids and steroid derivatives (androstenedione and cholesterol); nucleosides, nucleotides, analogue (inosine and 5-methyluridine and); and inorganic compounds (borate and phosphoric acid). Fructose was the single most predominant metabolite found in the seminal plasma of all bulls, followed by citric acid, lactic acid, urea, and phosphoric acid. Androstenedione, 4-ketoglucose, D-xylofuranose, 2-oxoglutaric acid and erythronic acid were among the five least abundant metabolites identified in bull seminal plasma. Partial-Least Squares Discriminant Analysis identified a distinct separation between high and low fertility bulls as regard to scores plot. In addition, metabolites with Variable Importance in Projection (VIP) greater than 1.5 included 2-oxoglutaric acid, fructose, phosphoric acid, D-mannitol, 4-ketoglucose, dulcitol and erythronic acid. Among these compounds, metabolites with the highest VIP score were 2-oxoglutaric acid (VIP = 2.17) and fructose (VIP = 2.1). Heat-map analysis, based on VIP score, and univariate analysis indicated that 2-oxoglutaric acid was significantly lower ($P < 0.05$) and fructose was significantly higher ($P < 0.05$) in seminal plasma from the high fertility bulls as compared to those of low fertility bulls. Based on fructose network panel, D-sorbitol undergoes a non-reversible reaction to produce fructose, which is converted to beta-D-fructose-6-phosphate and D-fructose-1-phosphate. Furthermore, 2-oxoglutaric acid is involved in several reversible and non-reversible reactions, as it can be synthetized from L-glutamate, succinyl-CoA, 2-hydroxyglutarate, 2-methyl-3-oxopropanoate and 3-amino-2-methylpropanoate, among others. In conclusion, we have demonstrated that fructose, citric acid, lactic acid, urea, and phosphoric acid are the predominant metabolites in bull seminal plasma. We also have detected separation of metabolite profiles between high and low fertility bulls and determined that fructose and 2-oxoglutaric acid are biomarker candidates of bull fertility. Findings of the present study help advance our current understanding of the multifactorial and complex processes involving the physiology of male fertility.

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Pathological lesions incidence and global DNA-methylation in rat prostate under streptozotocin-induced diabetes and melatonin treatment

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Chronic hyperglycemia leads to higher production of reactive oxygen species which favors carcinogenesis. The association between *diabetes mellitus* type 1 (DM1) and prostate cancer is controversial. Melatonin has antioxidant, anti-inflammatory and antiproliferative properties. We aimed to analyze if diabetes induced by streptozotocin favors pathological lesions in rat ventral prostate. It was also examined if melatonin administration at low dosage prevents tissue alterations caused by diabetes and alters the prostate histology of healthy rats. Melatonin was provided in drinking water (10 µg/kg/day) from the 5th week of age until the end of experiment. Diabetes was induced at the 13th week by STZ (40mg/kg, ip). The euthanasia occurred at the 14th week (short-term) and 21st week of age (long-term). Stereological and morphometric analysis of prostate components,as well as the incidence and density of malignant and pre-malignant lesions were assessed. Immunohistochemistry reactions for α-actin, cell proliferation (PCNA), Bcl-2, glutathione S-transferase (GSTpi) and DNA methylation (5-methylcytidine) were performed. Melatonin did not elicited conspicuous changes in healthy animals. Diabetic animals presented higher incidence of atrophy (93%), microinvasive carcinoma (6%), proliferative inflammatory atrophy, PIA (13%), prostatitis (26%) and prostate intraepithelial neoplasia, PIN (20%) associated with an increase of 40% in global DNA methylation. Melatonin attenuated epithelial and smooth muscle cells atrophy, normalized the interacinar collagen organization specially at short-term diabetes and the incidence of PIN (11%), inflammatory cells infiltrates, prostatitis (0%) and PIA (0%) at chonic diabetes. However, these animals also presented microinvasive carcinoma. MLT even at low dosage was able to prevent pre-malignant lesions and normalize epithelium and smc cells atrophy. CEUA: 51/2011

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Postmortem changes in mouse testicular structure and its implications in morphological analysis: effects of collecting time and fixing

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The vital functions cease at the time of death of the animal, but several changes begin to occur immediately in the postmortem period. The speed at which they occur depends on external factors, and the time interval between death and autopsy is the most critical factor associated with altered postmortem tissue. Knowledge of these factors is essential for the success of the experimental evaluation. The histological investigation plays an important role in postmortem examination to detect lesions, since the autolytic process causes changes in tissues that can mask or induce experimental results. The testes are paired organs with endocrine and exocrine function, composed of two compartments: tubular and Intertubular. In the first compartment are the seminiferous tubules formed by tunica propria, lumen and seminiferous epithelium, being responsible for the production of sperm. In Intertubular compartment are found blood and lymph vessels, nerves and a variable cell population consisting mainly of fibroblasts, macrophages, sometimes mast cells, and Leydig cells that synthesize and secrete testosterone. Because of the great use of laboratory animals in experiments, the aim of this study was to evaluate the potential quantitative and qualitative changes in testicular tissue at different times after the euthanasia of animals. Twenty five male mice were used and their testes were collected immediately after euthanasia, and 5, 10, 15 and 20 minutes postmortem. The organs were fixed, embedded, cut and stained with toluidine blue, and then photodocumented. For testis morphometry was used grid of 266 points by analyzing 10 images per animal, being counted points on lumen, seminiferous epithelium, tunica propria and intertubule. It was made the volumetry of testicular components considering the percentage of each component and the weight of the parenchyma. As testicular density is equal to 1, the testis weight was considered to their volume. The tubular diameter and height of the seminiferous epithelium were also measured. For histopathology analysis was made the proportion of cells with and without vacuoles. There was an increase in the ratio of seminiferous epithelium from 10 minutes after the euthanasia, while the lumen ratios, tunica propria and intertubule showed no significant changes. There was an increase in lumen volume and tunica propria in 10 minutes after euthanasia and a reduction from 15 minutes. No change in tubular diameter and epithelial height over time the testicle was out of fixative solution. We observed the formation of vacuoles 5 minutes after euthanasia with growth and significant increases with the passing of time postmortem. This is the first study evaluating possible changes in testicular structure due to the delay in onset fixation, showing that this time is essential for quality of analysis. The testis compared with other organs, show to be more resistant to the onset of morphological and volumetric change and with the passing of time outside fixative solution, can observe that pathological conditions can be simulated, starting immediately after euthanasia of the animal.

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Seminal, Biometric and Morphometric Profile of Nelore Bulls with Unspecific Orchitis

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The biometric and seminal evaluation are broadly used in bulls selection, however, these methods are not effective to diagnose some reproductive diseases. The unspecific orchitis is characterized by discrete interstitial inflammatory infiltrate with lymphocytic predominance. In this context, in a study with 56 Nelore Bulls raised extensively in Mato Grosso do Sul state Brazil we selected ten animals diagnosed after histological analysis with unspecific orchitis (Orchitis Group = OG) and 12 with normal testis (Normal Group = NG). The animals were previously submitted to three seminal collect and measure of scrotal perimeter with 15 days interval. The sperm kinetics was subjectively evaluated by motility (%), vigor (1 - 5), and turbulence (1 - 4) and spermatic analysis was performed in semen sample stored in 10% formalin solution and was determined the percentage of the normal sperms and spermatic concentration. Representative fragments of testis were collected by orchiectomy and fixed in Bouin solution's by 24 hours. After fixation, the tissues were processed and stained with hematoxilin and eosin for histological and morphometric analysis. In morphometric analysis were measured: area of germinal epithelium (GE), area of tubular lumen (TL), area of Leydig cells (LC) and stromal area (SA). Statistical analysis was conducted by software BioEstat 5.3®. The non-parametric data was analyzed by Mann-Whitney test and parametric date by t test for independents samples. Data with variation coefficient more than 30% were subjected to angular (%) and logarithmic transformations. In animals of OG, histological analysis showed interstitial mononuclear infiltrate and low degree of degeneration of germinal epithelium. In measure of scrotal perimeter wasn't statistical differences ($P > 0.05$) between NG and OG, 32.08 ± 0.90 and 31.80 ± 1.22 (mean ± sd) respectively. The motility of NG was 66.41 ± 10.25 and 44.00 ± 22.82 in OG, and these results was statistically different. Wherever, the results of vigor, NG = 4.00 ± 1.00 and OG = 2.50 ± 2.00, and turbulence, NG = 2.50 ± 1.00 and OG = 1.50 ± 1.00 (median ± interquartile range) is not significant ($P = 0.09$ and $P = 0.07$ respectively). Regarding the percentage of de normal sperm, wasn't statistical differences between periods in two groups, but when compared NG with OG the significance was observed only third collection ($P < 0,01$), being NG = 86.00 ± 4.81 and OG = 25.50 ± 23.82 (mean ± sd). The spermatic concentration, NG = 3.34 ± 1.56 and OG = 1.77 ± 1.49 (mean ± sd), was statistically significant ($P < 0,05$). The results of morphometric measures was: NG: GE= 53.17 ± 2.66%, TL= 23.17 ± 3.35%, LC= 3,75±1,36%, SA= 19.75 ± 4.18 and OG: GE = 49.70 ± 1.53%, TL= 24.90 ± 4.68%, LC= 4.60 ± 1.58, SA= 21.20 ± 5.53. In these measurements, there was statistical significance only in area of germinal epithelium ($P < 0,05$). The results of present study suggest that the unspecific orchitis affect significantly the fertility of bulls, probably due to chronic testicular degeneration associated with this process, leading to decrease in the germinal epithelium area. New investigation about etiology of unspecific orchitis should be carried, given that wasn't possible to identify the agent in this study.

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The renal sexual segment of the rattlesnake *Crotalus durissus* (Linneaus, 1758): seasonal cycle and androgen responsiveness

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The renal sexual segment of the kidney (RSS) comprises the distal region of the nephron and can be found in many male Squamata reptiles. The RSS has a simple epithelium and the cells are characterized by the presence of sexual granules throughout the cytoplasm. It has been proposed that the secretions are involved with nutrition and activation of spermatozoa in the semen. Several studies on snakes confirmed that the RSS has a seasonal cycle and the hypertrophy of the segmen is related with the mating season. The rattlesnake (*Crotalus durissus*), a neotropical snake from the Viperidae family, is reproductively active from the late summer to the beginning of the winter in the southeastern Brazil. In this species, the spermatogenic activity starts in the spring, reaching a peak in the summer. Testicular regression has been observed since the fall until the winter. Hence, the present study was address to investigate the renal sexual segment cycle along the four seasons and the androgen responsiveness as well. Forty eight adult males were collected along the four seasons of the year (CEUA-UFMG protocol number 130/2015). The specimens were pre-anesthetized with dry ice, and euthanized with an overdose of barbiturate (thiopental 100 mg/kg) via intra-cardiac. Kidneys and testis were collected, weighed and fixed in Bouin solution. Samples were dehydrated in a sequence of alcohol and embedded in glycol methacrilate for histomorphometric evaluation, and in paraplast for histochemical (Periodic Acid Schiff - PAS and Bromophenol Blue) and immunohistochemical analysis (androgen receptor - AR). The slides were photographed and morphometry was performed using Image J Software. The preliminary results indicate a seasonal cycle of the RSS which is probably related to the seasonal variation of testicular activity. We observed an increase of RSS parameters (kidney-somatic index, tubular diameter, and epithelium height of the RSS) during the summer and fall when compared to the spring and winter. There were also significant differences in the nuclear diameter between the seasons. We observed the highest values for this parameter during the summer and the lowest values in the spring. These results indicate a higher activity of synthesis of the cells of RSS during the summer (before mating season), and the lower activity during the spring. RSS tubules were weakly reactive to PAS and Bromophenol Blue during the spring, showing low concentrations of glycidic and proteinaceous compounds. RSS lower activity in the spring would be associated to returning of the spermatogenesis, since the testes were regressed in the winter. PAS and Bromophenol Blue positive tubules predominate in the summer (before mating season), fall (mating season) and winter. The highest values of the gonadossomatic index in the summer correspond to the peak of spermatogenesis, and the lowest values found in the winter correspond to the testes regressed. Hypertrophy of the RSS in the winter is possibly related to the extended breeding season and/or opportunistic copulation. The early regression of RSS in the winter is observed, however, strong PAS and Bromophenol Blue reaction was maintained probably due the reduction of RSS tubules volume. AR positive cells were found in the RSS during the spring. Taken together, the results suggest that the RSS undergoes remarkable changes along the year and these functional modifications observed might be modulated by androgens. Further studies are being developing in order the better understand these mechanisms.

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The time of day affects the scrotal surface temperature measured by infrared thermography and rectal temperature in rats

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Infrared thermography is a non-invasive diagnostic method that allows to determinate by thermal mapping, the physiological state of a tissue or organ, which can contribute to a medical diagnosis. To validate infrared thermography as a research or clinical tool, it is essential to determine factors that may influence the measurement of scrotal surface temperature (SST). Some relevant factors are already stated for an accurate thermographic measure as environment temperature and humidity and the distance between the camera and the target. Thereby the aim of this study was to determinate if the time of the day interferes on SST, measured by infrared thermography and to determine the difference between SST and rectal temperature in rats. The thermographic imaging protocol was standardized to reduce interferences in the measurements creating a reliable pattern. All research was conducted in a temperature and humidity controlled room (20°C and 60% humidity). Scrotal temperature image was performed by thermographic imaging using an infrared camera (Flir®, Infra Cam TM, FLIR Systems Inc. Portland, EUA), held 60 cm from the testicles. Rectal temperature was performed using a clinical digital thermometer after the thermography measurement. Twenty-four Wistar rats were used, aging 70-days-old and weighting 242.6 ± 16.0 g. The SST and rectal temperature were measured at two times of the day (9 am and 2 pm). The images were analyzed by Flir Quick-Report 1.2 SP2 Software (2009, FLIR Systems Inc.). The SST and rectal temperatures were analyzed by R stat software 3.2.2 using Shapiro–Wilk and t test was used with 5% ($p \leq 0.05$) significance level. The results were expressed in means and standard deviation. The mean of SST and rectal temperature were lower ($p < 0.001$) at 9 am than at 2 pm (SST: 28.2 ± 0.7 ºC and 30.8 ± 0.7 ºC; rectal temperature: 36.5 ± 0.3 ºC and 37.3 ±0.4 ºC, respectively). Additionally, the SST is 7 to 8 °C below rectal temperature in rats. We concluded that there are interference of the time of the day on SST and rectal temperature in rats, making necessary to standardize the time of the day to use infrared thermography tool to research. (CEUA- FMVZ – UNESP, Botucatu protocol 46/206).

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Bacterial resistance to antibiotics commonly included in extenders for cryopreserved bull semen

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Artificial insemination with frozen semen is one the most commonly used reproductive techniques in dairy production. Cryopreservation results in reduced sperm viability compared to fresh semen, mainly characterized by declined motility. Another factor that may affect negatively sperm viability is excessive growth of bacteria present in either the bull's prepuce microflora and external genitalia or that are introduced during semen collection and processing. Thus, antimicrobials are added to extenders to minimize such effects, which are regulated by the World Organization for Animal Health (OIE) and the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA). However, the indiscriminate inclusion of antibiotics in extenders may result in increased resistance of microorganisms. This study aimed to identify the sensitivity or *in vitro* resistance of microorganisms to antibiotics recommended by the MAPA in a bull semen production center. Bacteria were isolated from samples of fractions A and B of freezing extender, of artificial vaginas, of fresh, cooled and packaged semen, and of the flexible tube filling machine, comprising all processing steps of ejaculates. From such steps, 297 microorganisms were isolated and identified by Vitek®. Among them, 56 isolates were tested against antibiotics recommended by the MAPA through the disk diffusion method according to NCCLS (2015). After identification, the optical density of the isolates solution was adjusted by spectrophotometer, with wavelength of 540 nm to 0.5-0.6. Isolates were plated in the surface of petri dishes (150 x 20 mm) containing BHI agar and the discs were impregnated with antibiotics added in the following sequence: 10 µg gentamicin; 10 µg streptomycin; 10 µg penicillin; 30 µg tylosin; 60/30 µg spectinocin/lincomycin; and 30 µg lincomycin. The plates were kept at 35°C for 24 h. After, the inhibition zone was read using a ruler. From the 297 isolates, 24 bacterial types were identified, among which: *Eschericha coli, Enterobacter aerogenes, Staphylococcus aureus, Staphylococcus warneri, Staphylococcus simulans, Staphylococcus sciuri, Staphylococcus capitis, Rhizobium radiobacter, Enterococcus faecalis, Leuconostoc mesenteroides ssp cremoris, Sphigomonas paucimobilis, Aeromonas salmonicida*e, *Aerococcus viridans*. Among the 56 microorganisms tested, only *Staphylococcus epidermidis* was sensitive to both tylosin and lincomycin, whereas the other microorganisms were resistant to these two antibiotics. All isolates were resistant to penicillin, 22 (39.3%) were sensitive to spectinomycin/lincomycin, 43 (76.8%) were sensitive to gentamicin and only four (7.1%) were sensitive to streptomycin. The results indicate that tylosin, lincomycin, penicillin and streptomycin, which are commonly used in processing of bull semen, have low efficiency, due to the great percentage of resistance observed in the tested isolates (> 90%). Further studies should be performed to adjust the exact dose of each antibiotic and to investigate new antimicrobial combinations for use in semen processing.

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Characterization of the reproductive cycle of surubim-do-Paraíba females

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The surubim-do-Paraíba, *Steindachneridion parahybae*, is a species of fish endemic, natural of river Paraibuna South basin, São Paulo/BR, which is on the list of species of Red Book of Brazilian Fauna, therefore considered in critical extinction conditions. For this reason, it has aroused interest in cryopreservation of your gametes, in order to preserve the genetic material. Currently the project is being developed in order to cryopreserve surubim-of-Paraíba oocytes. However, little is known about the reproductive traits of females of this species, limiting only to macroscopic morphological analysis of ovaries. Thus, the aim of this study was to characterize the oocyte stages of surubim-do-Paraíba and period of the reproductive season in which they occur. Four females were anesthetized and decapitated in each collection over a year (Dec/14; May/15; Sep/15) for removal of the gonads with the following purposes: morphology of the ovaries (female body weight, gonad weight, visual features) and; morphology of the oocytes (ovum count/g, size and stage). Stereomicroscope images catches were made, they were measured through Belview 7. Descriptive statistics (mean and standard deviation, SD), average test (ANOVA) and frequency diameters were performed using Statistica Software 7. In Dec/14 the females F1 and F2 were undergone hormonal induction and extrusion, so the average diameter of their oocytes was the higher (1.87 ± 0.06 and 1.79 ± 0.11). The average diameters of the other females were also high (F3 1.49 ± 0.28; F4 1.42 ± 0.26), with well-vascularized ovaries, the oocytes with seemingly homogeneous size and very uniform color, featuring the mature stage. On May/15 only one female showed oocytes, but with reduced size diameter (1.05±0.09) in oocyte degeneration process, this is common on the ovarian regression period; while the other females may be considered at resting period, where the ovaries exhibit reduced size and absence of well-defined oocytes. Sep/15 can be marked by ovarian maturation period, with averages of smaller diameters (F1 0.78±0.28; F2 1.29±0.27; F3 1.24±0.27; F4 0.90±0.22), and the ovaries are filled, but with oocytes in very different colors. The diameter of the oocytes was divided into classes, featuring the development stages: Class 0, absence of well-defined oocytes or with diameter oocyte maximum until 0.405 mm; Class 1, primary (0.405 mm < diameter < 0.86 mm); Class 2, cortical alveoli or pre-vitellogenic (0.816 mm < diameter < 1.224 mm); Class 3, vitellogenic (1.224 mm < diameter < 1.632 mm) and; Class 4, maturing (diameter > 1.632 mm). Mature oocytes were obtained only after hormone injection and extrusion. Regarding the frequency of each class, it is observed that primary oocytes were present mostly while collecting September (11%) and may also be observed pre-vitellogenic oocytes (12%) and to a lesser extent vitellogenic (6%). Oocytes maturing are prevalent (14%) in the ovaries in December and keeps well until February, a period ideal for inducing breeding surubim-of-Paraíba females. In May oocytes, when present in the ovaries (1%), are degenerating indicating atretic period, despite the same diameter, has distinct morphological features of the pre-vitellogenic oocytes. Thus we can conclude that the reproductive period in this species have been November until February, with one month variation (beginning or end); diameters of each oocyte development stage, thereby facilitating the use of each of these stages of the species in cryopreservation protocols and frequencies at which they occur; and as a result, it was found that the species is spawning parceled type.

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DBP treatment during the masculinization programming window, but not right after it, causes break-up of seminiferous cords and formation of dysgenetic areas in rats

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Reproductive disorders are extremely common nowadays and some studies show that their incidence may be increasing in the last decades. These disorders include cryptorchidism and hypospadias in newborns and low sperm counts, testis germ cell cancer and hypogonadism in young adult males. These disorders are hypothesized to comprise a Testicular Dysgenesis Syndrome (TDS) in which environmental and lifestyle factors are clearly implicated as potential causes. Although the TDS disorders manifest in different life stages (at birth or young adulthood), there is strong evidence showing that they may have a common origin in fetal life, pointing to the importance of mother's lifestyle during pregnancy. As male reproductive development is a hormone dependent process, changes in the hormonal balance during fetal life, especially deficiency in androgen production and action during the masculinization programming window (MPW), can be related to these disorders. The MPW is thought to occur between 8 and 14 weeks in humans and from e15.5 to e18.5 in rats, a time period that is just after testis differentiation and seminiferous cord formation but before the differentiation of the reproductive tract which is highly dependent on androgens. Gestational exposure of pregnant rats to certain phthalate esters (such as dibutyl phthalate, DBP, which is a common environmental chemical) results in reproductive abnormalities in the male offspring that are very similar to TDS in humans, making this an excellent animal model for studying the fetal origins of human TDS. DBP treatment induces focal dysgenetic areas, which appear between e19.5-e21.5 and manifest as focal aggregation of Leydig cells and presence of ectopic Sertoli cells (SC), even when DBP treatment is initiated (e15.5) after completion of normal SC differentiation and seminiferous cord formation (e13.5-e14.5). There are some unexplained features about these ectopic SC: (1) they do not appear until beyond e19.5 (after cessation of DBP treatment); (2) DBP treatment during the period (e19.5-e21.5) when ectopic SC do appear, rather than during the MPW, fails to induce ectopic SC; (3) unlike normally differentiated SC, the ectopic SC do not form/initiate seminiferous cord formation during fetal life, but only later after birth. Our hypothesis is that the ectopic SC originate from breakdown of already formed seminiferous cords. To address this, time-mated female Wistar rats were treated daily with 750mg/kg/day of DBP in three different windows: full window (FW, e13.5-e20.5); masculinization programming window (MPW, e15.5-e18.5) or late window (LW, e19.5-e20.5). Fetal testis sections from the offspring were evaluated at several fetal ages, using triple immunofluorescence and stereology (n=6-12 per age/group). The results show that DBP treatment during the MPW produces more frequent and extensive dysgenetic areas, containing ectopic SC and germ cells (GC), while the LW treatment does not result in any dysgenetic areas. Additionally, the DBP treatment causes clustering of GC in the centre of the cords, especially in the FW and LW groups, while in the MPW group many GC still migrate to the basal lamina, as expected in normal testis development. The intensity of expression of smooth muscle actin, calponin and myosin in peritubular myoid cells at e21.5 in DBP exposed rats (MPW and FW) is reduced, suggesting an impaired basement membrane in these cords. Furthermore, we observed cords breaking up at e20.5 in the MPW group, releasing SC and GC to the interstitial compartment; we presume that this event gives rise to the focal dysgenetic areas observed in the FW and MPW DBP-treated testis. The mechanism for this still need to be investigated, and it might give us better understanding about how dysgenesis arises in human cases of TDS. Also, we show for the first time that a disruption can be induced after the testis is completely formed, and reinforced the critical importance of the MPW in this process.

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Key words: dibutyl phthalate; dysgenesis; masculinization programming window; testicular dysgenesis syndrome.

Endometrial immunostaining and gene expression of estrogen and progesterone receptors in non-cyclic mares after progesterone administration

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Administration of progesterone (P4) after estradiol is usually performed to prepare non-cyclic mares as embryo recipients, considering that in cyclic mares estradiol stimulates the expression of estrogen and P4 receptors. However, there are successful pregnancy reports after embryo transfer in non-cyclic mares treated only with P4. In this context, the objective of this study was to evaluate endometrial immunostaining and gene expression for estrogen receptor alpha (ERα), beta (ERβ) and progesterone receptors (PR) in non-cyclic mares, before and after administration of long acting progesterone (LA P4), without previous injection of estradiol. Endometrial tissue samples were collected from six anestrous mares immediately before the administration of 1,5g LA P4 (Sincrogest Injetável®, Ourofino, SP, Brazil) and five days after. Samples were divided in two parts and one was stored in 10% formalin for immunohistochemistry (IHC), while the other part was stored in liquid nitrogen for reversed transcription quantitative real-time polymerase chain reaction (RT-qPCR). The IHC technique was performed using ERα (1:100, mouse monoclonal SC-311, Santa Cruz Biotechnology), ERβ (1:100, mouse monoclonal PPG5/10, Abcam) and PR (1:100, mouse monoclonal PR-2C5, Invitrogen) antibodies according to peroxidase and DAB method. Staining intensity and distribution of the receptors in luminal epithelium, glandular epithelium and stroma were described by two researchers blinded to treatment. Changes in gene expression of ERα, ERβ and PR by RT-qPCR were calculated by mean quantification cycle (Cq) and then normalized for the endogenous reference gene B2M (β-2 microglobulin) to generate delta Cq values (ΔCq). Changes in relative abundance of the transcripts were calculated by the relative expression ratio (*R*) method. Before LA P4 administration, ERα immunostaining was apparently weak in the luminal epithelium, glandular epithelium and stromal nuclei. The opposite was observed for ERβ, which showed strong immunostaining in the epitheliums and stromal nuclei. Regarding PR, strong immunostaining was also observed in both luminal and glandular epithelium, and stroma. After LA P4 administration, ERα showed intense immunostaining in all cell types, mainly in the endometrial glands cytoplasm. Strong immunostaining was also observed in all cell types for PR. On the other hand, weak immunostaining was observed for ERβ in glandular epithelium, luminal epithelium and stromal nuclei. When gene expression was evaluated, similar results were observed. Administration of LA P4 altered ERα and PR expression in +1.46 times and +0.60 times, respectively. However, expression of ERβ was reduced in 2.12 times. In conclusion, expression for PR was detected before LA P4 administration and there was little variation after treatment. Interestingly, considerable variation was observed for ERα expression, as previously described when using estradiol followed by P4. Additional studies are needed to elucidate endometrial gene expression after estradiol and/or progesterone administration in non-cyclic mares.

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Environmental risk factors due to hormone use in assisted reproduction programs in cattle

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Reproductive biotechniques like artificial insemination at fixed time (FTAI), embryo transfer at fixed time (FTET) and ovarian super-stimulation (SOV) are used in large-scale cattle-rearing farms in Brazil. In 2014, an amount of 12,035,332 semen doses of beef and dairy bovines were traded in Brazil, 59% more than in 2009. This study aimed to assess the knowledge of cattle raisers and veterinarians on disposal of animal care solid waste (ACSW) and also determining possible water contamination due to washing of progesterone intravaginal inserts. Thus, we interviewed 65 breeders and 40 veterinarians. The questionnaire was based on socioeconomic issues on disposal of ACSW. Besides the surveys, waste analyzes were made on water from washings of progesterone intravaginal inserts. In addition, progestin analyses were carried out by high-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS). From the 65 farmers, 18.5% claimed they had received directions on how to discard ACSW, and veterinarians were the largest contributors to this guidance (91.6%). However, only one of the interviewed raisers had been properly disposed such material. Burning had been performed by 86% of the farmers. Washings of animal care devices had been held near housing for 64.4% of the cases, and near stockyards for the remaining 35.6%. Water disposal had been mainly made into cesspits (64.4%), followed by discard onto soil surface (35.6%). The amount of progesterone left over the wastewater of washings ranged from undetectable to 515 µg/kg, however, in Brazil there is not legislation on the subject. In this sense, we may conclude that there is lack of information and guidelines between veterinarians and farmers regarding properly disposal of ACSW from procedures with hormonal protocols of assisted reproduction programs in cattle, as well as concerning environmental contamination arising from the washings of intravaginal progesterone inserts.

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Evaluation of a surgical model to induce endometriosis in female rats

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The endometriosis is a chronic disease related with the proliferation of viable endometrial cells out of the uterus and it has high relevance in human health because it can causes infertility. Animals with estrous cycle don't develop endometriosis spontaneously, but the disease can be induced by ectopic transplant of endometrial tissue. Rodents can be an alternative model to the study of the disease, so this work searched to establish and to validate a surgical protocol to the induction of endometriosis in female rats. The study was conducted according to the Ethical Committee for Animal Research of the Univ Estadual Paulista (UNESP), (protocol 824-CEUA). Adult Wistar female rats (70 days) were maintained under conditions with controlled temperature and luminosity with ad libitum access to commercial diet and water in isolated boxes. The animals (n=20) were equitably distributed in a group of rats with surgical induction of endometriosis (G1) and a control group (G2). After anesthesia, it was done opening of the abdominal cavity by longitudinal incision in Alba line (about 2cm) to identify the right uterine horn. In G1 animals, 1/3 of the median portion of the uterine horn was separated and immersed in saline. Three transverse segments were inverted with surgical tweezers to expose the endometrium. The pieces were fixed in inner muscles wall of the abdominal cavity with catgut. After these procedures, the abdominal cavity wall and skin of the G1 and G2 animals were sutured and it was made post surgical clinical accompaniment. After 20 days the animals were killed and the implants (G1) or the uterine horn segments were processed in histopathological routine. The implants were identified adhered to the abdominal muscle layer and adhered to the intestinal loops in G1 group. Histologically the implants showed glandular arrangement internally covered by simple cubic epithelium and some had luminal leukocytes. These arrangements were inserted in the stroma with typical endometrial characteristics. Aspects of granulomatous chronic inflammatory answer and hemosiderin deposition were frequently observed. Occasionally it was also observed accumulation of xanthomatous cells. The morphological findings show lesions compatible with that expected to endometriosis, suggesting success in the procedure. Animal models allow controlled studies of the disease, minimizing or eliminating variations like age, menstrual cycle phases, diet, use of medication and environmental influence. The surgical induction in female rat is a fast and cheap procedure compared with primates or rabbits.

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Improvement of sperm quality after implementation of a HACCP system in a bull semen production Center

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Bull semen production centers (SPC) have satisfactory control of sperm quality, but commonly lack standardized quality control for hygiene procedures. This study assessed the impact of the implementation of a hazard analysis and critical control points (HACCP) system in a bull SPC, comparing semen quality and microbial counts on various steps of semen processing, before and after the HACCP implementation. Samples from artificial vagina (AV), flexible tubes from the straw filling machine (FT), fresh semen (FS), frozen semen (FZS), step-1 freezing extender (EA), step-2 freezing extender (EB) and packed semen (PS) were collected before and after HACCP implementation to evaluate counts of colony forming units (CFU). FZS sperm motility, membrane and acrosome integrity from both periods were also evaluated. The number of CFU/mL at distinct collection points was transformed to the logarithmic scale and subsequently compared between the periods before and after the HACCP implementation using analysis of variance, with comparison of means by the Tukey test. Post-thawing sperm motility, membrane integrity and acrosome integrity were compared between periods using the Wilcoxon ranks sum test for nonparametric data, also due to lack of normality. All analyzes were conducted with Statistix® (2013). Compared to the previous period, lower CFU ($P < 0.05$) were observed after the HACCP implementation, respectively, in samples collected from: AV (81.0 ± 19.0 and 19 ± 19.0); FT (2.5 x 106 ± 5.2 x 103 and 0); FS (41.800 ± 19.0 and 9.800 ± 4.100); and FZS (23.300 ± 4.100 and 7.300 ± 3.500). The CFU in samples from EA, EB and PS were similar in both periods ($P > 0.05$). Improvement after HACCP implementation compared to previous period were observed ($P <0.0001$), respectively, for post-thawing sperm motility (57.7 ± 1.3 and 33.7 ± 1.0), membrane integrity (85.3 ± 1.4 and 47.9 ± 1.6) and acrosome integrity (81.6 ± 3.6 and 61.4 ± 3.0). Thus, the implementation of a HACCP system in a bull SPC resulted in the production of high-quality semen doses with reduced microbial contamination.

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Leucocyte migration and amount of COX-2 protein in the susceptible mares endometrium treated with platelet-rich plasma

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The Post-Mating Induced Endometritis (PMIE) is characterized by the exacerbated influx of polymorphonuclear cells (PMNs) into the uterus and inability to eliminate intrauterine fluid after breeding, resulting in an unfavourable environment to embryo growth. The platelet rich-plasma (PRP) is an inexpensive and accessible product that has been used in the treatment of localized inflammations. Thus, the present study aimed to determine if the intrauterine infusion of PRP could modulate the uterine inflammatory response in susceptible mares. A total of 13 mares classified as susceptible to PMIE based on presence of intrauterine fluid 48 hours after artificial insemination (AI), more than 20% of polymorphonuclear cells (PMNs) 24 hours after AI and historic of less than 30% of embryo recovery rates were used for this study. The mares were inseminated with fresh semen in three consecutive cycles in a cross-over study design. PRP was prepared by single centrifugation protocol and just samples with a minimum concentration of 250.000 platelets/μL were used for treatments. The cycles were classified as control cycle (C): no pharmacological interference; PréT: 20 mL of PRP was infused 24 hours before AI; PostT: 20 mL of PRP was infused four hours after AI. Follicular dynamic was monitored daily by transrectal ultrasound and when a >35 mm follicle and endometrial edema at least grade 2 (min. 0 and max. 4) were detected, the ovulation induction was performed with deslorelin acetate (1mg, IM). AI was performed 24 hours after ovulation induction with the semen deposition (800 x 10^6 total sperm) in the uterine body. The percentage of PMNs in uterine cytology (CIT) was observed before and 24 hours after AI, using bright-field microscopy; in the biopsy (HIP) samples five fields were count and the average number of PMNs obtained, samples with an average of >17 PMNs cells were positive for endometritis; the number of COX-2 positive cells was evaluated by immunohistochemistry using a score system (0 – negative to COX-2; 1 – mild presence of positive cells; 2 – moderate presence of positive cells; 3 – severe presence of positive cells). The results were evaluated by Kruskal-Wallis test followed by Tukey-Krammer test, considering a 5% significance level. It was possible to observe that PMNs number decrease ($P < 0.05$) in CIT in both treated cycles (PréT & PostT) after AI (%, C – 46.6±6.3; PréT – 13.3±3.1; PostT - 18±5.2). The number of positive mares to endometritis in the HIP, also decrease ($P < 0.05$) in both treated cycles (Positive: C – 69%; PreT – 15%; PostT – 23%). Intensely ($P < 0.05$) positive COX-2 labelling was observed in the control (Score 2 – 30.7%; 3 – 69.2%) cycle compared to the PreT (1 – 15.4%; 2 – 84%) and PostT (1 – 30.7%; 2 – 53.8; 3 – 15.4) cycles. In conclusion, PRP is benefit to susceptible mares downregulating the PMNs influx into the uterine lumen and the amount of COX-2 protein in the endometrium after breed, becoming an option as a complementary treatment in these mares.

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Quality of inseminating dose of boar semen and its correlation with seminal bacterial population

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It is known that the bacterial contamination of the boar semen may reduce the seminal quality and to cause uterine disorders in the sow. Therefore, the understanding of the bacterial contamination of the semen and its outcome on sperm cell function is necessary to obtain high fertility rates. Thus, the objective was to identify the main bacteria present in the fresh and cooled boar semen and to correlate them with the seminal quality parameters. Twenty six ejaculates were collected from different boars and, immediately, samples were sending for microbiological analysis for bacterial isolation. Thereafter, the ejaculates were diluted in non-supplemented antibiotic media to obtain inseminating doses of $3x10^9$ cells/mL, which were stored at 15 ^oC for 72 hours. Another microbiological analysis was performed 72 hours later. Each microorganism identified was correlated with each sperm quality parameters by Pearson´s correlation test. It was observed that the UFC/mL values obtained from the cooled semen were numerically greater than those from fresh semen. *Sthaphylococcus spp.* (77% of ejaculates) and *Proteus mirabillis* (77%), *Burkholderia cepacia (*35%) and *Morganella morganii* (31%) were the most frequent bacteria found in fresh semen. In the cooled semen, there was an increase in *P. mirabilis* (96% of ejaculates) and reduction of *M. morganii* (31%) and *Staphylococcus spp*. (27%). In general, bacterial contamination has negative correlation with seminal parameters. The most harmful bacteria to affect the semen quality was *P. mirabillis* that showed negative correlation with spermatic motility and positive correlation with total morphological changes, sperm viability and percentage of whole acrosomes. By other hand, *B. cepacia* and *M. morganii* seem to be beneficial effect on the quality of the semen, since they affected positively the acrosome integrity and velocity of the fresh semen and sperm viability in the cooled semen. In conclusion, bacterial population affect the quality of inseminating doses stored at 15 ^oC for 72 hours. *P. mirabillis* is the most harmful bacterial for the boar ejaculates while *B. cepacia* and *M. morganii* seem to bring positive effects on the semen quality.

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Sexual dimorphism and perineal structure in *Coendou prehensilis* (Brazilian Prehensile-tailed porcupine)

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The *Coendou prehensilis* are mammals that belong to Rodentia order, which consists of five suborders: Sciuromorfos, which the squirrels belong; Castorimorfos where we find beavers, Myomorfos, which found the mice, Anomaluromorfos, Hare jumper, and Hystricomorfos, where we observe the capybara. Herein we investigated the perineal muscles contributing in the applied biotechnology development of reproduction that can be helpful even in captivity reproduction of these animals besides the free environment. One male and one female porcupine both adults found due to accidents on the roads were used. The animals were fixed with 10% formaldehyde and dissected in the perineum region. The only feature which allows us to differentiate males from females was the space between the anus and the genitals of the animals, the male has a higher space than the female. The perineum is an extra cavity region with lozenge definition, and this definition is formed by interpubic bones, pubic and the processes of the pubic and ischio. The male *Coendou prehensilis* perineum consists of five major muscles, three found arranged on the urogenital diaphragm surface (ischiocavernosus Muscles, M. bulbocavernosus and Mm. Bulboesponjosos) and two in the pelvic diaphragm (levator ani M. and M. penis retractor). The female's perineum is composed by (ischiocavernosus, bulbospongiosus, levator ani and external anal sphincter muscles). Both sexes have the ischiocavernosus muscles, Mm. Bulboesponjosos, M. levator ani and external anal sphincter. Studying the perineal region is very important, because understanding the function and location of each muscle we can contribute for animal reproduction and behavior during copulation helping to a better knowledge about reproductive potential of Erethizontidae rodents.

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Ultrasound diagnosis and macroscopic *post-mortem* evaluation of hydrosalpinx in dairy goats: a case report

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The oviduct plays a key role in the reproduction providing an ideal environment for the oocyte maturation, sperm capacitation, fertilization, and gamete and embryo transport. Hydrosalpinx is a pathology that can occur uni or bilaterally in the oviduct, affecting the reproductive function. In goats, it is considered of low frequency. The present study aims to describe ultrasound (US) images of two cases of hydrosalpinx associated with hydrometra in dairy goats. During the months of March and April of 2015, 20 Saanen goats aging 1 to 7 years, previously diagnosed with hydrometra by transrectal US (Mindray® M5Vet) scanning were monitored during their treatment. To reduce uterine contents, goats received three doses of 37.5 µg d-cloprostenol laterovulvar at 10 days apart (D0, D10 and D20). After the end of treatment, new US assessments were performed and it was noted that two goats of 2 and 3 years, weighting respectively 68.8 and 61.9 kg and body condition score of 4.5 (range 1-5), presented US images containing a large anechoic circumference (> 3 cm) laterally of the uterine horns. The initial image suggested the presence of ovarian cyst. However, due to the size of the observed structure, we opted for laparoscopy in the two females to assess the reproductive system. For the laparoscopic examination, the goats received 0.05 mg/Kg xylazine hydrochloride 2% and 5 mg/kg of ketamine hydrochloride 10% i.v. after water fasting and feeding of 24 hours. In both, there was an evidence of bilateral hydrosalpinx. Due to the reproductive history of infertility and the unfavorable prognosis in relation to reproductive activity, one of the goats was subjected to euthanasia according to ethical principles in animal testing of the Brazilian College of Animal Experimentation (COBEA). On both sides, the oviducts were dilated (about 6 mm at ampulla region) and filled with clear odorless liquid. The macroscopic analysis showed a cystic dilatation of each oviduct in the infundibulum region, with 10 cm (left) and 8 cm (right) in diameter. No malformation or segmental aplasia of the organ was detected. Adhesions were verified in both ovaries. It was concluded that the transrectal US exam associated with reproductive history represents an efficient tool in diagnosing hydrosalpinx in dairy goats.

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Addition of chlorogenic acid and vitamin E to swine cooled semen processed with Percoll

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Percoll is a sperm selection technique where in the centrifuging process is part of the methodology. However, this procedure causes negative effects on sperm motility due to the formation of reactive oxygen species (ROS). Although the technique is effective to select viable cells, the association of Percoll with antioxidants could minimize the production of ROS. Vitamin E (tocopherol) is a non-enzymatic and lipid-soluble antioxidant that protects the sperm cells from oxidative damage and chlorogenic acid is soluble and a phenolic compound which enhances the quality of insemination doses. The objective of this experiment was to evaluate the effects of adding vitamin E or chlorogenic acid on the quality of boar semen processed with Percoll and stored at 15°C for up to 72 hours. The randomized block design (ejaculates) with split plot in time (0, 48 and 72 hours of storage) with six treatments (processing or not with Percoll and three antioxidant systems - control, vitamin E and chlorogenic acid) and 12 repetitions was used. The semen was diluted in BTS (Beltsville Thawing Solution® - Minitub of Brazil LTDA) to form insemination dose of 40 ml containing 2.0 billion of sperm cells. Three insemination doses per ejaculate were processed. In one was added in a 4.5 mg/ml of chlorogenic acid, in another 400 ug/ml of vitamin E and the dose was used as control. Then, semen samples were subjected to centrifugation at 1000 × g at 37°C for 15 minutes, in test tubes containing two columns of Percoll (gradient of 45 and 90%). Following, 2 ml aliquots of each dose (processed and not processed in Percoll) were incubated in a water bath at 37°C. Microscopic evaluations were performed and the morphological changes, sperm viability and acrosome integrity were evaluated by microscopy optic, as well as, the kinetic parameters are measured by CASA system. The semen quality was negatively influenced ($P<0.05$) at 0, 48 and 72 hours of semen storage. There was no interaction ($P>0.05$) between Percoll and antioxidants in any of motility parameters, except ($P<0.01$) for linearity and rectilinear motion coefficients in the three evaluation times. For these coefficients, when chlorogenic acid was used showed good results ($P <0.05$) added insemination doses that have not been transformed by Percoll. The use of chlorogenic acid and vitamin E had no effect ($P> 0.05$) in semen parameters of sperm viability, acrosome integrity and morphological changes. The Percoll technique impaired ($P<0.01$) all motility parameters in all assessment times, however, it reduced ($P <0.01$) total morphological changes. There was no effect ($P> 0.05$) on the viability of sperm acrosomal integrity and using Percoll. Although the selection technique has influenced negatively the motility of sperm, it showed satisfactory results on the morphology of the sperm cells. Therefore, the addition of chlorogenic acid and vitamin E did not help in maintaining sperm quality after Percoll application, at 0, 48 and 72 hours of semen storage.

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Addition of Equex STM Paste to Tris extender increases the post-thawing longevity of collared peccaries (*Pecari tajacu* Linnaeus, 1758) sperm

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The Equex STM Paste (Nova Chemical Sales, Scituate, Inc., USA) is a detergent constituted by sodium dodecyl sulfate (SDS) that it is known for prolonging the post thawing sperm longevity in several species. Even if many advances have been achieved concerning the semen cryopreservation in collared peccaries (*Pecari tajacu*) in last decade, their sperm viability is largely affected after thawing, being recommended that the semen be used in artificial insemination within a maximum of 15 minutes. In order to improve the freezing protocol, we aimed to verify the effect of Equex STM Paste on the longevity of the collared peccary sperm after thawing. Twelve ejaculates were collected from mature male collared peccaries by an electroejaculation protocol standardized for the species. Ejaculates were immediately evaluated for volume, and sperm concentration. Total sperm motility (%) was assessed by a computer-assisted sperm analysis (IVOS 7.4G; Hamilton-Thorne Research, Beverly, MA, USA). Membrane functionality was evaluated by a hyposmotic swelling test using distilled water. Plasma membrane integrity was analyzed by epifluorescence microscopy using the probes propidium iodide and carboxyfluorescein diacetate. Ejaculates were then diluted in Tris-based extender plus 20% egg yolk and 3% glycerol, and divided in three aliquots. The first did not received any supplement, constituting the control group; the others were supplemented with 0.5% or 1.0% Equex STM® Paste. Samples were then packed in 0.25-mL plastic straws and frozen in nitrogen liquid. After one week, the straws were thawed in water bath at 37 °C and a thermal resistance test was conducted, in which sperm parameters were evaluated at 0, 15, 30, and 60 min. Differences among treatments were analyzed by ANOVA followed by Student's t-test ($P<0.05$). Immediately after thawing, no differences were verified among treatments that provided sperm motility values of 36.3 ± 5.8% for control, 32.5 ± 4.7% for Equex 0.5, and 36.9 ± 6.2% for Equex 1.0. After 30-min evaluation, groups containing Equex presented values for sperm motility (31.7±5.2% for Equex 0.5 and 35.6±6.1% for Equex 1.0) significantly higher than control group (18.1 ± 6.4%). Such difference was even more evident at 60 min when total sperm motility values were 11.2 ± 3.1% for Equex 0.5, 16.0 ± 4.3% for Equex 1.0 and 8.2 ± 3.4% for control group. Necessary to emphasize that semen from two individuals of control group presented no more movement at 60 min, while all the samples containing Equex in any concentration remains with sperm motility at this moment. Regarding evaluation of membrane functionality, plasma membrane integrity and sperm morphology, no differences were found among treatments in any evaluating time. Probably, the ability of SDS in solubilizing the lipid components of egg yolk and thus contributing to a better interaction of yolk phospholipids and sperm membrane would have contributed for prolonging the sperm longevity in samples containing the paste. In conclusion, addition of Equex STM Paste at 0.5% or 1.0% concentrations to Tris-egg yolk-glycerol extender increases the post-thawing longevity of collared peccaries' sperm for up to 30 min. This interesting result demonstrates the possibility of prolonging the use of the inseminating dose after the peccaries' semen thawing.

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Addition of resveratrol in boar insemination doses: impact on sperm membrane integrity and lipid peroxidation

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Imbalances of reactive oxygen species in mammal cells generate oxidative stress, which is deleterious to membranes architecture and sperm physiology. In this way, oxidative stress of boar spermatozoa could reduce the life spam of inseminations doses. Aiming to improve quality of cooled boar semen, antioxidants were usually added to semen extenders. Thus, this experiment was performed aiming to study the effect of addition of resveratrol (RVT) on plasma membrane integrity, acrossomal status and sperm susceptibility to lipid peroxidation on stored semen (17°C for 72 h). Thus, six ejaculates from six boars (n=36) were collected. After raw semen analysis, samples were extended in BTS medium supplemented with RVT, resulting in following treatments: T0 (BTS + RVT 0 mM), T1 (BTS + RVT 0.01 mM), T2 (BTS + RVT 0.1 mM) and T3 (BTS + RVT 1 mM). Treatments were cooled at 17 °C and evaluation was performed after 0, 24, 48 and 72 h for integrity of plasma membrane and acrosomal status. Sperm susceptibility to lipid peroxidation was performed by the technique of spontaneous thiobartituric acid reactive substances at 0 and 72 h. There was no interaction between time and treatment for TBARS assay ($p>0.05$); and there was no interference of the addition of RVT on spermatozoa susceptibility to lipid peroxidation (64.91 ± 4.52; 70.87 ± 6.30; 64.87 ± 4.68; 68.68 ± 3.74 ng of TBARs/sperm.10^6 to T0, T1, T2 and T3, respectively). There was an interaction between time and treatment for both plasma membrane and acrosomal integrity ($p<0.05$). The highest concentration of RVT (1mM) was deleterious for plasma membrane integrity in all of times assessed (0 hours: 91.89 ± 0.70^a; 92.22 ± 0.54^a; 92.26 ± 0.78^a; 84.54 ± 1.43^b / 24 hours: 88.84 ± 1.55^a; 90.09 ± 1.34^a; 90.06 ± 1.39^a; 71.81 ± 2.57^b / 48 hours: 89.03 ± 1.64^a; 89.77 ± 1.30^a; 88.97 ± 1.61^a; 59.70 ± 2.69^b / 72 hours: 88.26 ± 1.55^a; 88.56 ± 1.63^a; 87.26 ± 1.96^a; 62.52 ± 3.44^b; for T0, T1, T2, and T3, respectively for all times assessed). Similarly, the highest concentration of RVT increases acrosomal lesions when compared with T1, T2 and T3 at all times (0 hours: 89.04 ± 1.70^a; 91.46 ± 1.45^a; 92.04 ± 1.43^a; 84.80 ± 1.74^b/ 24 hours: 91.50 ± 0.87^a; 92.75 ± 0.97^a; 93.15 ± 0.88^a; 80.14 ± 1.82^b / 48 hours: 90.25 ± 1.52^a; 91.50 ± 1.25^a; 91.55 ± 1.46^a; 68.21 ± 2.38^b / 72 hours: 88.49 ± 1.73^a; 89.86 ± 1.59^a; 90.30 ± 1.42^a; 69.21 ± 2.92^b; for T0, T1, T2 and T3, respectively for all times assessed). In conclusion, despite have not affected spermatozoa susceptibility to plasma peroxidation, addition higher than 1mM of RVT on boar semen decreased plasma and acrosomal integrity. However, additional studies need to be performed to elucidate resveratrol effect on spermatozoa physiology.

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Addition of resveratrol on boar insemination doses decreases lipid peroxidation but increase fluidity of membrane lipids

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Lipid components located in the sperm membranes are deeply involved in sperm functions. The sperm cell metabolism spontaneously produces a plethora of reactive oxygen species (ROS). When ROS production exceeds the sperm's limited antioxidant defenses, a state of oxidative stress is induced affecting the peroxidation of sperm lipids and others sperm; functions. The production of low quantities of ROS plays an important role on normal sperm functioning, but disproportionate levels can impair sperm capacitation affecting negatively the quality of spermatozoa. Resveratrol (RVT) is a polyphenol compost with an intense capacity to inhibit ROS formation and consequently prevent excessive lipid peroxidation. The current study was design to evaluate whether the addition of the antioxidant RVT would influence positively membrane fluidity and lipid peroxidation of boar semen cooled at 17˚C for 72 h. Thus, six ejaculates from six boars (n=36) were collected. After initial raw semen analysis, samples were extended in BTS medium (50 x 10^6 spermatozoa/mL) and RVT was added, resulting in the following treatments: T0 (BTS + RVT 0 mM), T1 (BTS + RVT 0.01 mM), T2 (BTS + RVT 0.1 mM) and T3 (BTS + RVT 1 mM). Treatments were cooled and membrane fluidity and lipid peroxidation were accessed by flow cytometer after 0, 24, 48 and 72 h. There was no interaction between time and treatment for membrane fluidity ($p>0.05$). Addition of 0.01mM and absence of RVT (T2 and T1, respectively) were less harmful for sperm cells than 1mM (2875.21 ± 225.10^{c}; 3031.41 ± 195.55^{bc}; 3225.81 ± 202.43^{ab}; 3627.58 ± 271.90^{a}; data in mean fluorescence intensity [arbitrary units] for T0, T1, T2 and T3, respectively). Regarding to membrane lipid peroxidation, an interaction between time and treatment was observed ($p<0.05$). The highest concentration of RVT decreased peroxidation of membrane lipids only 72 h after cooling at 17˚C (0 hours: 3205.35 ± 93.80^{a}; 3249.28 ± 112.87^{a}; 3260.88 ± 120.51^{a}; 3104.03 ± 75.76^{a}; / 24 hours: 3351.11 ± 94.22^{a}; 3141.77 ± 67.66^{a}; 3237.14 ± 96.95^{a}; 3257.38 ± 127.47^{a}; / 48 hours: 3357.76 ± 121.28^{a}; 3349.78 ± 116.52^{a}; 3554.14 ± 158.30^{a}; 3393.38 ± 163.90^{a}; / 72 hours: 3244.30 ± 56.28^{a}; 3176.39 ± 36.15^{a}; 3160.24 ± 44.23^{a}; 2979.16 ± 39.17^{b}; data in mean fluorescence intensity [arbitrary units] for T0, T1, T2 and T3, respectively for each time). Concentrations higher than 1mM of RVT decreases membrane lipid peroxidation of sperm cells, but on the other hand, increases considerably membrane fluidity, impairing lifespan of sperm. In conclusion, despite the damage on sperm membrane lipid peroxidation have been decreased by addition of 1mM of RVT from 72 h, high concentrations of RVT alters the fluidity of membrane lipids increasing capacitation-like changes of sperm cells.

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Administration of equine Chorionic Gonadotrophin improves sperm quality in bucks during the non-breeding season

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Equine chorionic gonadotrophin (eCG) is a hormone that has FSH and LH activity widely used to induce ovulation in sheep. However, there is scarce information on its effects promoting reproductive activity in males. The aim of this study was to determine if the administration of eCG to goat bucks during the non-breeding season improves sperm quality of fresh semen (stage 1), of semen after the addition of an extender (stage 2), and after freezing-thawing (stage 3). Twenty adult bucks were adjudicated to two groups of 10 animals: a control group (group GCon, untreated bucks), and an eCG treated group (group GeCG). The GeCG bucks received 800 IU of eCG (Novormón, Syntex, Montevideo, Uruguay) on Day 0 (October 30), followed by 4 doses of 500 IU of eCG every 5 days. Semen was collected by electroejaculation on Days -14, -9, -3, 6, 21, 36 and 51. The quality of motility (scale 0-5) and percentage of motile sperm were determined on fresh semen, after the addition of a commercial extender (Andromed, Minitube, Germany) and after freezing-thawing in liquid nitrogen. The percentage of sperm with intact membrane was determined only on fresh semen and after the addition of an extender. Data were compared using an ANOVA for repeated measures including as main factors the treatments, the date of collection, the stage, and their interactions. The average data of fresh semen collected before Day 0 in each animal was included as a covariate. The quality of motility was greater in GeCG than in GCon (2.52 ± 0.08 vs 2.13 ± 0.08; respectively; P = 0.004) bucks. Also the quality of motility decreased along the stages in both groups (stage 1: 2.59 ± 0.09; stage 2: 2.29 ± 0.09; stage 3: 2.09 ± 0.09; P = 0.003), but there was no interaction of treatments with the days of collection or the stage. The percentage of motile sperm tended to be greater in the GeCG than in GCon (58.3 ± 1.50 vs 54.6 ± 1.51; respectively; P = 0.10). There was an interaction in the percentage of motile sperm between treatment and days (P = 0.03), being greater in GeCG than GCon on Days 6 (55.6 ± 2.72 vs 44.9 ± 2.77; respectively) and 21 (64.3 ± 2.78 vs 56.1 ± 2.72; respectively). The percentage of motile sperm declined throughout the stages (stage 1: 65.4 ± 1.78; stage 2: 62.1 ± 1.85; stage 3: 41.9 ± 1.79; P < 0.0001) without effects of treatments. The percentage of sperm with intact membrane did not differ between groups and did not present interaction between groups and stages, but there was an interaction between treatment and days (P = 0.009), being greater in GeCG than GCon on Day 6 (64.1 ± 3.95 vs 48.8 ± 4.17; respectively). Also the percentage of sperm with intact membrane declined throughout the stages (stage 1: 75.9 ± 2.54; stage 3: 33.8 ± 2.53; P < 0.0001). The effect of eCG in sperm was observed shortly after administration, and also ended immediately after the treatment finished. As spermatogenesis requires approximately 48 days in bucks, it can be speculated that the treatment had positive effects on spermatozoa and not in germinal cells. Therefore, the effect may be mainly mediated by changes in seminal plasma. In conclusion, the administration of eCG to goat bucks during the non-breeding season improved the sperm quality.

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Alternative methods for semen image capture of microscope

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The application of technology in national livestock is becoming frequent and associated with production systems, increased productivity levels, making the most efficient and sustainable creation. Advances are notorious when there is the use of simple management tools and technology. The computer programs for the evaluation of sperm kinetics may be more objective and more repeatable printing on the observations compared to the subjective analysis. Computer analysis (Computer-Assisted Sperm Analysis - CASA) allow a precise and accurate kinetics of sperm, perfecting the process of evaluation of semen. One of the biggest challenges for the analysis of sperm cells is the selection and standardization of the best image capture method. Thus, this study aimed to test three low-cost image capture methods, in order to facilitate the future use of the software in development. We used 20 samples of frozen bovine semen for making images. The straws (0.5ml) were thawed at 37 ° C / 30 seconds and hot plate kept until the movement od analysis. With the aid of a micropipette, semen 10μL were deposited between slide and cover slip and evaluated bright field microscopy with a device-camera (1.3 MP Coleman), a digital camera (Coolpix L340, Nikon) and a cell phone camera (5C iPhone, Apple), positioned in the eye. 20 seconds of video have been made to the device for each sample, and from them, sewed up photos to 10 seconds of each video. It held the count of sperm cells of photos to compare the range and accuracy of each image capture device, comparing them with the analysis of a technician. The results were submitted to analysis of variance and the means compared by Tukey test at the 5% significance level. The average sperm count in the image of each sample was 145.10 ± 101.28 for Microscope Camera (Coleman), 17.90 ± 11.54 for the digital camera (Nikon) and 407.80 ± 307.20 to cell phone camera (iPhone). The latter differed from the others ($p < 0.05$). The broader scope of the microscopic field was obtained by phone (iPhone). The best result for the definition / picture clarity was obtained by the microscope camera. The mounting system is simple and easy, with the support of specific camera to the microscope found in virtual store on the internet. Support for the mobile phone is provided for general use and has been adapted for this use. It is assumed that all kinds of images can be used by software. However, a specific setting must be performed for each one with a different algorithm for converting the number of sperm counted for sperm concentration of the sample. We conclude that there is great variation in the performance of different types of image capture, and the camera phone had the best performance.

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Bovine oocyte prematuration with cyclic adenosine monophosphate modulators: in vitro performance and semi-quantitative lipid evaluation

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Oocytes resume meiosis spontaneously when subjected to *in vitro* maturation (IVM). Cyclic adenosine monophosphate (cAMP) inductors have been used for artificial blocking of meiotic resumption to improve developmental competence of IVM oocytes. We tested a prematuration system that uses forskolin (FSK) and 3-isobutyl-1-methylxanthine (IBMX) to maintain higher concentrations of cAMP in the oocyte and sustain transzonal projections with gap junction communication within the cumulus-oocyte complex (COC). This system has been proved to prevent spontaneous meiosis resumption that usually occurs in the conventional IVM systems. Lipid accumulation in oocytes and IVP-embryos has been related to lower developmental potential and cryopreservation inability, especially when compared to their *in vivo* counterparts. We aimed to evaluate the effect of the in vitro prematuration system in the rates of blastocyst and on embryo quality by the semi-quantitative lipid content in oocytes and blastocysts from three systems: prematuration, conventional IVM and the laboratory control. Bovine immature oocytes were subjected to the prematuration system, in which COCs were cultured in the first 2h in TCM199 media with FSK (100 μM) and IBMX (500 μM), followed by 24 h of conventional IVM [TCM199, rhFSH (0.1 IU/mL), bovine serum albumine (4 mg/mL), pyruvate (0.011 g/mL) and amikacin (16.67 mg/μL) – Pre-IVM group]. Simultaneously, COCs were cultured for 24 h with the same IVM media described above for the conventional IVM (Con-IVM group). There was a third group as laboratory control cultured with the same media of the conventional IVM with the addition of 10% of fetal calf serum (FCS - Lab-Ctrl group). Matured oocytes were equally fertilized and cultured to the blastocyst stage in serum-free media, except for the Lab-Ctrl group where culture media was enriched with 2.5% of FCS. To evaluate the lipid content, we collected oocytes at different stages: immature, after 2h of prematuration and matured oocytes of the three different groups (Pre-IVM, Con-IVM and Lab-Ctrl). After assessing the blastocyst rates (day 7-8 after fertilization), expanded blastocysts from all groups were collected as well. Samples (oocytes and blastocysts) were fixed in formaldehyde (10% v/v) and stained in 1% Sudan black B/70% ethanol (w/v). The relative amount of lipid content was estimated by a digital image of each sample and examined in Image J software (v.1.41). Images were converted to a gray-scale pattern and for each structure we calculated the gray intensity per volume (arbitrary units/μm^3). There was no difference related to rates of cleavage (data not shown) or blastocyst (percentage in relation to oocyte number ± standard error of the mean) among groups (26.52±3.86, 22.22±4.45 and 32.89±4.99, respectively to Pre-IVM, Con-IVM and Lab-Ctrl). Lipid semi-quantification of oocytes indicated that immature oocytes had the lowest lipid content ($P<0.05$). After 2h of prematuration the lipid content was no different from that of immature oocytes. Among matured oocytes, Pre-IVM and Lab-Ctrl groups had higher lipid content compared to Con-IVM group ($P<0.05$). In blastocyst the lipid content from the Con-IVM group was higher than the Pre-IVM group ($P<0.05$). Lab-Ctrl blastocyst presented intermediate lipid content and was no different from the other groups ($P>0.05$). We can infer that the prematuration system did not affect the in vitro performance (blastocyst rates), but was able to affect the lipid accumulation differently in oocytes and blastocyst, in what seems to be a compensation mechanism during the preimplantation embryo development in vitro.

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Buserelin administered at heat detection or at Artificial Insemination moment effect in Brown Swiss cows` conception rates

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The present study aimed to evaluate the effect of the GnRH analog buserelin acetate (Sincroforte™, Ouro Fino, Cravinhos, SP, Brazil) used at the time of heat detection or at the artificial insemination (AI) moment, on the conception rate in Brown Swiss cows, with ranging daily milk production of 10 to 15 L. Sixty (60) animals were used, randomly distributed in one of the three groups: Group I (n=20) 0.01mg of buserelin acetate were administered, IM at the moment of heat detection; Group II (n=20) 0.01mg of buserelin acetate were administered, IM at the moment of AI; and, Group III (n=20) were administered 1mL of saline solution, IM at the moment of AI. All cows were inseminated 12 hours after the heat onset (observed twice daily, detected with observation of mount acceptation), by the same technician, using frozen semen from certified breeder. The gestation diagnosis was performed 45 days after the AI by transretal palpation associated to an ultrasonographic examination, performed by a specialized veterinary. The obtained data were analyzed using Fisher`s Exact Test, with 5% of significance level. The conception rate of the group I was 85%* (17/20), of the group II was 65% (13/20) and of the group III was 60%* (12/20). The statistical analyses permit to infer of the superiority tendency observed between group I and group III (*p=0.0776), but no difference were observed between groups II and I or between groups II and III (p>0.1). These results permit to conclude that the administration of buserelin acetate at time of heat detection in Brown Swiss cows lead to an increased conception rate.

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Comparative pregnancy rate after timed-artificial insemination of heifers previously cycling, ressincronized and post-induction of cyclicity

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The aim of this study was to compare the pregnancy outcome after a protocol for timed artificial insemination (TAI) in three different categories of cyclic heifers: previously cycling and submitted for the first TAI, non-pregnant to the first TAI and ressincronized for a second TAI, and cycling after a treatment to induce cyclicity and submitted to TAI. For that, 1,273 Nelore heifers with average body weight (BW) 312.0 ± 0.83 and average body condition score (BCS) 3.19 ± 0.01 from two farms located in Mato Grosso State, Brazil were used. All heifers were evaluated by ultrasonography (ovaries and uterus) and transretal palpation (uterine diameter) immediately before the onset of the TAI protocol (D0). Heifers classified as "previously cycling" were in their first evaluation and had uterine maturity (grade 3 and 4 in a 4-points scale) with or without a corpus luteum (CL). Heifers classified as "ressinc" were subjected to TAI 40 days before and were non-pregnant at this evaluation. Heifers classified as "induction" were pre-pubertal 40 days before, treated to stimulate cyclicity (10d of progesterone - P4 - device and 0,5 mg estradiol cypionate at device removal), and were considered cyclic (presence of CL and/or uterine maturity) in this subsequent evaluation. They were all subjected to the same TAI protocol. On D0 they received a P4 device (Cronipres® Mono Dose 1g, Biogénesis Bagó, Argentina), 1 mg estradiol benzoate (Bioestrogen®, Biogénesis Bagó, Brazil) and 75 µg D-Cloprostenol (PGF2α, Croniben®, Biogénesis Bagó, Brazil) IM. On D8, the device was removed and PGF2α (same dose given on D0), 200 IU eCG (Ecegon®, Biogénesis Bagó, Brazil) and 0.5 mg estradiol cypionate (Croni-Cip®, Biogénesis Bagó, Brazil) IM were administered. Also on D8 heifers were painted with chalk on their tailheads, and removal of chalk was used as an indication of estrus. TAI was performed by a single veterinary 48h after device withdrawal (D10) and concomitant with estrus determination by visual analysis of tail-paint score. Semen of two Aberdeen Angus bulls was homogenously distributed between groups. Pregnancy diagnosis was done 40d after TAI (Mindray DP2200VET). Data was analyzed by logistic regression (PROC GLIMMIX from SAS). There was no effect of BW (P = 0.28), BCS (P = 0.18), farm (P = 0.11) and bull (P = 0,86). However an effect of estrus was detected and heifers that showed estrus had greater pregnancy per AI (P/AI) than those without estrus, regardless of category (57.8% vs 45.5%; P = 0.0019). Similar P/AI was observed for "cyclic" [51.4% (334/650)], "ressinc" [51.8% (225/434)] and "induced" [57.7% (109/189); P = 0.92] heifers. The three studied categories had similar P/AI probably because they all had uterine maturity already established on D0 of the TAI protocol.

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Comparison between Tris and powdered coconut water (ACP-109c) extenders on the cryopreservation of epididymal sperm from Spix's yellow-toothed cavies (*Galea spixii*)

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The Spix's yellow-toothed cavies (*Galea spixii*) are wild rodents currently used as an alternative source of animal protein by communities of Northeast Brazil. Due to their high reproductive rate, they are largely indicated to be used as experimental models for other endangered hystricognath rodents, especially for the development of assisted reproductive techniques as the sperm cryopreservation. In this sense, this study aimed to evaluate the use of Tris and powdered coconut water (ACP-109c) extenders for the cryopreservation of epididymal sperm of cavies (*Galea spixii*). For this purpose, we recovered the testis-epididymis complexes from four mature individuals. The complexes were dissected and the epididymal sperm were collected by the retrograde washing method using Tris or ACP-109c in each epididymis from the same individual. Samples were immediately evaluated for motility and vigor, viability by the use of a Brome phenol blue stained smear, and the plasma membrane integrity by using the propidium iodide and carboxyfluorescein diacetate fluorescent probes. Already, the number of recovered sperm was calculated by the multiplication of sperm concentration, obtained through the Neubauer counting chamber, and the recovered volume. After analysis, samples were diluted with the same extender used for sperm recovery, e.g. Tris or ACP-109c, plus 20% egg yolk and 6% glycerol, on a final concentration of 100 x 10^6 sperm/mL. They were then refrigerated to 5 ºC, packed in 0.25-mL plastic straws, and stored in liquid nitrogen. After one week, samples were thawed in a water bath at 37°C/1 min and reevaluated. Data were expressed as mean and standard error. Comparison between extenders was conducted by Student's t test ($P < 0.05$). Fresh samples collected by the use of ACP-109c presented 87.5 ± 4.3% motile sperm with vigor 4.7 ± 0.25, being 85.5 ± 1.0% viable and 88.2 ± 3.2% with intact membrane, in a concentration of 632.5 ± 89,6 10^6 sperm/mL and a volume of 1.31 ± 0.08 mL. No differences were found between these samples and those collected in the use of Tris, that presented 95.0 ± 0.0% motile sperm, 5.0 ± 0.0 vigor, 77.78 ± 6.9 % viable sperm, 79.0 ± 7.5% intact membrane and a concentration of 517.5 ± 101.2 10^6 sperm/mL and a volume of1.18 ± 0.06 mL. After thawing, we verify that sperm characteristics were better preserved in the use of Tris extender ($P < 0.05$), that provided 65.0 ± 12.9% motile sperm with vigor 3.5 ± 0.6, being 65.8 ± 8.67% viable and 50.3 ± 18.8% cells with intact membrane. On the other hand, the use of ACP-109c as extender provided only 12.5 ± 11.9% motile sperm with vigor 1.8 ± 0.5, being 25 ± 7.1% viable and 39.8 ± 14.1% membrane integrity. Probably, Tris presented a more efficient conservation than ACP-109c due to its buffering action and capability of reducing the sperm fructose metabolism contributing to the conservation of cell energy. In conclusion, we demonstrated that Tris diluent was more efficient for the cryopreservation of epididymal sperm from Spix's yellow-toothed cavies (*Galea spixii*) in comparison to the powdered coconut water (ACP-109c) extender. These information are valuable for the formation of germplasm bank with genetic material derived from hystricognath rodents.

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Comparison of methods for recovery of canine epididymal sperm with ACP-106c

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The epididymal sperm are morphologically viable, with fertilization capacity, an alternative to animals that can no longer copulate, as well as high pet breeding value or great affection, which for some reason need to be spayed or even that may death. Epididymal sperm canines are commonly recovered by the tail slicing method due to epididymal size, but it can lead to cutting blood vessels, contaminating the sample with red blood cells. Other techniques described are retrograde flushing and compression by means of hemostats. Recently, in 2015, investigators described a modification of the compression technique being performed by glass slide. Thus, we decided to compare three recovery techniques to epididymal sperm canines in ACP-106c® extender. To achieve this goal, twenty-two mongrel dogs originated from the Environmental Control Center in Garanhuns underwent orchiectomy. After bilateral orchiectomy, testis and epididymis were placed in plastic bags containing saline sterile 0.9% added with penicillin (100 IU / ml) and streptomycin (0.1mg/ml), and immediately transported to the laboratory in cooler. We used a total of 33 complex testes,-epididymides, which were dissected out and washed with warm saline (37 ° C) containing streptomycin and penicillin, for removal of the epididymis. In the slicing method, the tail dissected was sliced into 3 ml of the ACP-106c in a petri dish for 10 minutes to remove the sperm sample. In retrograde washing method, the contours of the epididymal duct were broken and, where necessary, more than one incision was made to facilitate cleaning, obtaining by applying pressure to the vas deferens until the contents of the tail exit through a cut made at the junction with the body of the epididymis. The pressure was generated with a syringe injected with ACP 106c®. For the compression method by slide, incisions were made in pampiniform plexus and the blood drained before dissection of the epididymis. Two hemostats were placed on the body of epididymis and vas deferens (most proximal possible) in order to prevent loss of epididymal spermatozoa throughout the manipulation. After that, epididymis was placed in a Petri dish, positioned at an angle of 45°,containing 300µl ACP-106c and were then held four compressions using a glass slide, towards the cauda epididymis to the vas deferens. After recovery, the sperm samples were submitted to total motility assessment (%), vigor (0-5), concentration (million sperm / ml) and functionality of the membrane by hiposmotic test (%). The data were submitted to the Shapiro-Wilk normality test and not follow a normal distribution.Thus, the treatments were compared using the nonparametric Wilcoxon test ($P < 0.05$). Regarding to motility, vigor and percentage of functional sperm by hiposmotic test, there was no significant difference between the groups, with the following values: Motility (54.5% ± 11.3, 55.4% ± 13.7, 65.0% ± 9.7), Vigor (2.2 ± 0.7, 2.3 ± 0.7, 1.7 ± 0.7), spermatozoa with functional membrane (79.8% ± 13.0% 79.3 ± 10.5 69.8 ± 16.4%), for the methods slicing, retrograde washing and compression, respectively. However, it was observed that the method by compression with glass slide showed better sperm concentration (65.8 ± 20.2 x106 sperm / mL) compared to two other methods, slicing (32.8 ± 10.7 x106 sperm / ml) and retrograde washing (10.7 ± 3.5 x106 spermatozoa / ml). It was also observed that the slicing method was superior to retrograde washing. The results of motility and vigor were similar to work done by other authors. Despite the sperm concentration is the only parameter where difference was observed, it has a crucial importance because it will determine the insemination dose, which may reflect the at fertilization rate. In addition the compression method by glass slide showed be pratical, and a minor contamination with red blood cells when compared to the slicing method. Therefore, we can recommended the compression method for recovering canine epididymal spermatozoa using ACP-106 extender since a higher sperm concentration can inseminate a larger number of bitches.

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Computer evaluation of actin cytoskeleton integrity in criopreserved ovine sperm with vegetal extracts

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Many of the biochemical and functional damage observed in criopreserved sperm cells are related to steps involved in the freezing and thawing. Temperature changes cause an increase in reactive oxygen species (ROS) leading to oxidative stress and membrane damage, which in turn is regulated by the actin cytoskeleton. The polymerization and depolymerization of actin is profilin mediated and the structural changes that occur in the cytoskeleton are reflected in spermatic motility and viability, and therefore in the fertilising capacity. Sheep sperm are very sensitive and suffer severe damage to these changes. From this, it is proposed as an alternative to the use of natural antioxidants, derived from plant extracts. So, to understand and analyze the behavior of protein structures we rely on new computational tools such as Molecular Dynamics, which provides the ability to perform thermodynamic simulations. Classical molecular dynamics of protein was used for structural analysis of sheep profilin immersed in a solvent consisting of water, sodium chlorine and potassium ions to achieve electrical neutrality of the system, and glycerol as an agent which increases the viscosity of the system and favors the freezing-point depression. To control the temperature, the thermostat Nosé -Hoover was implemented and the interaction of Van der Waals was changed in the Gromos force field (GROMOS suite programs version 5.0.1; GROMACS) configured for parallel multiprocessing. This force field modification was adjusted with the evaluation of experimental parameters analyzed. We evaluated three replicates from samples of six rams before and after freezing in a commercial diluent, which was enriched with aqueous extracts of *Chenopodium ambrosioides, Allium cepa, and Rosmarius officinalis*, at three different inclusion levels (2.5g, 5g and 10g) and under three different incubation times at 37°C (0, 1, 2 and 3 hours). Data were analyzed using ANOVA Kruskal-Wallis ranges, Dunett and Tuckey test were used to compare media groups. Statistical difference was found in sperm fresh sheep diluents at time 0, 1 hour and 3 hours (T0, T1 and T3), while for the thawed sperm there were differences between the diluent at time 0 and 3 hours (T1 and T3; $P < 0.05$). As for the response membrane no statistical difference was found between the different diluents incubation time 0 and 2 hours (T0 and T2) with ($P > 0.05$); but in the time of 1 hour (T1), if statistically significant difference between the control diluent and the diluent enriched Epazote 5% (Ep 5 g) ($P = 0.021$) was found and the incubation time of 3 hours (T3) between the control group and the diluent enriched with Rosemary extract 2.5% (Ro 2.5g) in fresh sperm. While, in the thawed sperm-enriched Ep 5g sample was statistically different ($P = 0.002$). We conclude that the use of the *Chenopodium ambrosioides* enriched diluent (5%) improved parameters and membrane response feasibility. Otherwise the cryopreservation process alters the actin cytoskeleton and profilin in sheep sperm , which is reflected in the sharp decrease in fluorescence patterns of the actin cytoskeleton . We hypothesize that the regions 90 ~ 95, 115 ~ 120 and 135 ~ 138 of profilin are more susceptible to breaking and affect the integrity of the actin cytoskeleton in ovine sperm.

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Correlation analysis of the testicular volume and seminal quality with "fatted" phenomenon in captive squirrel monkey (*Saimiri colinsi* Osgood, 1916)

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In species of neotropical primates of the genus *Saimiri,* the breeding season is related to a photoperiod, the rainfall, the latitude, the temperature, behavioral and physiological processes, as the "*fatted*" phenomenon is exclusive for the genus, being characterized by seasonal increase in body size, and that relate to the spermatogenesis in this monkeys. Although previous research on the genus *Saimiri* reported a testicular increase in size, increased sperm concentration and semen volume during the breeding season, and these factors are related the "*fatted*" phenomenon, no information on *S. collinsi* has been reported. Thus, the aim of this study was to verify the correlation between total testicular volume and semen parameters of *S. collinsi* ejaculates and verify the correlation with the "*fatted*" phenomenon in this animals. The 6 adult male *S. collinsi* (age > 5 years) were provided by the National Primate Center (CENP, Ananindeua, PA, Brazil). Semen samples were collected by electroejaculation (June 2015 to May 2016). The testis were measured with a caliper and the total volume was calculated from the ellipsoid equation: $V = 4/3\pi\ ab^2$, where V = volume, *a* = half the length of the testis, and *b* = half the width of testis. The analyzed semen parameters were the seminal volume, sperm concentration, motility, sperm vigor and plasma membrane integrity. All data are expressed as mean ± standard error of the means (SEM). To compare the sperms parameters between the periods ("*nonfatted"* and "*fatted"*) Students' test was applied, and the Kendall rank correlation coefficient was used to measure correlation between total testicular volume and sperms parameters. Statistical significance was obtained whenever $P < 0.05$ The mean (± SEM) of testicular volume (mm^3), semen volume (µl), sperm concentration (x 10^6 sperm / ml), motility (%), vigor (0 - 5 score) and plasma membrane integrity (%) were: 25.048 ± 1.547; 106.548 ± 11.20; 61.056 ± 14.06; 61.806 ± 4.72; 3 ± 0.19 and 66.57 ± 3.53 for "*nonfatted"* period, and 39.191 ± 1.961; 265.909 ± 41.240; 46 ± 16.775; 76.429 ± 6.802; 3 ± 0.02 and 43 ± 7.37 for "*fatted*" period, respectively. There was a statistical difference with the increase in the total testicular volume ($p = 0.0001$) and seminal volume ($p = 0.0001$) compared in the periods. In the "*fatted"* period there was a lack of correlation for between total testicular volume with the seminal volume ($\tau = 0.076$; $p= 0.62$), sperm concentration ($\tau = - 0.50$; $p= 0.01$), motility ($\tau = - 0.110$; $p= 0.58$), vigor ($\tau = -0.099$; $p= 0.62$) and plasma membrane integrity ($\tau = 0.14$; $p= 0.47$). Likewise, in the "*nonfatted"* period, the seminal volume ($\tau = 0.249$; $p= 0.02$), sperm concentration ($\tau = - 0.119$; $p= 0.30$), motility ($\tau = 0.124$; $p= 0.28$), vigor ($\tau = 0.032$; $p= 0.78$) and plasma membrane integrity ($\tau = 0.037$; $p= 0.75$) were not correlated with testicular volume. Thus, although there is no correlation between total testicular volume and seminal characteristics in which the animals were in "*fatted*, there was an improvement in all sperm parameters in this period. These data may be used in future studies to elucidate the reproductive physiology of the genus *Saimiri* and select breeder assisted reproduction programs.

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Cryoprotectants toxicity in *Steindachneridion parahybae* oocyte in the early stages of development (Pisces, Siluriformes)

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The surubim-do-Paraíba, *Steindachneridion parahybae* is a species of South American fish threatened with extinction. For this reason, has aroused interest in cryopreservation of gametes his, with the genetic material conservation order. Currently a project is being developed in order to cryopreserve oocytes surubim-do-Paraíba. In the present study, the aim was investigated the cryoprotectants toxicity in immatures oocytes. Two females were anesthetized and sacrificed for collecting ovaries. The initial oocytes were separated enzymatically (collagenase [0,4%]). Approximately 30 oocytes were incubated at primary, pre-vitellogenic and vitelogenic stages using Hank's (HBSS) media for 30 minutes at room temperature, containing the following cryoprotectants in different concentrations: glucose [0,25M and 0,5M], methanol [1M and 2M], ethylene glycol [1M and 2M]. Negative control (NC), only in HBSS media, and positive control (CP), with the oocytes kept dry, were carried out. Three replicates for each treatment and control were used. Evaluations were made by feasibility tests: membrane integrity staining in 0.2% Trypan blue (TB); and maturation *in vitro* to verify the germinal vesicle breakdown (GVBD). The results showed that the toxic effect of the cryoprotectant sucrose and methanol increases with increasing concentration; The cryoprotectants used in different concentrations did not differ statistically from the CP, and presented viability (TB) statistically superior to CN, except for ethylene glycol 2M. As for GVBD test, ethylene glycol 1M showed the highest viability, not statistically different from the other treatments, unless the CN and methanol 2M. We conclude that, for initial oocytes may be used all cryoprotectants tested in different concentrations. This may indicate that for this specie, the initials stages have shown a lower toxicity than the mature stage, for the same cryoprotectants and concentrations (reported results). This reinforces the need for more studies using these earliest stages in freezing test of oocyte surubim-do-Paraíba.

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Deslorelin and histrelin in reproduction of tambaqui (*Colossoma macropomun*)

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Hormonal induction of teleost fish maintained in captive is a very useful technique for teleost fish reproduction. The most used exogenous hormone treatment in fish is the pituitary carp extract (PCE) however has a high cost and do not pass through any sanitary control. The GnRH presents some advantages over PCE, as the way it induces the beginning of the hypothalamic pituitary gonadal axis, stimulating the fish to synthesize their own natural gonadotropin. Synthetic molecules mimic some types of GnRH, and there are some synthetic analogues available in the market, but is necessary experiments to evaluate them in fish reproduction. Therefore, the aim of the present study was to evaluate two synthetic molecules analogues to GnRH, deslorelin and histrelin, in the induced reproduction of *Colossoma macropomum* (Tambaqui). Three females, weighting from 7.5 to 9kg, were selected by evaluation of their curved and soft cealomathic cavity and hyperemic and edematous urogenital papilla. In addition central position of the germinal vesicle (CPGV) was evaluated and only female that presented 70% of oocytes in this condition was induced (two females). Three males, weighting from 3 to 4.5kg, that were liberating sperm under soft compression of cealomathic cavity were selected. Breeders were maintained in polyethylene boxes with thermostats that kept water at 27°C. Both male and female were induced with two doses of histrelin or deslorelin in concentrations of 1% at first dose (priming dose) and 4% at second dose (inductive dose), in a 12-hour interval, independent of the weight, the total volume used was standardized to 1mL. Females started to spawn with 10 hours after first hormone injection, however tambaqui females just start reproduction after 6 hours of the second injection of the hormone, so we decide suture urogenital papillae in order to stop de output of oocytes, thinking that this extra time could improve egg quality, and gave time for males to prepare for the fertilizating time. At that this moment, the males were induced with one hormone dose and after 7 hours it initiate the spawn. The sperm was collected at same time as the oocytes were extruded. Females extruded 0,800 and 1,200g of oocytes with deslorelin and histrelin, respectively. It was observed that with deslorelin, 70% of oocytes have central position of germinal vesicle (PCVG), and with histrelin, 50% have PCVG, indicating that oocytes didn't maturate properly. Males have extruded only 1 ml of semen independent of the hormone. Motility of the sperm was 70% and motility duration after activation was 15 seconds for all males. Fertilization was performed, but the fertility rates were 0%, probably due to the quality of eggs. We conclude that the tested hormones promote an anticipation of the spawn in Tambaqui females, nevertheless, the sperm had good quality. These hormones could be an alternative for reproduction of Tambaqui, due its capacity to anticipate spawning in females with a low concentration/volume for induction, independent of the breeder weight. This is the first study that indicates the induction of spawn of Tambaqui during breeding season with deslorelin and histrelin. However more work is being perform to determine a protocol for use of these hormonal preparation in the field.

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Effect of deslorelin or histrelin treatment on germinal vesicule migration of *Astyanax fasciatus*

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Gonadotropin releasing hormone (GnRH) is a hypothalamic hormone released according to environmental alterations in fish. This hormone is responsible for pituitary stimulus to induce the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both of these pituitary hormones are responsible for gonadal stimulus to steroidogenic and gamete production. Artificial reproduction in fish is consisted of hormonal induction of spawn in females and spermiation in males. This occurs because teleost fish cannot complete its reproduction cycle in captive due to the lack of the process that induce pituitary to produce FSH and LH in nature. One of the characteristics that are important to observe during the artificial reproduction in females is the position of germinal vesicle. During the gonadal maturation of female, the germinal vesicle migrate from central position of the oocyte to peripheral position due to the increase of oestradiol. Synthetic preparations of GnRH analogues have been tested in teleost fish, however the result are still controversial, and probably because of the amount of molecules that hypothalamus produce naturally, which is hard to repeat with exogenous hormone induction. The aim of this study was to test histrelin and deslorelin (synthetic analogues of GnRH) in the migration of germinal vesicle in *Astyanax fasciatus* oocyte (Lambari do rabo vermelho). Forty (40) Lambaris` females were divided into four groups: G1 (reveived two doses of histrelin 1%), G2 (received two doses of histrelin 5%), G3 (received two doses of deslorelin 1%) and G4 (received two doses of deslorelin 5%), between doses interval was of 12 hours in each group. A urethral probe size 8 was used to oocytes recovery in order to proceed its evaluation. The probe was introduced through urogenital papilla and when reached the gonads were made a gentle negative pressure with the aid of a syringe, which pulled out the oocytes into the probe. After recovered, the oocytes were placed on a slide with Serra solution (60 mL de alcohol 90ºGL; 30 mL de formalin and 10mL de acetic glacial acid). The oocytes were recovered into two moments using this technique, the first one 12 hours after the first hormone injection and the other one at the end of the artificial reproduction. The results were submitted to variance analysis (ANOVA, $p<0.05$), using PROC GLM of SAS 9.3. It was not observed statistical differences between the evaluated variables ($p>0.05\%$). At the beginning of the treatments, no oocytes were retrieved, but it was observed that Lambaris submitted to deslorelin 5% (G4) and histrelin 1% (G1) had a tendency ($p>0.05$ but <0.1) to produce more oocytes with central position of germinal vesicle. For the groups deslorelin 1% (G3) and histrelin 5% (G2) the frequency of central and peripherical position of germinal vesicle presented a relation o 1:1. After the second hormone application were observed a higher tendency for peripherical position of germinal vesicle for deslorelin and histrelin 5% (G4 and G2, respectively) and central position for deslorelin an estrelin 1% (G3 and G1, respectively). Fish submitted to histrelin 5% (G2) and deslorelin 5% (G4) spawn, with a fecundity rate of 50%. It was concluded that deslorelin 1% and histrelin 5% could be used at the beginning of the treatment to induced reproduction of Lambari fish, however the second hormone injection must be of 5%, for better results.

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Effect of frame acquisition rate and different viewing chambers on equine sperm kinetics parameters measured by the SCA®

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Computerized analysis system of semen (CASA) is an important tool to evaluate sperm kinetics, providing accurate information, objective and high repeatability. However, some factors might affect motility and speed measured by the *Sperm Class Analyzer*® (SCA®). The acquisition frame rate and the type of viewing chamber have been reported among those which may influence the results considerably. This research was carried out in two experimental phases. The first experiment was conducted to evaluate the effect of acquisition of 25 frames/second with a frame rate of 25Hz, 30Hz or 50Hz on equine sperm kinetics parameters measured by the SCA®. The second experiment was conducted to evaluate the effect of different types of viewing chamber or slides that consisted of Slide-coverslip, Leja®10 µm and Leja®20 µm on equine sperm kinetics parameters measured by the SCA®. The semen of Mangalarga Machador stallions were diluted in BotuCrio® Extender (Biotech®, Botucatu, SP), packed into 0.5 mL straws, cooled (-0.5 °C/min), maintained at 5 °C for 30 min and then frozen (-10 °C/min) using a TK4000® (TK Freezing technology LTDA, Uberaba-MG). Immediately after thawing (46 °C/20 s), sperm motility and speed characteristics assessed by the SCA® (v.4, Microptics S.L, Barcelona, Spain) were: Total Motility (%), Progressive Motility (%), Velocity Curvilinear (VCL-µm/s), Velocity Straight Line (VSL-µm/s), Velocity Average Path (VAP-µm/s), the percentage of Fast, Medium and Slow sperm, the Linearity (LIN%), the Straightness (STR%), the Oscillation Index (WOB%), the Amplitude of Lateral Head Displacement (ALH-µm), the Beat Cross Frequency (BCF-Hz) and Hyperactive. At least three randomly selected microscopic fields were scanned for each sample. The frame acquisition rate did not affect the equine sperm kinetics parameters assessed by the SCA® ($P > 0.05$). The type of viewing chamber used had influence over the parameters of progressive motility, Medium, Slow, VSL, LIN, STR, WOB and Hyperactive ($P < 0.05$). Total motility, Fast, VCL, VAP, ALH, BCF did not differ among the viewing chambers tested ($P>0.05$). Progressive motility, Medium and VSL were greater for Slide-coverslip than the Leja®20 µm ($P<0.05$), but for progressive motility, Medium, VSL and Hyperactive were similar to the Leja®10 µm ($P>0.05$). Based on these results, a frame acquisition rate of 25 frames/second with a frame rate of 50 Hz was chosen for further experiments. The results did not indicate the ideal acquisition frame rate to assess the kinetics parameters of equine cryopreserved sperm. Slide-coverslip may be used to evaluate equine sperm motility and speed by the SCA®.

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Effect of myo-inositol on epididymal bovine sperm motility subjected to cooling at 5°C for 48 hours

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The recovery and cryopreservation of epididymal sperm is an important genetic resource when animals of high genetic value or great appraisement suffers an accident or have died. Although cryopreservation is often used, it has important limitations such as reduced motility and sperm integrity parameters. Myo-inositol is an antioxidant and osmotic regulator that could ameliorate the negative effects of cryopreservation on epididymal sperm. Thus, the objective of this study was to evaluate the effect of different myo-inositol concentrations on sperm cell obtained directly from bovine epididymis. Sperm cells were recovered from 33 mongrel cattle testicles/epididymis complexes obtained in slaughterhouses by the flotation technique. To prevent individual variation on sperm cell parameters, a pool of five animals was prepared for each treatment group to a final concentration of approximately $80x10^6$ sperm/mL. Each biological replicate was further divided into one control group (Tris-egg yolk extender without myo-inositol) and three experimental groups: G1, G2 and G3, which were prepared by adding 5, 15, and 30µM of myo-inositol to the extender, respectively. The samples were subjected to evaluations of subjective motility, integrity of plasma membrane and functionality of the plasma membrane after 0h (T0), 24h (T24) and 48h (T48) of incubation at 5 ºC. Four replicates were performed for each treatment group. ANOVA followed by Tukey's post-hoc test was performed to compare differences in the variables with respect to incubation time and myo-inositol concentrations, with a significance level of 0,05. A significant decrease in sperm motility was observed ($p<0.05$) in the G3 compared to the other groups irrespective of incubation time ($p>0.05$). No differences were observed ($p>0.05$) in integrity and functionality parameters among the experimental groups. Myo-inositol is the precursor of phosphatidylinositol, which stimulates sperm cells to maintain cell membrane potential and regulate control of intracellular Ca^{2+} ion balance. The high concentration of myo-inositol in the G3 may have potentiated cellular metabolism and consequently increased formation of reactive oxygen species (ROS) causing oxidative stress resulting in damage to the mitochondrial membrane. On the other hand, the release of Ca^{2+} by the endoplasmic reticulum can promote hyperactivation and early capacitation by further increasing energetic consumption. In conclusion, the addition of myo-inositol at a concentration of 30 mM negatively interferes in the total motility of sperm cells obtained from bovine epididymis subjected to cooling at 5 °C for 48 hours.

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Effect of oxygen tension on in vitro viability and development of dog follicles enclosed within the ovarian cortex

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Oxygen concentration has been shown to influence in vitro viability and growth of ovarian follicles. The present study examined the effect of oxygen tension on in vitro development of dog follicles within the ovarian cortex Ovaries were obtained from prepubertal (age, 2.5–6 months) and adult (age, 8 months to 2 years) bitches, and ovarian cortical fragments were recovered. The cortices were then incubated on 1.5% (w/v) agarose gel blocks within a 4-wells culture plate (three cortical pieces/well) containing Eagle Minimum Essential Medium (MEM) supplemented with 4.2 μg/ml insulin, 3.8 μg/ml transferrin, 5 ng/ml selenium, 2 mM L-glutamine, 100 μg/mL of penicillin G sodium, 100 μg/mL of streptomycin sulfate, 0.05 mM ascorbic acid, 10 ng/mL of FSH and 0.1% (w/v) polyvinyl alcohol in humidified atmosphere of 5% or 20% oxygen concentration. Ovarian follicles within the tissues were process for histology and assessed for follicle density, viability (based on morphology) and diameter immediately after collection (Control) or after 2 or 5 days of *in vitro* incubation. Apoptotic cells were assessed using TUNEL assay. Comparison among fresh and culture treatment group were performed using ANOVA or Kruskal-Wallis rank sum test. There were no differences ($P > 0.05$) in follicle density among group at Day 2 of in vitro culture. However, the density of follicles within cortices cultured in 20% oxygen for 5 days significantly reduced compared to the Control and those incubated in 5% concentration. The viability of cultured follicles decreased ($P < 0.05$) compared to the Control after 2 days incubation, and this value further reduced ($P < 0.05$) in 20% oxygen group at Day 5. The diameter of follicles at all developmental stages, except the primary follicle cultured with 5% oxygen concentration was significantly larger than those incubated in 20%. There were no differences in the percentages of apoptotic follicles between the two treatment group ($P>0.05$). Nevertheless, after 5 days of culture, the percentage of TUNEL positive cells of primordial follicles decreased significantly ($p<0.05$) in 5% oxygen group. In sum, our findings demonstrated that 5% oxygen level was superior to 20% concentration in sustaining in vitro viability of dog follicles enclosed within the ovarian cortex.

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Effect of the *in vitro* addition of docosahexaenoic acid (DHA) and insulin-like growth factor 1 (IGF-1) on the quality of cryopreserved stallion semen

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Good semen quality after the freeze/thaw process is important for later use of cryopreserved equine semen in artificial insemination. A way to improve semen quality is by adding some substances into extenders. Docosahexaenoic acid (DHA) is an omega-3-polyunsaturated acid which has been shown to improve the integrity of the sperm cell membrane during the temperature changes. Insulin-like growth factor 1 (IGF-1) is a protein hormone that helps mainly glucose and other energy sources to enter into sperm cells. This is the first report using the *in vitro* addition of DHA and IGF-1 simultaneously in stallion semen. The aim of this study was to assess the effect of the *in vitro* addition of DHA and IGF-1 to frozen-thawed stallion semen. Three ejaculates from each stallion (two Connemara Pony and one Selle Francais) were collected between February and March 2016 with a rest interval of at least 3 days and all ejaculates were processed individually. Semen collections were conducted at a commercial stud in Ireland using an artificial vagina. Following collection, the gel fraction was removed and total motility was assessed (minimum total motility of 70% was used). Each ejaculate was diluted in a 1:1 ratio using INRA 96 extender (IMV Technologies, L'Aigle, France) and centrifuged at 1000 *g* for 10 min at 32°C. The pellet was diluted to 100 x 10^6 sperm/mL in Gent freezing extender (Minitüb, Tiefenbach, Germany). Semen was then cooled to 4°C for 2 h, packed into 0.5 mL straws, frozen in a programmable freezer and stored under liquid nitrogen at -196°C. Straws were thawed at 37°C for 30 sec. Vitamin E was added at 0.02 mMol to prevent lipid peroxidation. DHA was added at 0 or 1 ng/mL and IGF-1 at 0 or 100 ng/mL resulting in four treatments, namely: DHA0, $DHA0_{IGF\text{-}1}$, DHA1, $DHA1_{IGF\text{-}1}$. Semen was incubated at 32°C until analysis were completed. After 30, 60 and 120 min of incubation total motility and progressive linear motility were assessed using Computer Assisted Sperm Analysis Software (CASA; Sperm Class Analyser, Microptic, Spain). After 30 min viability and acrosome integrity were assessed using flow cytometry (Guava easyCyte 6HT-2L, Merck Millipore, USA). Data were examined for normality of distribution, tested for homogeneity of variance and analysed using an Analysis of Variance (ANOVA) or repeated measures ANOVA in the Statistical Package for the Social Sciences (SPSS; version 22.0, IBM, USA). The final statistical model employed, included the main effects of treatment, incubation period and treatment x incubation period interaction. Post hoc tests were conducted using the Tukey test and $P<0.05$ was deemed to be statistically significant. All results are reported as the mean ± the standard error of the mean (s.e.m.). There were no effects of the addition of DHA and IGF-1 to thawed semen on any of the *in vitro* analysed parameters ($P>0.05$) with an overall post-thaw total motility of 18.4 ± 2.61%, progressive linear motility of 4.8 ± 1.25%, viability of 23.4 ± 4.99% and acrosome integrity of the live population of 97.8 ± 0.55%. There was an effect of incubation period on total motility and progressive linear motility ($P<0.05$), whereas total motility and progressive linear motility decreased over time. There was no interaction between treatment and incubation period in both parameters ($P>0.05$). Contrarily, other researchers using *in vitro* addition of DHA in bull and boar semen have found improvements in sperm quality, especially in motility characteristics. Similarly, some studies have concluded that the *in vitro* addition of IGF-1 to ram, boar and buffalo semen increases the fertilization capacity of spermatozoa. The *in vitro* addition of IGF-1 to stallion semen was shown to promote the longevity of spermatozoa and supplementation of DHA in the diet improved the sperm mobility characteristics in stallion cooled and frozen semen. Probably stallion spermatozoa need a higher concentration of DHA and IGF-1 when they are added simultaneously into the extender than the concentrations tested in this experiment. More research should be conducted in order to test other concentrations of the combination of DHA and IGF-1 to optimize equine semen quality. In conclusion, the *in vitro* addition of DHA and IGF-1 had no effect on the quality of frozen/thawed stallion semen. The authors gratefully acknowledge support from FAPEMIG, Brazil.

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Effect of the preparation of bovine sperm before intracytoplasmic sperm injection on rate embryo production

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During ICSI, plasma membranes as well as the acrosome are introduced into the oocyte. This fact has been suggested to prevent the male pronucleus formation. Thus, the objective of this study was performing two pre-treatments on bovine sperm before ICSI to increase the embryo production. After thawing, the semen was washed 3 times using washing medium and subjected to two treatments before ICSI: T1- immobilization and breaking of sperm plasma membrane with the injection pipette in 10% PVP medium; T2 incubation of sperm with denuded oocytes for 2 h in fertilization medium supplemented with heparin. Subsequently, each spermatozoon was isolated and immobilized with the injection pipette. After ICSI, the oocytes were activated with double incubation in 7% ethanol medium, with an interval of 4 hours between the first and second activation. Fluorescein Isothiocyanate conjugate with Lectin from Arachis Hypogaea and Propidium Iodide (FITC-PNA-PI) were used to verify the sperm acrosome condition. For statistical analysis was applied Anova and T test at 5%. The sperm pre-incubation with oocytes and medium with heparin (T2) produced more sperm with acrosome reacted than when they were only centrifuged in washing medium (77.33 ± 2.51% vs. 53.66 ± 3.05%, respectively to T2 and T1; $P < 0.05$). There was no difference between the cleavage rate in D2 between treatments. However, the pre-incubation with oocytes in fertilization medium with heparin (T2) was more efficient in producing blastocysts in D7 (25.65% vs. 13.43%, respectively to T2 and T1; $P < 0.05$). Therefore, we believe that the injection of sperm with reduced acrosomal and plasma membrane ruptured facilitated the decondensation of sperm nucleus and consequently increased the embryo production. This approach can be used to improve production of bovine embryos by ICSI.

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Effects of fibroblast growth factor 8 (FGF8) on glycolytic activity of bovine cumulus cells during *in vitro* maturation

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The oocyte regulates cumulus cells differentiation and function via secretion of paracrine factors members of the transforming growth factor β (TGF-β) and fibroblast growth factor (FGF) families. In the mouse, BMP15 and FGF8 cooperate to increase glycolytic activity of cumulus cells (Sugiura *et al.*, 2007. Development. 134(14): 2593-2603). In this study, we tested the hypothesis that FGF8 increases glycolytic activity and abundance of mRNA encoding glycolitic enzymes [platelet phosphofructokinase (*PFKP*) and lactate dehydrogenase A (*LDHA*)] and the glucose transporter 1 (*GLUT1*) in bovine cumulus cells undergoing *in vitro* maturation (IVM). Follicles 3-8 mm in diameter were aspirated from slaughterhouse bovine ovaries, and grade 1 and 2 COCs were selected, pooled in groups of 20 and submitted to IVM in 4 well plates containing 400 µl of TCM 199 supplemented with pyruvate (22 µg/ml), amikacin (75 µg/ml), FSH (1 µg/ml), BSA (4 mg/ml), and graded doses of FGF8 (0, 1, 10 and 100 ng/ml). IVM was performed at 38.5 °C and 5.5% CO_2 for 6 and 9 hours. Glucose and lactate concentrations were measured in the medium by dry chemistry (VITROS®Fusion 5.1 FS – Johnson & Johnson, Ontario, Canada) after 9 hours of culture. Messenger RNA abundance was assessed by real time RT-PCR after 6 hours of culture. Cumulus cells were mechanically separated from oocytes, total RNA was extracted using RNasy kit Qiagen® and reverse transcribed Oligo-dT and Ominiscript. Relative expression values were calculated using *CYC-A* as the housekeeping gene. FGF8 did not alter lactate production but decreased glucose uptake at 100 ng/ml. FGF8 also decreased *GLUT1* mRNA abundance at the same concentration (100 ng/ml), but did not alter the expression of *LDHA* or *PFKP* mRNA. In conclusion, the present data suggest that FGF8 decreases glucose uptake in bovine cumulus cells undergoing IVM by mechanisms involving reduced expression of *GLUT1*.

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Effects of follicle stimulating hormone (FSH) and amphiregulin (AREG) on meiosis dynamics and embryo production of bovine oocytes cultured *in vitro*

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In vivo, the maturation of the cumulus oocyte complex (COC) is triggered by the ovulatory peak of LH, which induces germinal vesicle breakdown (GVBD) and progression of nuclear maturation to metaphase II (MII), *in vitro* FSH provides this process. The LH surge induces the secretion of epidermal growth factor (EGF)-like peptides, amphiregulin (AREG), epiregulin (EREG) and betacelulin (BTC) that bind to EGFR leading to Cx43 phosphorylation through the MAPK/ERK pathway closuring of gap junctions communications prevents the transfer of cGMP and cAMP from cumulus cells to the oocyte. The decrease of cGMP levels augments the activity of phosphodiesterase 3 (PDE3) on cAMP, which is hydrolyzed and in low concentrations fails to inhibit via phosphorylation the maturation promoting factor (MPF), thus leading to meiosis resumption. This study aimed to compare meiosis dynamics in oocytes cultured with FSH or AREG and embryo production, testing the hypothesis that the direct stimulation with AREG speeds up nuclear maturation and improves blastocyst rates. The COCs were aspirated from bovine ovaries obtained at a slaughterhouse, and submitted to IVM for 20, 22 and 24 hours in TCM 199 containing Earle's salts, L-glutamine, NaHCO3, supplemented with BSA (0.4%), amikacin (75µg/mL), pyruvate (22µg/mL) and 10^{-1} UI/ml of r-hFSH or 100ηg/mL of AREG. At the end of the IVM, oocytes were fixed and stained with Hoechst 33342 to assess meiotic stage, or submitted to IVF after 24 hours of cultured. Data were transformed to arcsine and groups compared by the Student t test, considering values of $P<0.05$ as significant. AREG promoted progression to MII at equivalent rates from 20 to 24 hours of culture (75.35%; 72.06% and 77.41% respectively), whereas FSH promoted MII at a lower rate at 20 hours (24.57%) increasing at 22 and 24 hours (64.52% and 65.76% respectively), presenting statistical difference between AREG and FSH after 20 hours of culture (P=0,0198). Embryo production after 24 hours of maturation with AREG or FSH promoted equivalent blastocyst rates (31% vs 31.33% respectively). The present data demonstrated that AREG was able to progress meiosis and more rapidly than FSH however, when tested on embryo production AREG and FSH group were similar. Although, no differences were observed on embryo production, the achievement of an *in vitro* system able to mimic physiological mechanisms has an important role on research related to oocyte physiology.

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Efficiency increase in the embryo recipients results with resynchronization protocols

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The aim of this study was to evaluate the effectiveness of a resynchronization protocol on pregnancy rate in crossbred heifers used as embryo recipients. This experiment was conducted on a farm located in southwest Minas Gerais. Experiment 1: Aimed to evaluate the efficiency of 1 mg of estradiol benzoate (EB) in the synchronization of follicular development wave. 40 crossbred females divided into two groups were used. G1 - 1mg of BE; and G2 - 1 ml saline. Changes in follicular dynamics were accompanied by ultrasound. The 1mg BE Application proved effective (95%) in synchronization of follicular development. Experiment 2, it was produced 368 embryos in vitro (PIV) using donor Gir (Bos taurus indicus) and sexed semen of the Dutch race. The transfer of embryos was synchronized through an embryo transfer protocol at fixed time (FTET). D0: it was implanted an intravaginal progesterone device – PID plus 0.5 mg of estradiol benzoate - BE intramuscular (IM); D8: Remove PID plus 0.5 mg of estradiol cypionate - EC MI and 0.125 mg of D-cloprostenol. On the day of embryo transfer (D17) recipients were evaluated by ultrasound (Mindray® - M5). Fourteen days after embryo transfer (ET) recipients were randomly divided into two groups: G1 (n = 184) initiation of resynchronization protocol by inserting a PID application of 1 mg of EB and G2 (n = 184) it was not treated. This procedure was developed to evaluate the effect of estradiol application in early pregnancy. In D39 all recipients passed via pregnancy diagnostic ultrasound (US). In G1 pregnant only removed the PID. Eight days later, all recipients were reassessed by ultrasound for the diagnosis of pregnancy. In females pregnant of the G1 was only removed the DIP (D39) and females do not pregnant in this group received 0.5 mg of EC and 0.15 mg of D-Chloprostenol intramuscular and PID was removed. Ten days later females were again evaluated and those capable were inovulated. Already in G2 the beginning of the second FTET protocol only begins after the diagnosis of non-pregnancy (D39), repeating the same procedure D8. It was compared by X2 pregnancy rate after the first and second transfer embryos and the number of pregnant recipients at 30 and 45 days. The pregnancy rate after the first TE had no difference between the groups (P> 0.05 to 52.2 vs. 48.9% for G1 and G2, respectively). This result confirms that the insertion of a DIP application of 1 mg estradiol benzoate cows in early pregnancy does not interfere with the maintenance of pregnancy. There was no difference in pregnancy rates after the second TE (P > 0.05 to 47.9 vs. 48.8% for G1 and G2, respectively). This result shows that the resynchronization does not interfere with the fertility of the recipient. It follows that the resynchronization protocol used does not affect the maintenance of early pregnancy in recipient and may provide a pregnancy advance and allow pregnancy receiving a short period of time.

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Estimated weight of pig fetuses by ultrasonography

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In swine, the search for ever greater pregnancies often generates piglets of low birth weight, contributing to increased waste in the squad and low weight at slaughter, consequently, significant damage to the productive system. Thus, the early estimates of fetal weight pigs, reflects in time for implementation, too early technological actions in order to reduce the economic losses, and assist in the improvement of the production of the pig industry. This study aimed to estimate the weight of piglets at different ages by means of ultrasound in obstetrics. To this end, we used 15 gilts from a commercial line with appropriate health status during a trial period of four months. Gilts were divided into three groups: Group I consists of pregnant females were slaughtered at 50 days (n = 5), Group II consists of pregnant females were slaughtered at 80 days (n = 5) and Group III consists of pregnant they were slaughtered at 106 days (n = 5). The fetal biometry measurements taken by means of ultrasound, as well as fetal weights were taken after slaughter from each of the experimental groups of females, were used to develop an equation that would predict fetal weight based on the variables ultrasound. For this we used a multiple linear regression model. From equation found, it was possible to estimate fetal weights and validate the reliability with 95% and 92% of weight variation.

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Evaluation of IGF -1 concentration in the activation of ovine pre-antral follicles after in situ culture

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The in vitro culture of small ovarian cortex fragments (*in situ* culture), where the primordial follicles are mainly present, aims to study the activation and growth of follicles. The base medium composition is critical to the success of this growth, and may be influenced by addition of different substances, such as hormones and growth factors. These factors operating in the development of ovarian follicles, highlight the IGF-I, insulin and FSH, play a clear role in the control and regulation of folliculogenesis in several species. Therefore, this study aimed to verify the best concentration of IGF-1 and FSH for follicular activation in culture of pre-antral follicles enclosed in ovarian tissue of sheep. The ovaries were obtained from ovine abattoirs (n = 60), and cut in small fragments. One fragment of each pair of ovaries was preserved (fresh control), and the others were cultured in vitro for 8 days at 39 °C and 5% CO_2. The culture was performed using α-Minimum Essential Medium added supplements (α-MEM +) as base medium, and the culture groups were divided in pure α-MEM + (culture control), and α-MEM + added with 10, 25, 50, 75 or 100 ng/mL of IGF-1 or FSH. After culture, all fragments were submitted to usual histology technics, and stained with hematoxylin-eosin. To assess the optimal concentration of each factor, 30 pre-antral follicles were counted in each section and classified in primordial (PL), primary (PO) and secondary (SE) follicles. The evaluation was carried out in triplicates and different concentrations were compared to cultured and fresh control groups. Data were analyzed by ANOVA. The results showed that, after 8 days culture, the number of SE was greater in 50 e 75 ng/mL of IGF-1 groups than fresh control ($P < 0.05$), however, only 50 ng/mL was greater than culture control. The concentration of 50 ng/ml of IGF-1 also promoted an increase ($P < 0.05$) in the follicular activation, compared to 25 ng/mL. Regarding to FSH, there was no significant difference between the treatments. Thus, it can be concluded that α-MEM + culture medium added of IGF-1 in small concentration promotes follicular activation of ovine pre-antral follicles, and FSH alone do not have any action to activate primordial follicles *in situ* cultured. Further studies are now being performed, including association between the factors and immunohistochemistry for evaluation of follicular apoptosis after culture.

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Expression and regulation of bta-mir-222 in bovine antral follicles

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MicroRNAs (miRNAs) are small noncoding RNA molecules, composed by 19-25 base pair that control the expression of important genes in physiological processes, including reproduction. Among these miRNAs is found miR-222 that is related to cellular development, reproductive system development and function and possibly in regulating of luteinizing hormone receptor (LHR) in cattle (Hossain *et* al., 2009) .Superovulation with exogenous gonadotropins affects oocyte and embryo quality as well granulosa cells differentiation. The LHR expression in bovine granulosa and theca cells is crucial for the transition of FSH/LH-dependency in antral follicles and previous data showed that ovarian superstimulation with FSH increases LHR mRNA abundance in bovine granulosa cells. Thus, the aim of present study was investigate the effects of superstimulation with FSH or FHS combined with eCG on the *Bos taurus* miR-222 (bta-mir-222) abundance on granulosa and theca cells from Nelore cows (*Bos taurus indicus*). Additionally, was investigated the presence of miR-222 in several fetal or adult bovine tissues. Nelore cows were submitted to two superstimulatory protocols: P-36 protocol (n=10) or P-36/eCG protocol (replacement of the FSH by eCG administration on the last day of treatment; n=10). Non-superstimulated cows were only submitted to estrous synchronization (n=10). The animals were slaughtered 12 hours before the expected endogenous LH surge. The theca and granulosa cells were harvested from follicles and submitted to total RNA and miRNA extraction. The mRNA abundance of bta-mir-222 was measured by real time RT-PCR using bovine specific probes. Effects of the superstimulatory treatments were tested by ANOVA and the mean values compared with orthogonal contrast ($P<0.05$) indicated significant difference). Data are presented by mean ± SEM. The bta-mir-222 was expressed in the major adult and fetal bovine tissues measured (ovary, testicle, spleen, liver, kidney, hart and brain). Granulosa cells from Nelore cows submitted to P-36 protocol showed lower levels ($P<0.05$) of bta-mir-22 expression (0.08±0.03) when compared with non-superstimulated cows (0.34±0.13), but did not differ from granulosa cells cows submitted to P36/eCG treatment (0.14±0.06; $P>0.05$). However, miR-222 expression did not differ between theca cells from non-superstimulated cows and cows submitted to P-36 and P-36/eCG protocol. In summary, the bta-miR-222 is express in various bovine tissues, including adult ovary. The ovarian superstimulation protocol did not regulate the miR-222 expression on theca cells, but the P-36 protocol down regulates it on granulosa cells.

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In vivo manipulation of Nile tilapia male germline cell by lentiviral gene transfer

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The spermatogonial stem cells (SSCs) are the foundation of the spermatogenic process and due to their capacity of continuously transmit the genetic information to the subsequent generation, these cells represent an important and appropriate target for fish germline modification. In the present study, we investigate the feasibility of obtaining genetically modified germ cells through in vivo lentiviral infection of Nile tilapia SSCs. Lentivirus particle were produced using the pLenti6.3/V5™-TOPO® vector containing the DsRed2 gene, which expresses red fluorescent protein (RFP) under the control of the human cytomegalovirus immediate-early promoter. For in vivo transduction lentivirus particles ($6.5x10^5$ TU/mL) were injected into the testis of nine sexually mature Nile tilapia through the common spermatic duct that opens in the urogenital pore. Twenty-four hours, four days and one weeks post-transduction the expression of RFP was analyzed in the fish testis by fluorescence microscopy. Additionally, semen samples were obtained by abdominal massage and submitted to flow cytometry two and three weeks after infection. In all different period investigated we could observe widespread expression of RFP fluorescence in the transduced testis. DsRed2 mRNA expression was also detected in the fish gonads. Through histological analysis, we found very few fluorescent spermatozoa in the seminiferous tubules lume. Flow cytometric quantification showed that the percentage of RFP positive spermatozoa ranged from 1% to 9,5%. Although preliminary, these data suggest that viral transduction represent a suitable method to introduce genes into fish male germline cells and to obtain gametes carrying the trangene. Therefore, these findings provide the first step in establishing a system that will allow the in vivo genetic manipulation of fish SSCs, representing an important progress towards the production of transgenic fish lines and new biotechnologies in aquaculture.

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Induction of reproduction in *carassius auratus* (kingio) using two different synthetic GNRH analogues

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The *Carassius auratus* (goldfish) is a member of the Cyprinidae. It is related to important ecological and genetic models, for example fathead minnows and zebrafish. Perhaps the most significant scientific advances resulting from research on goldfish are related to neuroendocrine signaling and how the brain regulates growth, feeding and reproduction. In goldfish, the gonadotrophs cells located in the proximal pars distalis are directly innervated by GnRH neurons and are responsible for the liberation of luteinizing hormone (LH) which is responsible for ovulation. The aim of the present study was to determine if deslorelin or histrelin (synthetic analogues of GnRH) are able to induce spawn of goldfish. Two males and four female were induced with deslorelin or histrelin 1% (preparing dose) and 4% (indutory dose) using the protocol of two applications with 12 hours interval, males and females were maintained together during the procedure. Water was maintained at 27°C and a light:dark period of 14:10. After 6 hours of the second hormone aplication was performed the extrusion of gametes. The female that received deslorelin liberate 1 g of oocytes, however under the light microscope they were degenerated, which was confirmed by the fertilization rate of 0%. However, after one week both females naturally reproduced, and the fertilization rate was of 80%. In conclusion, probably the hormone promotes the reproduction of Carassius auratus but egg quality was poor. After one week of the induction female reproduce, however normally they reproduce in intervals of 1 month. So the hormone could induce natural reproduction, however, more researches need to be performed to determinate the best concentration rates and if those hormones can actually induce the anticipation of natural reproduction.

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Influence of *in vitro* system on miRNAs expression and their regulated pathways in bovine cumulus cells

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Dynamic variations in mRNAs and proteins in oocytes and cumulus cells symbolize an important component of successful embryo development. Oocyte maturation occurs concomitantly with oocyte chromatin condensation and is associated with widespread transcriptional decrease. Even though the oocyte becomes transcriptionally inactive, cumulus cells remain active and are capable of transfering mRNA molecules to the oocyte through the transzonal projections (TZPs). Small non-coding RNA called miRNAs can block translation of target mRNAs regulating several pathways. MiRNAs present in cumulus cells can influence the meiotic progression and developmental competence of the oocyte; however, it is not well understood how the *in vitro* process affects miRNA levels. Therefore, the aim of this study was to identify differentially expressed miRNAs in cumulus cells (CC) from immature, *in vivo* and *in vitro* maturated bovine cumulus-enclosed-oocytes (COC) and identify the pathways regulated by these miRNAs. For this, total RNA from three different pools (3 per group) of CCs from 20 immature, or 10 *in vivo* and *in vitro*-matured COCs were obtained in order to allow miRNA extraction using a combination of TRizol reagent (Invitrogen) and the miRNeasy kit (Qiagen). Reverse transcription was carried out using the miScript PCR System (Qiagen). For RT-qPCR analysis of the 251 bovine miRNAs, a total of 100 ng of cDNA was used per sample. Data were normalized with the geometric mean of 3 endogenous control genes (RNU43 snoRNA, Hm/Ms/Rt U1 snRNA and bta-miR-99b). Expression levels were calculated using the $2^{-\Delta Ct}$ method and data were tested by ANOVA and compared by Tukey's test at 5%. A total of 251 miRNAs were detected in the CC, with 7 exclusively present in the immature group (miR-345-5p, miR-369-5p, miR-377, miR-485, miR-599, miR-885, miR-935), 7 in *in vitro*-matured cumulus cells (miR-106a, miR-124b, miR-369-3p, miR-410, miR-433, miR-493, miR-764) and 11 in *in vivo*-matured ones (miR-126-5p, miR-133b, miR-150, miR-17-5p, miR-22-3p, miR-30a-5p, miR-376c, miR-424-3p, miR-483, miR-487a, miR-542-5p). We used mirPath DIANA tools to determine enriched pathways regulated by these miRNAs. Functional enrichment of immature-exclusive miRNAs indicate the regulation of several pathways, such as: TGF-beta (20 genes), cell cycle (23 genes), FoxO signaling pathway (24 genes), phosphatidylinositol signaling system (11 genes), progesterone-mediated oocyte maturation (17 genes), adherens junction (14 genes), gap junction (10 genes), HIF-1 (18 genes), ErbB (15 genes) and p53 signaling pathway (14 genes). Regarding *in vitro*-exclusive miRNAs, the targeted pathways include TGF-beta signaling pathway (40 genes), adherens junction (40 genes), fatty acid elongation (10 genes), FoxO signaling pathway (64 genes), gap junction (38 genes), cell cycle (51 genes), progesterone-mediated oocyte maturation (40 genes), ErbB (36 genes), fatty acid degradation (16 genes) and HIF-1 (44 genes). Finally, the miRNAs exclusively identified in the *in vivo* group were related to genes involved in adherens junction (46 genes), TGF-beta signaling pathway (43 genes), cell cycle (68 genes), ubiquitin-mediated proteolysis (81 genes), oocyte meiosis (58 genes), FoxO signaling pathway (69 genes), fatty acid biosynthesis (4 genes), focal adhesion (100 genes), p53 signaling pathway (40 genes) and mTOR signaling pathway (35 genes). Therefore, cumulus cells derived from *in vivo*- and *in vitro*-matured COCs have uniquely expressed miRNAs. The specific roles of these signatures miRNAs on oocytes fate are still unknown, however important signaling pathways are differentially regulated suggesting potential implications of the *in vitro* culture conditions on oocyte competence.

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Influence of media and cooling time on the subsequent development of *in vitro*-produced bovine embryos

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The feasibility of cooling as hypothermic preservation method has been asked recently, even after decades of its first report, since cryopreservation, although routinely used in genetic improvement programs, inevitably causes damage to embryonic cells, reducing pregnancy rates, it is not the most appropriate method of storage for short period of time. Thus, the aim of this study was to investigate the effects of different media and cooling times on the development of *in vitro*-produced bovine embryos by assessing embryo quality, blastocyst re-expansion and hatching rates. Therefore, oocytes obtained from ovarian collected from an abattoir, with three or more layers of compact cumulus cells and homogeneous cytoplasm were selected for *in vitro* maturation (IVM). Oocytes (20-25) were matured in Medium 199 (M5017, Sigma-Aldrich Co., St. Louis, MO, USA), supplemented with 2.62 mM of sodium bicarbonate, 0.2 mM sodium pyruvate, 100 IU ml^{-1} penicillin and 10% FCS, plus 5 mg L^{-1} LH and 1 g ml^{-1} of FSH in an humidified atmosphere, at 5% CO_2, in air, at 38.8 °C. After 24h of IVM, the oocytes were transferred to TALP medium plus 30 µg/ml heparin, 2 mM penicillamine, 1mM hipotaurine and 250 mM epinephrine. Semen was thawed in water at 37 °C/30s and the sperm was selected in discontinuous gradient of Percoll®. After selection, the sperm was co-incubated with oocytes for 20 hours under the same conditions of culture adopted to IVM. After *in vitro* fertilization (IVF), presumptive zygotes were denuded by successive pipetting, transferred to SOFaa culture medium and cultured under the same conditions of IVM and IVF steps, for 7 days (D7). On D7, blastocysts were equally divided in number and quality into the following experimental groups: control, embryos kept in SOFaa medium, in an incubator at 5% CO_2, in air, at 38.8 °C, for 6 and 48h; cooled group, embryos kept at 5 °C in Medium 199 supplemented with 2.62 mM sodium bicarbonate, 0.2 mM sodium pyruvate, 100 IU ml^{-1} penicillin, 25 mM HEPES and 50% of FCS for 24 (M199-24h) and 48h (M199-48h) or in commercial BotuEmbryo® medium (Botupharma, Botucatu, Brazil) for 24 (BE-24) and 48h (BE-48h). For cooling, the embryos (15 to 20 per group) were washed in the respective medium and transferred to tubes containing 500 uL of medium and stored for 24 and 48 hours in BotuFlex® shipping box (Botupharma) previously equilibrated at 5 °C. After cooling, the embryos were cultured in SOFaa medium, in an incubator at 5% CO_2, under the same conditions and times adopted for the control group. At 6 and 48h of culture, cooled embryos was assessed for viability, i.e., absence of blastomere breakdown and atypical color, and at 24 and 48h, the re-expansion and hatching rates, respectively. At 6h of culture, the highest percentage ($P < 0.05$) of cooled embryos classified as morphologically viable was observed in BE-24 group (91.81%), differing significantly from M199-48 group (54.81%), which showed a lower average of embryo viability. At the end of the post-cooling culture (48h), embryo viability did not differ between blastocysts subjected to cooling, though lower percentages of viable embryos was observed in M199-48 group (35.53%), differing significantly ($P < 0, 05$) from the control group (100%). Among the cooled embryos, was no effect of the medium and cooling time on re-expansion and hatching rates, however, at 48h post-cooling culture, blastocysts cooled for 48 hours (7.54% and 22.98% for the M199 and BE, respectively) showed significantly lower hatching rate ($P < 0.05$) than the control group (77.52%). In conclusion, under the experimental conditions adopted, no potential adverse effect of medium and cooling time was observed among cooled groups on the viability, re-expansion and hatching rate of bovine blastocysts after 48h of hypothermic storage, although M199-48 and BE-48 groups had lower hatching rate compared to control group. The embryo transfer produced by the proposed methodology can confirm their effectiveness in establishing of similar conception rates to those obtained with fresh transferred embryos. Financial support: FAPESP # 2013/15905-9.

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Influence of microbiological activity over the quality of cooled *Oreochromis niloticus* semen

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Semen cooling for fish is a technique that maintains spermatic feasibility for hours or days after collection. A possible limiting factor to this technique is the presence of bacteria in the diluting medium, or even in the contamination of the semen during collection. This work aims at evaluating the possible effects of bacterial microbiological activity over the quality of cooled *in natura* semen of Nile tilapia *Oreochromis niloticus*, diluted in BTS™ and coconut water. The work was conducted in November of 2014, using 18 male (320 ± 110 g) tilapia, of the UFLA variety, originated from the fish farm at the Federal University of Lavras (UFLA). The animals were stored one fish per box of 250 L. The fish were collected (after 24 h fasting), carefully cleaning the urogenital papillae in order for there to be no contamination from water, feces or urine. Subsequently, we performed massage over the coelomic cavity, in the craniocaudal direction, to remove the semen by means of 1 mL sterilized syringes. The collected semen was stored in Eppendorf tubes, with open lid, in Styrofoam boxes with artificial ice (9-11°C), and transported to the Laboratory of Veterinary Sciences of UFLA, for posterior refrigeration (4-6°C). Since the amount of semen was insufficient for the evaluations (on average 0,33 mL), a pool was used for every two animals, totalizing nine semen analyses. In addition to the *in natura* semen, samples of fresh semen were diluted in BTS™ (5%) and in sterilized coconut water (Nosso Coco), evaluating spermatic motility in room temperature (time zero) ad after cooling (up to 72 hours), by light microscope (400x), with samples from 5 µL (semen) activated by 50 µL water tank (1:10). Semen fresh and diluted besides of pure solutions (BTS™ and coconut water), were plated per method *Myles Y Misra*, in petri dishes with nutrient solution (blood agar) for bacteria and placed in an oven plates (37 °C for 24 h) to colonization of the bacteria. Samples (in BTS™ and coconut water) were diluted 1:10 (Semen: the total volume), giving a final concentration of 0.62 x 10^8 sperm/mL. The plating was repeated in the material cooled (4-6 °C) until completion of the 72 hours. Triage verified the following bacterial colonies: *Streptococcus*, *Staphylococcus*, coccobacillus, and bacillus. In average, coconut water presented no bacterial colonies, while BTS™ presented 110 CFU/mL. These values are due to the use of commercial and sterilized coconut water, while BTS™, done in laboratory, was contaminated during manipulation. The *in natura* semen presented high spermatic motility (84 ± 5%) at time zero, and low motility (49 ± 22%, 43 ± 24% e 24 ± 8%) at times 24, 48 and 72 hours of refrigeration, respectively, with low bacterial contamination in the initial and final times (3-6 CFU/mL). The semen diluted in coconut water presented low spermatic motility (29 ± 22%) at time zero. Therefore, it was not evaluated at other times. This semen presented low bacterial contamination, indicating only 1 CFU/mL at the initial time. The semen diluted in BTS™ presented high spermatic motility (94 ± 7%, 83 ± 9%, 81 ± 11% e 72 ± 12%) at times 24, 48 and 72 hours of refrigeration, respectively. This semen presented great increase in bacterial colonization, from 11 CFU/mL, at the initial time, to 61 CFU/mL, at the final time of evaluation. The study indicates that seminal quality was not affected by the bacterial colonies present in the solutions, since, despite the BTS™ being the most contaminated medium, it maintained spermatic quality for three days (72 h).

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Influence of selenium and vitamin E on fresh, refrigerated and frozen semen in dogs

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New methodologies have been developed seeking to maximize pregnancy rate in female dogs created in commercial kennels, and also in order to maintain the quality of canine semen after dilution, refrigeration or freezing. One of the main factors that generate damage to sperm is oxidative stress, to minimize sperm damage, selenium and antioxidants like vitamin E are administered, by oral administration, seeking to improve the quality of semen.The objective was to study the effect of vitamin E and selenium, by oral administration, in the quality of fresh, refrigerated and frozen semen in adult dogs French Bulldog breed.Semen samples were collected from 5 adult dogs, French Bulldog breed, being 2 semen drawing before the daily oral supplementation with vitamin E and selenium (ESE®) and semen drawing at 20, 40 and 60 days after the beginning of oral supplement.The ejaculated samples were diluted in TRIS - fructose citric acid (3.28 g TRIS-hydroxy-methyl-amino-methane, 1.78 g of citric acid monohydrate and 1.25 g of D – fructose, dissolved in 100 mL of distilled water and added of 20% egg yolk and 6% of glycerol.The characteristics evaluated in fresh semen were: volume (mL), color, appearance, concentration ($x10^6$/mL), sperm motility (%), vigor (1 to 5) and morphology (%).For refrigerated and frozen semen were analyzed: sperm motility (%), vigor (1-5) and morphology (%).Diluted semen samples were centrifuged at: 1500 g / 10 min and pellets formed by sperm of each ejaculated, detached from the tube wall were diluted homogeneously in the diluent TRIS type up to the final volume of 1.5mL.After that, packaged in 0.5mL French straws,kept under refrigeration at 5ºC/4h, placed in nitrogen vapor at -120ºC/15 min, and dipped in liquid nitrogen at -196ºC and then stored on identified rachis and stored in liquid nitrogen container until the time of thawing in water bath at 37°C/30 sec for semen microscopic analysis.Data from fresh, refrigerated and frozen semen were statistically analyzed by analysis of variance and the average compared by 5% of Tukey test.Fresh semen sperm concentration differed ($P < 0.05$) between the samples, rising after 40 days after the beginning of oral supplementation with selenium and vitamin E.For the vigor better score ($P < 0.05$) was observed at collection 4, in 40 days after the beginning of oral supplementation to dogs.For fresh and refrigerated semen, the total defects, defects of head, acrosome and tail did not differ ($P > 0.05$) between the samples.Total sperm defects and minor head and tail defects did not differ ($P > 0.05$) between the samples in post-thawing.Regarding the acrosome defects after thawing, there was a significant reduction ($P < 0.05$) in samples performed 40 and 60 days after the beginning of oral supplementation with selenium and vitamin E. The managed supplement, by oral administration, containing selenium and vitamin E, influenced beneficially raising the sperm concentration in fresh semen and decreasing the acrosome defects in frozen semen.Oral administration of supplementation with selenium and vitamin E is recommended for improving the quality of fresh and frozen semen in dogs.(CEUA/UNOESTE/Protocol#2101/2014).

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Influence of vitamin E in extenders in refrigerated and frozen semen in dogs

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Researches have been conducted in order to maintain the quality of the fresh, diluted, refrigerated or frozen semen for artificial insemination purposes in dogs. The objective was to study the influence of the vitamin E addition in types of extenders in the quality of the fresh, refrigerated and frozen semen in dogs of French Bulldog breed. The 25 samples colected by digital manipulation were performed on 5 adult dogs, five on each dog. The characteristics evaluated in fresh semen were: volume (mL), color, aspect, concentration ($x10^6$/mL), sperm motility (%), vigor (1-5) and sperm morphology (%). For refrigerated and frozen semen, motility, vigor and morphology were analyzed. Each ejaculated was fractionated in 4 equal parts and diluted in the ratio 1:1 in the following extenders: 1 – TRIS Fructose Citric acid + 200mM of vitamin E; 2 – TRIS Fructose Citric acid; 3 - coconut water (ACP-106®) + 200mM of vitamin E; and 4 – coconut water (ACP-106®). The four aliquots of semen, diluted in the four respective extenders were centrifuged at 1500g/10 min and the "pellets" formed of sperm from every ejaculated were resuspended homogeneously with the four extenders to the volume of 1.5mL and filled into 0.5mL French straws kept under refrigeration at 5ºC/4 h after placed in a nitrogen vapor at -120ºC/15 min, and immersed in liquid nitrogen at -196ºC in the sequence stored in identified racks and stored in liquid nitrogen container until the time of thawing in a water bath at 37ºC/30 seconds for microscopic semen analysis. Data from fresh, refrigerated and frozen semen were statistically analyzed by analysis of variance and the average compared by Tukey ($P < 0.05$). For fresh semen diluted in the four extenders, in pre-cooling curve, there was a difference ($P < 0.05$) for defects in the sperm head, between TRIS + vit. E (7.59±4.01%) and TRIS (10.48±5.42%). In the post-cooling curve to 5ºC/4 hours, for the four extenders, there was no difference ($P > 0.05$) between the evaluated characteristics. For frozen semen with TRIS and thawed at 37ºC/30 sec, there was difference ($P < 0.05$) for the major sperm defects, being the top average (26.62 ± 5.52%) compared to the other three extenders. For minor sperm defects in frozen semen with TRIS, there was difference ($P < 0.05$) with a lower percentage of incidence (16.23 ± 2.02%) compared to other extenders. There was difference ($P < 0.05$) with increase of total defects in frozen semen with the extender ACP + vit. E, compared to other extenders. It is found that the compositions of the extenders diverge from each other, resulting in variations in the results related to the maintenance of the quality of semen characteristics. Attention should be paid for what purpose the extenders within the refrigeration or freezing biotech will be used. In the present study, we found that the microscopic analysis of the spermatic motility and vigor in frozen semen with the ACP extender is hampered due to the lower transparency of this extender in relation to the TRIS extender. We conclude that the TRIS + vit. E extender it is the most recommended to dilute the fresh semen for the purpose of immediate artificial insemination due to lower presence of the sperm head defects. For refrigeration, the four extenders are recommended, with similarity in semen characteristics maintenance. For frozen semen the indicated extenders are the TRIS, TRIS + vit. E, and the extender ACP. The addition of vit. E in these extenders did not provide improvement of refrigerated and frozen semen, with optional use of it. (CEUA/UNOESTE/Protocol#2101).

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Infrared thermography in the reproduction in dairy cattle

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The digital infrared imaging thermography is a non-invasive imaging exam of easy performance accurately to measure temperatures of the areas of the animals body. The objective was to study the physiological variations of the surface temperature of the mammary gland, vulva, pelvis, thorax and abdomen with digital infrared imaging thermography in negative dairy cattle with California mastitis test (CMT) in different seasons, and the influence of climatic factors in these temperatures. 18 Holstein cows, pregnant and non pregnant were used, negative for the California Mastitis Test, grazing on *Urochloa decumbens* pasture, receiving 2 kg of corn / animal / day, mineral mix and *ad libitum* water. Infrared thermography was performed every 30 days, for five months: January, February, March, April and May with thermographic camera (E40®, FLIR, Sweden) in the areas of the body: vulva, mammary gland, pelvis, abdomen and thorax. The thermal images (thermograms) were processed using the Flir Tools 2.1® program. The climatic factors: room temperature and relative humidity were monitored with globe thermometer (ITitwtg 2000®, Instrutemp, Brazil). Data were analyzed by analysis of variance and the average compared by 5% Tukey test. For room temperature data, relative humidity, rectal temperature and for the areas of the body we used Pearson correlation to 5%. For climatic factors, among the samples, room temperature and relative humidity, there was a significant difference ($P<0.05$). To the skin surface temperatures of the body areas examined: vulva, pelvis, ischium, abdomen, thorax and mammary gland, among the samples, there were differences ($P<0.05$) with lower temperatures of the areas in March and May samples, compared to the months of January, February and April. The average temperatures of the surfaces of the examined areas vary for the vulva between 33°C and 38°C; ischium, pelvis and abdomen between 30°C and 37°C; teats between 28°C and 37°C; Cistern of mammary quarter between 32°C and 38°C; and thorax from 31°C to 37°C. There were significant correlations ($P<0.01$) between: rectal temperature x room temperature (0.49); Rectal temperature x relative humidity (- 0.37). To the surface temperatures of all areas of the body examined, there were significant correlations ($P<0.01$) with the room temperature between 0.73 and 0.85; and between - 0.57 and - 0.75 for the relative humidity. The animals showed no behavioral change during the thermography examination. In dairy cattle it is recommended the use of infrared thermography as a routine test to measure the temperatures of the areas of the body. Climatic factors, room temperature and relative humidity influence the rectal temperatures and the body surfaces of the cows. The areas of the body examined by infrared thermography showed different temperatures, in the same data collection, showing physiological temperature variations that assist in the clinical evaluation of each of the areas examined. The thermographic images were saved and processed easily, quickly and in a practical way, recommending the use of thermal imaging by infrared for routine as imaging test complementary to the clinical examination of the mammary gland and of the body areas in dairy cattle.

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Is there a difference in the display of the vascularity of pampiniform plexus and testicular parenchyma using different frequencies in the color Doppler ultrasound?

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Ultrasonography is an indirect, non-invasive method used for disorders diagnostics, especially in the reproductive tract. The B-mode ultrasonography is presented by the intensity of echoes from different points of gray shades, which may present more or less echogenic or with higher or lower resolution, color mode (Doppler) is an important tool for the evaluation of testicular pathologies associated with blood perfusion. The analysis are dependent on what structures are evaluated (deeper or superficial) for such different frequencies may be used for this purpose. However, there are no studies about the ideal frequency at analysis on vascularization of reproductive male structures. Thus, the present study aimed to evaluate whether there differences in the display of the vascularity on parenchyma and pampiniform plexus of bovine testicles using different frequencies through of ultrasonography in color mode (Doppler). To this end six Nellore breed bulls (Bos indicus) with a mean age of 34 (± 0.89) months old were used. To analyze the vascularization display of the testes a Doppler ultrasound device M5Vet® model (Mindray, China) was used with a linear transducer (Model Mindray 7L4s). The testis were evaluated both, right and the left, as regards: vascularization of the pampiniform plexus (VPP) by assigning a score from 1 to 5, with 1 being less vascularized and 5 more vascularized; vascularization of the testicular parenchyma (VTP) by assigning a score from 0 to 4, with 0 being no visible vascularization in the image and 4 several large vessels in the image, at frequencies of 5 Hz, 7.5 Hz and 10 Hz. Were also made analysis of the quality of images (ranking of the image - RI) observing the picture quality as a whole; and image quality of the vascularization (vascularization ranking - VR) only observing the display quality of the vasculature. These were obtained through rankings, assigning scores ranging from 1 to 3, with 1 being the lowest image quality (resolution) and 3 to bigger or better image quality (resolution). This ranking was done on both plexes and both parenchymas and all for all frequencies (5 Hz, 7.5 Hz and 10 Hz). For all variables, grades were given by two trained observers in a double-blind model, with the means analyzed in the end. Data were submitted to analysis of variance considering the three different frequencies using MIXED procedure of Statical Analysis System (SAS, 2004). The significance level was ≤5%. There was no differences between the data from VPP and VTP in any of the frequencies showing that the vasculature score did not differ in terms of frequencies. However, RI and VR presented significant difference. RI on plexus (RIPL) showed better image at 5 (RIPL Right = 2.50 ± 0.31, RIPL Left = 2.41 ± 0.23) and 7.5 (RIPL Right = 2.25 ± 0.21, RIPL Left = 2.33 ± 0.27) in relation to 10 (RIPL Right = 1.25 ± 0.17, RIPL Left = 1.25 ± 0.11) Hz. But when observed in the parenchyma (RIPA) the frequency of 7.5 (RIPA Right = 2.58 ± 0.20, RIPA Left = 2.41 ± 00.23) showed better visualization than the 10 (RIPA Right = 1.50 ± 0.22, RIPA Left = 1.50 ± 0.12) Hz. With this information, we can infer that for viewing the image as a whole would be the best frequencies of 5 and 7.5 Hz. On the other hand, when we observe the data from the vascularization ranking of the plexus (VRPL), we found that the frequencies of 7.5 (VRPL Right = 2.25 ± 0.28, VRPL Left = 2.25 ± 00.11) and 10 (VRPL Right = 2.50 ± 12.25 , VRPL Left = 2.41 ± 0.30) Hz were better in the visualization than the 5 (VRPL Right = 1.25 ± 0.11, VRPL Left = 1.33 ± 0.21) Hz. In the analysis of vascularization ranking of the parenchyma (VRPA) the frequency of 10 (VRPA Right = 2.90 ± 0.10 VRPA Left = 2.90 ± 0.10) Hz were greater than the frequencies of 7.5 (VRPA Right = 2.25 ± 0.17, VRPA Left = 2.00 ± 0) and 5 (VRPA Right = 1.08 ± 0.08, VRPA left = 10.01 ± 0.10) Hz. By the end, we can conclude that the frequency of 7.5 showed a better visualization of the black and white image, however the vascularization visualization was better at the frequency of 10 Hz, this should be the frequency of choice when performed testicular hemodynamic analysis.

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Monitoring of the uterine environment of repeat breeder Holstein cows during estrus and diestrus by endometrial cytology, histopathology and transcription of inflammatory mediators

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Repeat breeders are cows that have normal estrous cycles, with no clinical abnormalities in the reproductive tract, but fail to conceive after at least three successive services. In herds with conception rate of 50%, it is estimated the occurrence of 12% of repeat breeders. Repeat breeding is a multifactorial problem involving extrinsic factors associated with herd as well as factors intrinsic to the individual animal. This syndrome leads to expressive economic loss for dairy producers due to increased calving interval and increased culling rates. The aim of this study was to investigate the occurrence of subclinical changes in the endometrium of repeat breeder Holstein cows. Endometrial inflammatory response was evaluated by assessing inflammatory cells and transcription of the immunomodulators: CXC chemokines ligands, namely, CXCL6 (also known as granulocyte chemotactic protein 2, GCP-2) and CXCL8 (interleukin 8, IL8), IL10, and prostaglandin synthases E (PGES) and F (PGFS) during the estrus and diestrus. Twenty-one pluriparous Holstein cows were submitted to estrous synchronization, and separated into two groups according to the reproductive history and number of inseminations between endometrial samples collections and next pregnancy. Six cows were considered repeat breeders (mean 7.2 ± 1.6 inseminations between last parturition and the time of collection of endometrial samples, and 8.7 ± 1.6 inseminations until the next pregnancy) and they formed the Group 1. Five cows became pregnant with up to three successive inseminations and composed the control group, Group 2 (no previous inseminations and 1.8 ± 0.8 later). Endometrial samples were obtained on average 383.2 ± 40.0 and 81.2 ± 14.4 days postpartum for cows from Groups 1 and 2, respectively. Monitoring of the uterine environment was performed by cytobrush technique and uterine biopsies in the 10th and 22nd days after initiation of hormone protocol, in the estrus and diestrus, respectively. Cytological endometritis was characterized by the presence of more than 5% of polymorphonuclear cells in the uterine smear. Two endometrial samples were collected on right uterine horn in estrus phase and two on the left horn in the diestrus. One sample was collected for histopathology and the other was processed for RNA extraction, cDNA synthesis and qRT-PCR. GraphPad Instat version 3.10 was used for statistical analyses. This experimental protocol has been approved by the institutional committee on experimental animal use (CETEA/UFMG - Protocol#49/2011). Endometrial cytology of Group 2 had significantly more ($P \leq 0.05$, Student's t test) polymorphonuclear cells in estrus (4.8 ± 3.3) than in diestrus (1.9 ± 3.1). There was no significant difference ($P < 0.05$, Student's t test) between endometrial cytology of repeat breeders in the phases of estrus and diestrus, nor between samples from Groups 1 and 2. According to the occurrence of inflammatory infiltrates in the histopathology, one repeat breeder showed slight endometritis at the estrus, and two cows from each group had slight endometritis at the diestrus. Two repeat breeders had moderate endometritis at the diestrus. Concerning endometrial transcription of immunomodulators, repeat breeder cows had significantly higher levels ($P \leq 0.05$, Mann-Whitney's test) of CXCL6 when compared to the control group in both phases of the estrous cycle. Endometrial transcription of CXCL8 in repeat breeders was higher ($P \leq 0.05$, Mann-Whitney's test) than control cows only at the estrus. No significant differences ($P > 0.05$, Teste de Wilcoxon) were found between phases of the estrous cycle in the Groups 1 and 2. There were significant ($P < 0.05$, Spearman correlation) and high ($r \geq 0.70$) correlations between endometrial transcription levels of CXC L6 and CXCL8 in the estrus and diestrus. Increased endometrial transcription of neutrophil chemoattractants such CXCL6 and CXCL8 can have a negative effect on fertility leading to repeat breeding. Increased neutrophil influx to the endometrium during the estrus is likely to be detrimental to sperm, which may impair the fertilization. In addition, changes in the uterine environment during the diestrus may affect the establishment of pregnancy. However, in the present study we found no association between endometrial transcription levels of inflammatory mediators, cytological and histopathology results.

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Overexpression of pluripotency factors OCT4 and SOX2 and cell sorting influence on *in vitro* induced reprogramming in cattle

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Reproductive biotechniques such as *in vitro* embryo production and somatic cell nuclear transfer may greatly contribute for fertility improvements, to enhance animal production or else to contribute to a better understanding of the underlying mechanism involved during initial embryonic development. The generation of induced pluripotency models (induced pluripotent stem cells, or iPS) made it possible to study the process of *in vitro* reprogramming in a more solid and precise manner. *OCT4* and *SOX2* are fundamental genes for the acquisition and maintenance process of cellular pluripotency. Recently, it has been reported that both factors may have a huge influence on the regulation of some imprinted genes known to be important for the process of cellular reprogramming. Therefore, this study aimed to generate an *in vitro* experimental model where the above transcription factors were studied together or separately regarding their influence on cellular reprogramming. For that, three bovine fetal fibroblasts cell lines (bFF1, bFF2 and bFF3) were cultured in Iscove's Modified Dulbecco's Media (IMDM), 10% fetal bovine serum (FBS), penicillin/streptomycin (100 U·ml−1 penicillin and 100 µg·ml−1 streptomycin) in 5% CO2 at 38,5°C. The fibroblasts were transduced with lentiviral vectors containing human OCT4 or SOX2 cDNAs (Papapetrou et al., PNAS 4;106(31) 2009). Five days after transduction the fibroblasts were analyzed trough cell cytometry and positive cells were sorted. One fibroblast lineage expressing OCT4, SOX2, both (OCT4+SOX2), none (control) was further submitted to fluorescence activated cell sorting (FACS) and, together with a non-sorted and non-transgenic control, the groups were submitted to in vitro induced reprogramming and production of iPS cell colonies. Induction into pluripotency was realized by transducing fibroblasts with polycistronic excisable vector containing the murine cDNA of OCT4, SOX2, c-MYC and KLF4 transcription factors (OSMK, STEMCCA vector). Five to six days after transduction the cells were replated onto mouse inactivated fibroblast feeder layers (MEFs) and further cultured in iPS media: KnockOut DMEM/F-12 medium with 20% KnockOut Serum Replacement (KSR), 0.5 mmol L−1 L -glutamine, 1% NEAA, 0.1 mmol L−1 β-mercaptoethanol, 1% penicillin/streptomycin and 10 ug/mL basic Fibroblast Growth Factor (bFGF). The results of fluorescence analysis by flow cytometry were 79.8%, 22.7% and 18.7% for OCT4, 10.2%, 4.2% and 3.9% for SOX2, and 1.3%, 0.4% and 0.2% for OCT4+SOX2 respectively for bFF1, 2 and 3, and the average was 40.4% for OCT4, 6.1% for SOX2 and 0,63% for OCT4+SOX2 groups. bFF1 was the only lineage presenting a post-sorting recovery that enabled its use for pluripotency induction. Interestingly, non-sorted cells generated biPS colonies with an efficiency of 0.0675% (27 colonies per $4x10^4$ transduced cells) while sorted cells (control non transgenic, OCT4, SOX2 and OCT4+SOX2 expressing cells) did not generate biPS cells (0 colonies per $4x10^4$ transduced cells/group). We conclude that the cell sorting procedure clearly had a detrimental effect on *in vitro* reprogramming efficiency and next steps will focus on possible epigenetic and viability analysis to further dissect the mechanisms of pluripotency acquisition *in vitro*.

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Performance of the Gir and Girolando race donors during lactation and dry period on the *in-vitro* production system of embryos: Preliminary Results

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The *in-vitro* production of embryos (IVPE) has been the technique of choice in dairy herds. The possibility of using sexed semen with satisfactory results made it so utilizing this bio-technique in dairy animals increased by allowing a larger number of females to be born. However, many times, in dairy farms the oocyte donors are in lactation, which can negatively influence the results. Therefore, there are necessary procedures to be carried out in this period to maintain levels of optimum performance. Thus, in present work there was a purpose to evaluate the parameters: the number of oocytes, the quantity of blastocysts, and the rate of blastocysts in cows of the Gir and the Girolando race (Gir X Holstein) during lactation and the dry period. Ninety-one follicular aspirations (OPU) were performed in the period from April 2013 to April 2016, for a total of 20 aspiration sections. The data analyzed was from animals of the same flock. Seven of the Gir race and five of the Girolando race, which were submitted to the same management at the property localized in the town of Tumiritinga in the state of Minas Gerais, Brazil. At this location, we varied rainy periods and dry periods . Since April through October the season is dry and from November to April the season is rainy. The provided diet during the lactation period was the base of corn silage and was focused with 24% of crude protein. The proportion of the diet was based in function with the daily milk production, with reference to 1 kg of concentrate for every 2 liters of milk produced, differing the provided diet during the dry period, in which the animals were maintained in a grazing system (Cynodon ssp. e Brachiaria brizantha) and mineral supplementation at ease. The *in-vitro* production of embryos was performed by the same laboratory and all the steps were done according to the protocol from the company responsible for the production of embryos. A normality test was performed to verify the distribution of variables and so the test was not performed parametric to Kruskal-Wallis, considering the level of significance of $p < 0.05$. The production of viable oocytes (mean ± standard error) were 23.5 ± 3.6 for the breastfeeding period and 21.9 ± 1.8 for the dry period. The quantity of blastocytes produced for the lactation period was between 6.4 ± 0.9 and between 6.3 ± 0.6 for the dry period. The rate of blastocytes was already 26.4 ± 1.6 and 30.2 ± 1.8, respectively, for the breastfeeding period and the dry period. The comparison between the average was always performed in between the same donor considering the two periods. The result differs in some literature work that demonstrate the difference in production between animals that aspirate during lactation versus the dry period. However, in recent studies the donors during the dry period had offered a specific diet and approximately sixty days before the planned due date, these animals were submitted to a pre-partum diet, and after starting lactation they were offered another diet with the objective to meet the needs of these animals in function of lactation. Therefore, even during lactation, the donors included in this work maintained the potential of donors for receiving the proper diet for the period who were at the time during aspiration. Thus, oocyte donors from dairy races can be aspirated and submitted to the in-vitro production of embryos with satisfactory results, even during the lactation period, provided the diet is adjusted.

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Pregnancy and mortality rates after exposure of *in vitro*-produced nelore embryos to forskolin

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This study aimed to evaluate the effects of treatment of *in vitro*-produced Nelore embryos with Forskolin, a drug that stimulates lipolysis, on the production of embryos, conception rate, mortality rate (including early embryonic death, abortions and stillbirths) and weight at birth of the calves. Oocyte donors Nelore cows were aspired with ultrasound aid and COCs (n=584) were matured *in vitro* for 22h at 38.5°C and 5% CO_2 in air in 100 µL droplets of TCM199 medium with 10% FCS and hormones. Subsequently, the oocytes were fertilized with semen of one Nelore bull previously tested for fertility, and presumptive zygotes were cultured *in vitro* in SOFaa (Control group) or SOFaa supplemented with 5.0 µM Forskolin (Forsk group) from day 6 to 7 of the culture (Day 0 = day of IVF). The culture was conducted at 38.5 °C and 5% CO_2 in air for 7 days. Produced blastocysts (n=234) were transferred to Nelore recipients previously synchronized with the following protocol at fixed time: introducing intravaginal progesterone device (CIDR, Zoetis-Pfizer, Brazil) and application of 2 mg Estradiol Benzoate (Bioestrogen®, Biogénesis Bagó, Brazil) on Day 0; removal of the device and application of 150 µg of D-cloprostenol (Croniben®, Biogenesis Bagó, Brazil) and 250 IU ECG (Novormon®, Zoetis-Pfizer, Brazil) on Day 8; application of 1 mg Estradiol Benzoate on Day 9; transfer of embryos on Day 17 without heat detection in animals presenting corpus luteum. The pregnancy and mortality rates, and weight at birth were analyzed using generalized linear mixed model framework with repeated measurements, in the Imea4 package in R v3.2.5 software. The blastocyst production rate was similar (P>0.05) between the Control (36.26±3.33%) and Forsk (36.10±2.62%) groups. The conception rate after transfer of embryos was similar (P>0.05) between the Control (39.17±4.89%) and Forsk (43.10±5.02%) groups. Similarly, the mortality rate did not differ (P>0.05) between the control group (14.75±4.54%) and Forsk (14.52±4.47%). There was no difference in birth weight (P>0.05) between the Control (39.79±0,35 kg) and Forsk (39.30±0,46 kg) groups, and the differences observed in weight of the calves were attributed only to the effect of sex, and the males were weighed on average 1.8±0.6 kg more than females (P<0.05). According to these results, treatment with Forskolin, commonly used in an attempt to reduce cytoplasmic lipid accumulation in *in vitro*-produced embryos aiming to improve their cryotolerance does not affect embryo development, pregnancy and mortality rates, and weight at birth.

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Pregnancy rate and litter size in Border Collie breed females inseminated with fresh semen

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The aim of this study was to evaluate the conception rate and litter size of bitches Border Collie breed artificially inseminated with fresh semen. Seven bitches were inseminated, and four of them with two pregnancies and three bitches, only one pregnancy. The dogs were aged between 23 and 51 months (mean=33.73; standard error=2.74), and all were deemed clinically healthy, and fertility history. The inseminations were performed after the blade vaginal cytology present more than 80% of superficial cells, whether there is the presence of erythrocytes and vaginal bleeding and females presenting male taking behavior. In all females were two inseminations at intervals of 48 hours. The semen donor males were evaluated clinically, including serologic evaluation for brucellosis and herpes virus. The semen of the male was collected by digital manipulation of the penis in the presence of a female in heat. Immediately after collection, the semen was kept in a water bath temperature of 37°C and evaluated macroscopically and microscopically. They were evaluated color parameters, volume, motility, vigor, concentration and morphology. To perform the artificial insemination, semen was considered suitable as presented the following characteristics: higher motility of 70%, minimum vigor of 3 and at least $200x10^6$ concentration of sperm. The used artificial insemination was intravaginal. The pregnancies were confirmed by transabdominal ultrasound, approximately 30 days after the last insemination. The conception rate in this study was 100 % (11/11). For the 11 pregnancies, the estimates of mean and standard error for litter size was 8.54 ±0.56 puppies. The study shows that Intravaginal Artificial Insemination technique with fresh semen quality has favorable results of prolificacy in female Border Collie breed.

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Pregnancy-associated plasma Protein-A improves oocyte maturation and modulates the pattern of transcription in bovine *in vitro*-produced embryos

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The Insulin-like Growth Factor (IGF) system has been demonstrated as an important factor to cumulus oocyte complex (COC) maturation and early embryo development. Studies demonstrate benefits of IGF-I addition during the *in vitro* maturation of oocytes in different species, including cattle. The IGF availability is controlled by two mechanisms: IGF Binding Proteins (IGFBPs), which inhibit IGF actions; and Pregnancy-Associated Plasma Protein-A (PAPP-A), responsible to cleavage the IGFBP and to release free IGF. The aim of this study was to investigate the effects of PAPP-A on cellular aspects of *in vitro*-matured COCs and further, to explore their impacts in the blastocysts' yield and pattern of transcription. Bovine COCs (5 replicates/20 COC per replicate) from ovaries obtained in a local abattoir, predominantly from Nelore cows, were aspirated and matured in serum free medium in the absence (control group) or presence of 1 ng/mL (P1 group), 10 ng/mL (P10 group) or 100 ng/mL (P100 group) of PAPP-A. After 24 h of *in vitro* maturation (IVM), we assessed the meiosis progression by Hoescht staining and DNA fragmentation (apoptosis) by TUNEL assay, Matured COCs (5 replicates/20 COC per replicate) from each group were fertilized and cultured until blastocyst stage. On day seven, the blastocyst rate was analyzed and pools of three blastocysts were submitted to assess embryonic gene expression. The transcription pattern of 91 genes were analyzed by RT-qPCR using Taqman® assays in the HD-Biomark System® (96.96 dynamic array IFC) in four pools for control, P1 and P10 groups and five pools for P100 group. The statistical analysis was tested by ANOVA using PROC GLM procedure of SAS (SAS, 9.2, SAS Inst., Cary, NC, USA). Individual differences were analyzed through pair-wise comparisons (SAS). The addition of PAPP-A did not affect apoptotic rates: 42% (control), 50% (P1), 55 % (P10) and 45 % (P100). Moreover, the presence of PAPP-A during IVM did not affect the percentage of oocytes in metaphase II as compared to control group; however, it increased the number of oocytes with complete extrusion of the first polar body in the P100 group (90%) when compared to P1 group (71%). Also, no differences were demonstrated on embryo yield: cleavage rate (83%, 84%, 84% and 79%, in control, P1, P10 and P100 groups, respectively) and blastocyst rate (12%, 15%, 16% and 17%, in control, P1, P10 and P100 groups, respectively). However, the embryonic pattern of transcription was affected, demonstrating higher mRNA abundance of *DNMT3A* in the P1 and P100 when compared to control and P10 groups; higher mRNA abundance of *ATF4* and *POU5F1* in the P100 as compared to control group; higher mRNA abundance of *STAT3* and *PAF1* in P100 than the P10 group and higher mRNA abundance of *TFAM* in P100 then the control and P1 groups. Taken together, these data demonstrate an important role of PAPP-A in the control of oocyte maturation and further effects on embryonic gene expression. Specifically, the addition of 100 ng/mL of PAPP-A during the IVM of bovine COCs was able to positively influence the meiosis progression and affected the abundance of important genes related to embryo quality, possibly, by increasing the IGF bioavailability, which is known to support embryo development and improve embryo quality.

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Preovulatory follicle diameter and its correlation with reproductive efficiency in *Bos taurus indicus* cows submitted to timed artificial insemination

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There are several factors that influence the success of timed artificial insemination (TAI) programs in *Bos taurus indicus* cows, among which location, herd, parity and body condition score can be mentioned. The size of the preovulatory follicle (POF) influence pregnancy conception rate (CR) at artificial insemination. The current study aimed to evaluate the diameter of the ovarian POF at the time of TAI in multiparous *Bos taurus indicus* cows correlating it with the conception rate (CR). One hundred and forty-five, calved up to 65 days were used and subjected to a single protocol: day 0 (d0) implantation of 1.0 mg of progesterone + application 2mg of estradiol benzoate; d8 implants were removed and applied 500 mcg of D-cloprostenol + 300 IU of equine chorionic gonadotropin, and 1 mg estradiol cypionate; on d10 the POFs were mesuared using ultrasound and proceeded to TAI with frozen semen. On d35 after TAI the pregnancy diagnosis was performed. The conception rate at TAI was 62.7% and the average POF diameter was 15.1 mm in pregnant cows and 13.7mm in non-pregnant cows ($P = 0.03$). Four categories were stablished according to the diameter of the POF: 8.0 to 11.0 mm; 11.1 to 14.0mm; 14.1 to 17.0mm and 17.1 to 20.0mm, resulting respectively in 37.0; 75.7; 66.6 and 72.7% CR. The diameter of the POFs were still partitioned in other two categories ≤11.0mm and ≥ 11,1mm resulting in CR of 36.8% and 72.0% ($P< 0.01$) respectively. Eleven animals ovulated before TAI, with CR of 36.4%. It is concluded that the diameter of POF at TAI have positive correlation with CR; POF > 11.1mm are strong indication of greater reproductive efficiency.

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Protein profile of seminal plasma of squirrel monkeys (*Saimiri collinsi*) and capuchin monkeys (*Sapajus apella*) obtained by one-dimensional polyacrylamide gel electrophoresis: a preliminary study

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Neotropical primates *Saimiri* sp. and *Sapajus* sp. present the semen as a consistent coagulum after ejaculation. This represents one important barrier for semen cryopreservation success. Besides, the elements related to this characteristic are poorly understood in these species. The aim of this study was to separate the protein components of seminal coagula from *Saimiri collinsi* and *Sapajus apella* by one-dimensional gel electrophoresis. The experimental animals were provided by the National Primate Center (CENP, Ananindeua, PA, Brazil). Seminal samples from 9 adult males *S. collinsi* (age > 10 years) and 8 adult males *S. apella* (age > 5 years) were collected by electroejaculation. Seminal proteins extraction was performed from different seminal groups, as follow: (T1[liquid fraction of fresh semen (only *S. collinsi*)]; T2 [coagulum dissolved in Tris/NaCl (150mM Tris + 20mM NaCl) + NaOH3M]; T3 [suspension of macerated coagulum in Tris/NaCl (150mM Tris + 20 mM NaCl) at 37°C]; and T4 [T3 remaining coagulum liquefied in Tris/NaCl (150 mM Tris + 20 mM NaCl + NaOH 3M)]. Each treatment was centrifuged (1000 x *g* / 20min) and the supernatant separated. The protein concentrations of the samples were estimated by spectrophotometry (NanoDrop 2000c). A 30µg aliquot of each sample was placed separately into wells ("slots") in one-dimensional electrophoresis apparatus (Amersham ECL Gel Box). The electrophoretic run (54mA 160V 20W) in gradient polyacrylamide gel (8-16%) and the molecular weight of the proteins were determined from a standard molecular marker (12/225kDa - 250µl - GE Healthcare). The separated proteins of *S. collinsi* seminal samples showed molecular weight ranging of 76kDa to 12kDa, and of *S. apella* ranging of 12kDa to 102kDa. In both species, 76kDa protein bands were present, being the molecular weight of the protein semenogelin isoform (Sg). In humans, this protein was reported to be the most abundant component of the seminal coagulum. As described in *Macaca mulatta*, this protein forms a tangle, which retains soluble proteins. It is supposed that the low concentration of Sg facilitates the release of soluble proteins, as verified by similarities between the sequences corresponding to the bands T1 and T3 in *S. collinsi*, where semen is more fluid. The identification of proteins related to seminal coagula from *S. collinsi* and *S. apella* favors the development of appropriate extenders, making possible the use of semen in biotechnologies of reproduction, such as semen cryopreservation.

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Proteome of bovine cumulus cells as related to oocyte *in vitro* maturation

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The objective of the present study was to evaluate the proteome of bovine cumulus cells (CC) from cumulus-oocytes complexes (COCs) before and after 24 h of *in vitro* maturation. Ovaries were collected from a near abattoir and COCs were aspirated from follicles 3-8 mm of diameter. Grade I COCs were selected and two groups of 10 COCs were arranged (immature and mature). The CC from the immature group were mechanically removed and frozen in ammonium bicarbonate and immediately lyophilized for proteome analysis. The COCs from the matured group were put in TCM199 media supplemented with 10% fetal bovine serum, 0.2 mM Na pyruvate, 50 µg/ml gentamicin, 0.5 µg/ml FSH and 0.5 µg/ml LH under mineral oil and incubated in 5% CO_2 and 90% humidity for 24 h. After maturation, CC were collected and processed as described above. Samples were solubilized in 100 µl 1% triton-X 100 and sonicated at 4°C for 15 minutes. For protein electrophoresis, 30 mg of each sample were subjected to 15 % SDS-PAGE and gels were stained with Coomassie Blue G-250, scanned and analyzed with Quantity One software (Bio Rad, USA). Proteins were identified by ESI-MS/MS and Mascot software v 10.0. Protein expression difference was set at 4.0-fold. *In silico* protein interactions were investigated using Cytoscape v 3.4.0. Major proteins expressed in all CCs were 78 kDa glucose-regulated protein, alpha-2-HS-glycoprotein, alpha-enolase, beta-actin, elongation factor 1-alpha 1, endoplasmin, glyceraldehyde-3-phosphate dehydrogenase, heat shock protein HSP 90-beta, 6-phosphofructo-2-kinase, protein disulfide-isomerase A6, pyruvate kinase PKM, tubulin beta-2B chain and vimentin. Protein 78 kDa glucose-regulated protein (GRP-78) appeared more expressed in immature CC while Alpha-2-HS-glycoprotein (Fetuin-A) and vimentin (VIM) had greater expression in mature CC. As evidenced by *in silico* analysis, protein GRP-78, which plays a role in facilitating the assembly of multimeric protein complexes inside the endoplasmic reticulum, interacts with several bone morphogenetic proteins (BMP), which are multi-functional growth factors that belong to the transforming growth factor beta (TGFbeta) superfamily. Specifically within the interactions, BMP-6 seems to be a potential oocyte secreted factor, involved in the prevention of premature luteinization in cumulus cells via enhancing 17β-estradiol synthesis. Process needed during early stages of maturation, and probably regulated in its early protein assembly by GRP-78. Protein Fetuin seems to interact with bone morphogenetic protein receptor type-1 and 2, BMP and activin membrane-bound inhibitor homolog, a negatively regulator of TGF beta signaling, hemojuvelin, a BMP co-receptor, and bone morphogenetic protein 2, involved in activation of map kinase activity. Such interactions suggest the existence of a well orchestrate process involving cumulus cells proteins during early oocyte and embryo development. In addition, VIM, a class-III intermediate filament, has been reported with greater expression during *in vitro* development of bovine embryo and it is crucial for nuclear reprogramming in cloned embryos. VIM showed several interactions with proteins involved in structural oocyte and embryo formation. Identification of proteins expressed in cumulus cells will allow the search for molecular markers of oocyte quality. Moreover, this study sets the foundation for finding and understanding the proteins in cumulus cell that modulate oocyte competence and that are potentially related to early embryo development.

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Quality evaluation of cat's epididymal spermatozoa chilled to -1ºC and 4ºC

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In cryopreservation of domestic cat's semen, chilling at 4ºC apparently avoids the thermal shock and irreversible loss of sperm cells. However, studies demonstrating the behavior of feline's sperm cells at different chilling temperatures were not found in the available literature. The aim of this study was to evaluate the quality of fresh domestic cats spermatozoids obtained from epididymis tail, and compare after chilling at -1ºC and 4ºC for 24 to 48 hours. Twenty nine adult cats were used, mixed breeds, in good nutritional status, weighting 2 to 6 kg, in the period from november 2015 to february 2016. The study was conducted in two stages: in the first was used testes of 14 cats and the chilling temperature of -1ºC; the second stage was realized with 15 cats and spermatozoids were chilled at 4º C. After elective orchiectomy, spermatozoids were recovered from epididymis tail and diluted with ACP-117® medium. Spermatozoids quality was evaluated in three stages: fresh, after 24 hours and 48 hours of chilling. Spermatozoids kinetics was calculated by computer analysis (CASA, Hamilton-Thorne IVOS II®, Beverly, USA), concentration by Neubauer's chamber, morphology by Karras modified staining and membrane integrity by eosin negrosin staining. The results were analyzed using the R 3.2.5 software. The Friedman's nonparametric test was used to compare the times (fresh, 24 and 48 hours), with 5% of significance levels for all tests. There was no difference in motility when compared the two chilling temperatures (-1ºC and 4ºC). The average motility of the fresh spermatozoids, 24 and 48 hours after chilling were different even at -1ºC (67.5 ± 18.7%; 40 ± 20.9%; 29.2 ± 19.9%) as the 4ºC (70.4 ± 11.9%, 28.6 ± 21.7%; 17 ± 19.7%). However, it was visually remarkable the reduction of progressive motility in the chilling at 4°C, which was different in three stages (40.4 ± 9.4%; 12.7 ± 11.7%; 10.5 ± 6.5 %), whereas at -1°C this drop was less pronounced (27.6 ± 14.2%, 17.6% ± 13.5 and 12.2 ± 13%). In morphology, although there was no difference in the percentage of normal cells of the three times at -1º C (52.5 ± 20.3%; 42.3 ± 22.9%; 35 ± 23.3%) and at 4 °C (55.9 ± 43.1%; 43.1 ± 15.2%; 43.4 ± 18.6%), there was a significant increase in larger defects between the chilling at 24 and 48 hours - 1°C (28.4 ± 16.7%; 36.2 ± 22.9%). Nevertheless, 4° C chilling had significant increase in the sperm membrane damage (4.9 ± 3.1%; 8.6% ± 5, 12 ± 7.3%), and was not observed that damage cells when chilled at -1°C (7.1 ± 3.6%; 9.1 ± 5.4%; 10.1 ± 4.6%). The recovered spermatozoids from epididymis tails chilled at -1ºC showed acceptable results both in motility as in percentage of spermatozoids integrity. Therefore we conclude that the chilling temperature of -1 °C is effective to maintain the quality of sperm recovered from the epididymis, a new technique can be used in the cryopreservation of domestic cats.

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Rabbit fibroblast growth assessment and their interaction on scaffolds based gelatin-chitosan blended with Aloe vera and Snail mucus for skin regeneration

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Serious trauma, genetic skin disorders and chronic wounds might result in skin loss, with burns and scalds being major contributors to rapid, extensive, deep wounds with substantial areas of skin damage, frequently without the possibility of skin regeneration. Scaffold plays a critical role in the success of the living tissue reconstructs damaged by such traumas. Enhancement of cellular attachment, proliferation, and the organized development of native structures are all the attributes of a beneficial scaffold. The area of veterinary medicine, currently provides valuable tools to evaluate the performance of these materials regarding their interaction with cells responsible for healing, proliferation, epithelialization and tissue repair. Thus, this study provides a multidisciplinary work between the area of veterinary medicine and tissue engineering applied to biomedical applications, developing and in vitro testing gelatin-chitosan-based scaffolds where the porous architecture was achieved by freeze-drying. The potential for use of these scaffolds for tissue regeneration was evaluated in vitro using rabbit fibroblasts, in which the cell adhesion, proliferation, and morphology were investigated. The scaffold constructs were examined by SEM, histological analysis, and cell viability study. The SEM images showed that in general all types of scaffolds have good ability to facilitate cell growth and adhesion, forming well defined and visible structures, with populations of abundant agglomerates, which were growing and developing fibrous structures more visible towards the end of incubation period. The composite scaffolds supported fibroblast attachment and proliferation, demonstrating to be a good substrate for mammalian cell culture. A lot of fibroblasts in development was observed in the scaffold made from the mixture of Aloe vera and snail mucus. Apparently, the interaction of both components facilitates cell growth faster. Currently it began to assess histochemistry tissue and cell viability assays.

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Reproductive performance of beef heifers subjected to a timed artificial insemination protocol at 15 months versus heifers 27 months of age

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In intensive production systems (milk and meat), heifers should give birth up to 25 months of age and the delay in the occurrence of the first delivery results in economic losses. The age at first breeding of beef cattle impacts the efficiency of the production system and the earlier the mating, the greater the productivity of the cow and the herd, leading the animal to longevity in reproductive life. To reverse the low reproductive efficiency framework is necessary to reduce the age of heifers at their first farrowing. This work was carried out to demonstrate the feasibility of including heifers under 15 months of age on timed artificial insemination (TAI) protocols favoring the exploration of the reproductive life of this animal category. Ninety-two beef cross heifers (Red Angus x Nellore) were used and divided into two categories: 15 months (n=50) (N1) and over 26 months (n=52) (N2). Both groups were kept under the same nutritional management on native pastures in spring seasons, summer and early fall; in the late fall and winter months, the animals had access to cultivated oat pastures (*Avena sativa*) and ryegrass (*Lolium multiflorum*). Throughout the experiment the animals had access to water and mineral supplementation *ad libitum*. N1 and N2 underwent the same hormonal protocol for TAI (d0:US+P4 (1.9g)+BE (2.0mg); d7: PGF2α (12.5mg); d9: -P4+eCG (400IU)+ECP (1.0mg); d11: TAI; d39: Pregnancy Diagnosis; d135: Final Pregnancy Diagnosis) and were evaluated for weight, body condition score (BCS) and ovarian condition status (anestrus or cyclic). The average weight and body condition score were 261,8kg e 3,21 respectively for N1 and 366,2kg and 3,1 for N2. Pregnancy rates (PR) were 50.0% and 63.5% in N1 e N2 respectively afrter TAI, and 67.5% and 77.5% at the end of the breeding season. N1 e N2 showed statistical difference in the weight range and the ovarian condition status ($P < 0.0001$ and $P = 0.0365$) but there was no difference in pregnancy rates ($P = 0.4855$). In the N1 group there was strong positive correlation (0.85) between the weight of the animals and the BCS and between weight and pregnancy rate in TAI PR (0.70) and FINAL PR (0.70). A strong positive correlation was also found (0.70) between the weight and the cyclical condition of animals and moderate correlation found between the BCS and TAI PR (0.58) and FINAL PR (0.50). The nutrition of animals is of great importance in reproduction because weight and body condition are closely linked and are, along with the maturation of the hypothalamic axis, determinants of age at puberty. It is clear that the body condition of the animals is strongly related to ovarian activity and significant effect on pregnancy rates and fertility of animals submitted to TAI. The disadvantages of early mating, such as dystocia, can be avoided with proper handling and caring of animals, especially with nutrition and the right choice of breeder to mate with these smaller and younger heifers. The choice of heifers with better ECC and good weight (at least 60 % of adult body weight) for inclusion in TAI programs is a way to circumvent the adversities from early mating. It was concluded that the inclusion of crossed beef heifers at 15 months in the TAI protocols is viable, allowing reasonable pregnancy rates. The insertion of these young animals maximizes the reproductive potential of the animal category, generating more calves during reproductive life.

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Scrotal surface temperature before and after semen collection with digital infrared thermography in Nellore bulls (*Bos taurus indicus)*

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Reproductive and economic efficiency of extensive livestock depends on bull fertility in the field, and digital infrared thermography can help to identify thermal stress in animals in a non-invasive manner. This study aimed to investigate the surface temperature of the scrotum using digital infrared thermography before and after semen collection, and to determine the correlation of these temperatures with semen quality in Nellore bulls. Semen was collected from 80 Nellore bulls via electroejaculation, and semen samples were immediate analyzed of progressive sperm motility (M) 0–100%, mass sperm motion (MM) 0–5, and sperm vigor (VIG) 0–5. The sperm morphology (minor defects, major defects and total defects) was evaluated from 200 cells with a phase-contrast optical microscopy. Digital infrared thermograms of the scrotum were obtained, before and after collection of semen, and rectal temperature (RT) was determined. Each thermogram was processed and the following data were obtained, standardizing and adopting the following nomenclature: T1: spermatic cord temperature; T2: temperature of the dorsal third of the testicles; T3: temperature of the medial third of the testicles; T4: temperature of the ventral third of the testicles, and T5: temperature of the epididymal tails. Between the anatomical points analyzed (T1 to T5) there were significantly difference ($P<0.05$), where the means from T1 to T5 before electroejaculation were: T1 (36.91±1.56), T2 (35.70 ±1.54), T3 (34.74 ±1.57), T4 (33.87 ±1.64) e T5 (32.87±1.88). And the means from T1 to T5 after electroejaculation were: T1 (36.72±1.70), T2 (35.65±1.57), T3 (34.56±1.47), T4 (33.61±1.52) e T5 (32.77±1.57). There was no significantly difference ($P>0.05$) when compared the moments before and after electroejaculation, for the temperatures in each anatomic point. There was a correlation between RT × T1 ($r=0.51$; $P<0.05$); RT × T2 ($r=0.43$; $P<0.05$); RT × T3 ($r=0.37$; $P<0.05$); RT × T4 ($r=0.33$; $P<0.05$) and RT × T5 ($r=0.32$; $P<0.05$). There were correlations between T2 × total defects ($r=0.30$; $P<0.05$); T3 × minor defects ($r=0.35$; $P<0.05$); T3 × major defects ($r=0.30$; $P<0.05$) and T3 × total defects ($r=0.42$; $P<0.05$); T4 × minor defects ($r=0.30$; $P<0.05$), T4 × major defects ($r=0.28$; $P<0.05$) and T4 × total defects ($r=0.37$; $P<0.05$); T5 × major defects ($r=0.29$; $P<0.05$) and T5 × total defects ($r=0.28$; $P<0.05$). With these results we can see that the temperatures in the anatomical points of the scrotum will decrease in the dorsal-ventral axis, which indicates the adequate testicular thermoregulation, with difference of 4.04ºC before and 3.95ºC after electroejaculation. Significant positive correlations indicate that when there is an increase in rectal temperature, there is a rise in temperature of the scrotum. In the same way, the temperature of the scrotum when appears increased, there is an increase in minor, major and total defects, indicated by significant positive correlations. In conclusion, the digital infrared thermography, both before and after electroejaculation, provided accurate measurements of scrotal surface temperature and, as such, generated complementary information for seminal evaluation. Therefore, this technique is recommended for screening to detect adequate scrotal thermoregulation in the selection of breeding bulls. (CEUA/UNOESTE/Protocol#1920/2013)

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Semen collection by transrectal ultrasound-guided massage of the accessory sex glands is less stressful than electroejaculation in non-anaesthetised goat bucks

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Electroejaculation (EE) is a technique used for semen collection in small ruminants that were no trained to use an artificial vagina or that are still prepuberal, or during the non-breeding season. Although it is easy to apply, it induces pain and stress. Transrectal ultrasound-guided massage of the accessory sex glands (TUMASG) is an alternative technique for semen collection in small ruminants that occasionally require some electric pulses to collect sperm. Although to perform TUMASG procedures technicians need to be trained, it provokes less stress responses than EE in anaesthetised ruminants, however some sperm characteristics are better when semen is collected with EE. Moreover, TUMASG has not been tested yet without general anaesthesia. Thus, the aim of the present study was to compare the stress response and sperm characteristics of the semen collected with TUMASG or EE in non-anesthetized goat bucks. The semen was collected by TUMASG or EE in 10 conscious Gabon bucks. First, each procedure was applied in 5 animals, and one week later the other procedure was applied in the other 5 animals (total = 10 collections/technique from the same 10 animals). The length time and the number of electric pulses applied were recorded during each procedure. Cortisol concentration, glycaemia, concentration of creatine kinase, total protein, albumin and globulin, as well as white blood cell number (WBC) were measured before and after of each procedure. Besides, the sperm parameters (volume, sperm concentration, sperm mass motility (0-5), percentages of sperm motility, sperm progressive motility, sperm viability, acrosome integrity and normal morphology) were determined. More electric pulses were needed with EE than with TUMASG (23.4 ± 2.1 vs 1.2 ± 0.1, respectively; $P < 0.0001$), but TUMASG took more time than EE (487.5 ± 37.5 s vs 109.1 ± 12.3 s, respectively; $P < 0.0001$). Cortisol concentration was greater with EE than TUMASG (25.9 ± 1.2 vs 22.0 ± 1.2 nmol/L, respectively; $P = 0.04$). Concentration of creatine kinase, total protein, albumin and globulin increased similarly after both procedures (general means before: 78.4 ± 10.4 U/L; 88.8 ± 1.6 g/L; 26.5 ± 1.2 g/L; 61.9 ± 1.7 g/L; respectively; after: 82.9 ± 10.3 U/L; 92.3 ± 1.6 g/L; 28.1 ± 1.2 g/L; 63.5 ± 1.7 g/L; respectively). Glycaemia (general mean: 71.5 ± 1.6 mg/dL) and WBC (general mean: 8.9 ± 0.8 10^9/L) were not affected by the procedures. None of the sperm parameters were affected by TUMASG or EE. We concluded that although TUMASG took more time than EE, the former is less stressful, as it induced lower secretion of cortisol and needed less electric pulses than EE. In summary, TUMASG did not affect the sperm characteristic and provoked less negative effects on animal welfare than EE, thus it could be used to collect semen in non-anaesthetised small ruminants.

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Setting time of artificial insemination of superovulated lactating Holstein cows with sex-sorted sperm

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The results of the combination of sex-sorted sperm and superovulation (SOV) have not been very encouraging so far, due to the low quantity of sperms as well as their short life. In a previous study conducted in Brazil in Holstein cows, it was shown that using the protocol of SOV P36/Lh60 and inseminating with sex-sorted sperm at the 18 and 30 hours (interval of 12h between the two inseminations) after application of the inductor of the ovulationit is obtained an equal quantity of transferable structures than inseminating at the 12 and 24h with non-sorted sperm. The aim of this investigation was to adjust the hours of the artificial insemination (AI) in superovulated and inseminated cows with sex-sorted sperm to achieve better synchrony with ovulations, so in this way to increase the quantity of transferable embryosobtained. This adjustment evaluated 2 moments of AI in which the interval between both diminished to 6h. For this, 30 lactating Holstein cows previously superovulated once or twice were newly treated with the protocol P36/LH60 and, at insemination, divided at random in three groups: in the Control group (CG: IA18/30; n=10) the inseminations were carried out at the 18 and 30 h after application of the inductor of the ovulation (GnRH); in group 1 (G1: IA18/24; n=10) inseminations were made at 18 and 24 h after GnRH and in group 2 (G2: IA24/30; n=10) at 24 and 30 h. In all cases, each insemination was doing with 2.1 x 10^6 sex-sorted sperm. The results between groups were no different in the quantity of transferable embryos (1,9±0,6; 4,1±1,5; 1,3±0,4 respectively for groups Control, G1 and G2; p>0,05). In conclusion, the low number of cows in each group and the high variability of responses do not permit to conclude on real differences with some of alternatives evaluated. In this way, our proposal produced at least similar results to previous works. However, the numerical results lead to propose new studies to increase the number of animals treated and so, reevaluate these results.

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Software efficiency for counting bovine spermatic cells: preliminary results

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This study aimed to develop a software for bovine sperm cell count, from images taken under a microscopy. This program should be compatible with notebooks and PCs for home use, easy to use, in the Portuguese language and free. It is intended, with this software, standardize the sperm concentration analysis carried out by veterinarians during andrological examinations at field, cheapening the cost of acquisition of specific equipment. Twenty images of bovine semen were made by microscopy from thawed commercial semen doses. The 0.5 mL reed were thawed for 30 seconds in a water bath at 37 ° C. Semen drops were deposited on slides and Neubauer chamber both covered with cover slip for microscopy. The images were obtained from phase contrast microscope (Jenamed2) with 1.3 MP camera attached (Coleman). The software was developed from resources already available in an open source Java solution called ImageJ. The approximate count of the sperm contained in the image was possible through particle analysis capabilities. Initially, the video images were converted into frames and subjected to some treatments, using only 8-bit color and segmenting grayscale so that the software could do the analysis of the image particles. The 20 semen samples were analyzed by the technician in Neubauer chamber and by the software for spermatozoa count (from microscope image video). For statistical analysis, the results of the counts were subjected to analysis of variance (SAS, 2012) at a significance level of 5%. The average values of the sperm cell counts did not differ and were 145.10 ± 101.28 and 186.15 ± 107.41 by the technician and by the software, respectively ($p > 0.05$). The software was highly efficient for sperm cell count, being a convenient and easy to use solution. The CASA instruments have shown high levels of accuracy and reliability using different methodologies of classification that provide a great tool to improve our knowledge and ability to analyze sperm, making it essential to research, personnel training and standardization between laboratories. Regardless of the manufacturer, the different instruments are based on similar principles, but differ in terms of optics and software used to identify the sperm and the construction of the track, respectively. Our differential is the gratuity and ease of use, since it is a specific software for sperm analysis and available in Portuguese. In conclusion, the developed software showed the same efficiency as the count carried out by the technician.

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Software efficiency for counting buffalo spermatic cells: preliminary results

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The fertility varies among the buffalo bulls, same when these possess the minimum seminal qualities. In this context several laboratory methods for assessment of the semen have been used. These together could help to predict the fertility potential. Usually, sperm motility as well as concentration and morphology are estimated by the technician, what makes the results highly variable and inaccurate. The aim of this study was to develop a new software, compatible with notebooks and PCs, easily manipulated, in the Portuguese language and free. It is intended, with this software, standardize the sperm concentration analysis carried out by veterinarians during andrological examinations at field, cheapening the cost of acquisition of specific equipment. This program could help to eliminate de subjectivity of the results, turning then more accurate and reliable. Twenty two images of buffalo semen were made by microscopy from thawed semen doses. The 0.5 mL reed were thawed for 30 seconds in a water bath at 37 ° C. Semen drops were analyzed by CASA (Hamilton Thorne Research) and deposited on slides and Neubauer chamber both covered with cover slip for microscopy. The images were obtained from phase contrast microscope (Jenamed2) with 1.3 MP camera attached (Coleman). The software was developed from resources already available in an open source Java solution called ImageJ. The approximate count of the sperm contained in the image was possible through particle analysis capabilities. Initially, the video images were converted into frames and subjected to some treatments, using only 8-bit color and segmenting grayscale so that the software could do the analysis of the image particles. The 22 semen samples were analyzed by CASA, by the technician in Neubauer chamber and by the software for spermatozoa count. For statistical analysis, the results of the counts were subjected to analysis of variance (SAS, 2012) at a significance level of 5%. The values of the sperm cell count did not differ between groups ($p > 0.05$) and averages were 136.00 ± 68.19, 111.00 ± 67.90 and 120.00 ± 100.83 by CASA, by the technician and by the software, respectively. The software was highly efficient for sperm cell count, being a convenient and easy to use solution. The CASA instruments have shown high levels of accuracy and reliability using different methodologies of classification that provide a great tool to improve our knowledge and ability to analyze sperm, making it essential to research, personnel training and standardization between laboratories. Regardless of the manufacturer, the different instruments are based on similar principles, but differ in terms of optics and software used to identify the sperm and the construction of the track, respectively. Our differential is the gratuity and ease of use, since it is a specific software for sperm analysis and available in Portuguese. In conclusion, the developed software showed the same efficiency as the count carried out by CASA e by the technician.

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Sperm quality parameters of cryopreserved bovine semen and its relationship to pregnancy rate in timed-AI program

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Semen cryopreservation results in damage to sperm structure, and it is important to evaluate the semen batches before being submitted to timed artificial insemination (TAI). However, conventional semen evaluations are not always sufficient to identify batches that may result in lower pregnancy rate in the field, requiring a more thorough and accurate investigation. The aim of this study was to evaluate the relationship of bovine sperm quality parameters on pregnancy rate in a timed-AI program. Six frozen semen samples that were used in a program of artificial insemination were evaluated and classified in high fertility (HF, ≥ 55% pregnancy rate) and medium fertility (MF, < 55% pregnancy rate). Fertility data were obtained from Nellore cows (n = 654) in the same timed-AI program with a minimum of 100 inseminations per batch. Pregnancy diagnosis was made with a B-mode ultrassound (Aloka SSD 500), 30-45 days after insemination. Two semen straws from the every batch were thawed at 37ºC for 30 seconds, homogenized and immediately analyzed by the conventional analysis (motility, vigor, concentration and morphology). Semen was diluted in TALP medium to adjust sperm concentration to 10×10^6 sperm/mL, then analyzed for: total motility (TM), progressive motility (PM), average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN) and rapidly moving cells (RAPID) by the Sperm Class Analyzer software (SCA, Microptics, Barcelona, Spain). The percentage of cells that presented intact plasma membrane (IPM), intact acrosome membrane (IAM), high mitochondrial potential (HMP) and these characteristics simultaneously (designated IPIAH) were evaluated using fluorescent probes; for this sperm were suspended in TALP medium (150 µL) and incubated with 2 µL of Hoescht 33342 (0.5 mg/mL, Life Technologies), 2 µL of propidium iodide (0.5 mg/mL, Sigma), 20 µL of fluorescein isothiocyanate-labeled *Pisum sativum* agglutinin (FITC-PSA, 100 µg/mL, Sigma), and 6 µL of 5,5′,6,6′tetracloro 1,1′,3,3′tetraetilbenzimidazolil carbocianin iodide (JC-1, 153 µM, Life Technologies) per 8 min at 37°C in the dark. The reading was performed with an epifluorescent microscope (80i model, Nikon, Tokyo, Japan) at a magnification of × 1000, using a triple filter (D/F/R, C58420). Data were analyzed with the R software (version 3.3.1.) by analysis of variance. The pregnancy rates for HF and MF were 67.0% and 53.3%, respectively ($p<0.05$). The vigor was higher for MF ($p<0.05$) than HF. The number of sperm cell showing motility in straw for HF (25.6±6.6 sperm/straws) was higher ($p<0.05$) than MF (18.5±2.4 sperm/straws). Abnormal sperm was higher ($p<0.05$) for HF (29.2±5.77%) than MF (11.2±3.8%). While the TM and PM, LIN, STR were higher in MF ($p<0.05$), the other parameters VCL, VSL, VAP, ALH and BCF were higher in HF ($p<0.05$). Despite the differences between sperm conventional analysis for HF and MF groups, all the values are within the acceptable standard to maintain the fertility. Regarding the percentage of cells IPIAH for HF (49.7±7.1%) and MF (28.2±5.8%) significant differences ($p<0.05$) were observed. The percentage of cells IPM and IAM showed no significant differences ($p>0.05$). Interestingly, it was detected that the HF group (55.3±6.7%) exhibited higher APM ($p<0.05$) than MF group (33.03±5.3%). We concluded that the routine semen evaluation are useful in eliminating samples of very poor quality, but others characteristics are also associated with the fertilizing capacity of the spermatozoa. The method of semen analyses by fluorescent probes is more accurate and reliable to detect of real semen fertilization capacity in cryopreserved bovine semen than conventional analysis. Moreover, the relationship between field fertility and mitochondrial activity could be an interesting tool for predicting the fertility.

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The effect of different extenders on bovine epididymal spermatozoa submitted to 5°C for 48 hours

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Post-mortem spermatozoa recovery is an important technique for obtaining germplasm reserves from genetically valuable animals or endangered species. At the same time, cryopreservation can cause changes in spermatozoa cell morphology and reduce fertilization rate. Extenders commonly used in cryopreservation presents substances that allow the motility preservation and plasma membrane integrity, with varying protective effect depending on the animal species. Thus, the aim of this study was to evaluate the effect of different extenders on the bovine sperm cell obtained directly from the epididymis and subjected to cooling at 5°C during 48 hours. Twenty-six testis/epididymis complexes from crossbreed cattle were obtained from slaughterhouses. Spermatozoa cell were recovered by the flotation technique. To avoid variation in sperm parameters due to individual variation, pools of five animals per biological replicate were prepared to a final concentration of 80 x 10^6 spermatozoa/mL. Three extenders were prepared for each group: G1 group (Tris-egg yolk - classic extender); G2 group (Skimmed milk Molico® inactivated: 10g skimmed milk powder; 196mg glucose; 100mL of bidistilled water; solution incubated at 90 °C for 10 minutes, pH 6,5); and G3 group (Skimmed milk Molico® not inactivated: 10g skimmed milk powder; 196mg glucose, 100mL bidistilled water; pH 6.5). The samples were subjected to evaluations of subjective motility, integrity of plasma membrane and functionality of the plasma membrane after 0h (T0), 24h (T24) and 48h (T48) of incubation at 5 ºC. Six replicates were performed for each treatment group. ANOVA followed by Tukey's post-hoc test was performed to compare differences in the variables with respect to incubation time and extenders, with a significance level of 0.05. The alternative extenders were not capable of improving sperm cell parameters compared to the classical extender ($p<0.05$) as observed by better motility in G1 (T0: G1= 70,64±17,37^a; G2= 57,66±19,68^a; G3= 21,98±13,24^b; T24: G1= 60,66±19,43^a; G2= 31,44±5,58^{ab}; G3= 15,32±11,14^b; T48: G1= 48,62±9,72^a; G2= 33,84±2,45^{ab}; G3= 9,70±4,91^b). However, the inactivation of the alternative extender (G2) resulted in better sperm motility compared to the activated alternative extender (G3) ($p<0.05$). The egg yolk low-density fraction, mainly composed of low-density lipoprotein (LDL), is responsible for thermal shock resistance by delaying premature sperm capacitation caused by cryopreservation; the lactenin is a nitrogenous substance present in milk and presents spermicidal property that can explain the lower motility in G3. On the other hand, the lactenin is inactivated by temperature above 80 °C, as observed in G2, thus the heat released sulphydric lactoglobulins that form lactenin chelates and remove this toxic factor. Other explanation to the intermediate values of G2 compared to G1 can be due to the absence of buffer solution in the skimmed milk extender, because the epididymal sperm cells has inactive motility in acid pH, as observed in these groups. In conclusion, the Tris egg-yolk extender promotes better bovine epididymal spermatozoa motility subjected to cooling at 5 °C during 48 hours.

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The importance of inseminator in conception rate of FTAI programs in beef cattle

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Obtaining a good reproductive efficiency depends on new technologies such as artificial insemination, ovulation and estrus synchronization. Thus, the beef cattle can reach high levels of quality and productivity. This study aims estimated the effect of inseminator training on the outcome of the FTAI protocol in crossed Nelore cows. The animals (n = 40) used in this experiment had a body score of 2.5 to 3.5 (0-5) and no significant changes in the previous gynecological examination. Cows had the estrus cycle and ovulation synchronized by OvSynch method, which was first administration (D0) of 0.01 mg of buserelin acetate (Sincroforte®, Ouro Fino, Cravinhos, SP, Brazil), IM, in D7 application 0.25 mg of cloprostenol (Sincroforte®, Ouro Fino, Cravinhos, SP, Brazil), IM and a second application of buserelin, IM, in D9. At the time of FTAI held 16 - 18 hours after the second application of GnRH analogue using proven semen of a Nelore bull, the animals were divided into two groups, and in G1 (n=20) students of a course of artificial insemination were the inseminating, had no previous experience in cervical transposition procedure cows with semen universal applicator; G2 (n=20) experienced inseminating be used. The pregnancy diagnosis was performed by ultrasound 45 days after insemination with linear transducer of 7.5 MHz, B-mode, rectally, according to standardized procedure for cattle. Conception rates were measured in response to the FTAI. Data were analyzed by Chi-Square test, with 5% significance level. The conception rate in G1 was 25^{b}% (5/20) and G2 65^{a}% (13/20). There was a significant difference being the two study groups ($p < 0.05$). These data indicate the need for a qualified inseminator to that the technique results in effective pregnancy rate, and therefore, the absence of professional ability that can become a limiting factor in obtaining satisfactory results of conception.

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Treatment with cholesterol loaded cyclodextrin improves semen characteristics in rams, especially low freezeability semen, but does not increase pregnancy rates

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Despite scientific and technological advances, cryopreservation of semen still faces major obstacles, such as loss of post-thaw sperm viability due to changes in osmolarity and conformation that the sperm suffers. Concern about the quality of frozen ram semen has been the subject of extensive research, particularly through the high cryocapacitation rates of cryopreserved sperm, which results in reduced fertility when used in artificial insemination. Cholesterol loaded cyclodextrin has been widely used in various animal species, in order to increase the level of preservation of sperm membranes and motility and, therefore, fertility rates. Thus, this study aimed to analyze whether the cholesterol incorporated by cyclodextrin improves the semen quality after thawing and if it increases pregnancy rates. For this purpose we used 30 ejaculates from 6 White Dorper rams aged from 2 to 4 years, and 12 Santa Inês sheeps for inseminations. The semen collection was done through artificial vagina in conical tubes (37^{o}C). Fresh and thawed semen were evaluated for: motility parameters (CASA), sperm morphology (DIC), integrity of plasma (PI+H342) and acrosomal (FITC-PSA) membranes, mitochondrial potential (JC-1), production of free radicals (CellRox), lipid peroxidation (BODIPY) and cell membrane permeability (Merocyanine 540). Semen was divided into two aliquots, prior to cryopreservation, in which were added: only an egg yolk based extender (control - CON) to the first aliquot; and egg yolk extender with cholesterol loaded cyclodextrin (CHO-CLC) to the second aliquot. The cyclodextrin was prepared in the laboratory at a concentration of 2 mg of cyclodextrin/120 x 10^6 sperm. Samples were divided by freezeability based on the total post-thaw sperm motility and the percentage of reduction in total motility when compared fresh to post-thaw semen (only extender), being then divided into the high, intermediate and low freezeability groups. The inseminations of the sheeps were made by laparoscopy, using two semen straws from the same batch. Sheeps were divided in a crossover model. Pregnancy diagnosis were performed 30 and 45 days after. Data were analyzed using PROC MIXED procedure of SAS software. Means were separated by the Tukey test ($p < 0.05$) or non-parametric statistics (Kruskal-Wallis), when necessary. For analysis of pregnancy data was employed the GLIMMIX SAS PROC. The significance level was 5%. The CHO+CLC treatment proved to be more effective than CON treatments in preserving the parameters of motility (65.49±3.40 against 49.02±4.22%), plasma membrane integrity (26.25±2.42 against 11.97±1.42%), acrosome integrity (64.57±2.61 against 39.27±2.72%) and mitochondrial potential (50.83±4.54 against 33.97±3.70%). Concerning the freezeability, data showed that the treatment group had higher values of sperm preservation when compared with the control group in the low freezeability ejaculates. This was observed in the total (low = 14.47±2.84 vs. 47.01±6.54%) and progressive (low = 5.53±1.11 vs. 23.15±5.40%) motility, rapid cells (low = 5.06±1.06 vs. 23.07±6.40%), plasma (low = 6.23±2.78 vs. 14.92±4.26%) and acrosomal (low = 29.23±6.89 vs. 50.47±4.16%) membranes integrity and in the mitochondrial potential (low = 21.87±6.71 vs. 30.73±8.58%). There was no statistical difference ($p = 0.57$) in pregnancy rates between CHO-CLC (33.33%) and CON (45.45%) groups. Similarly when semen were divide by freezeability, low freezeability group showed no statistical difference ($p = 0.64$) to pregnancy rates between CHO-CLC (37.50%) and CON (50%) groups. Thus, we could conclude that treatment with cholesterol loaded cyclodextrin improves the frozen semen characteristics of rams, especially in low freezeability animals, however does not improve the fertility rates.

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Using smartphone camera for software to count bovine spermatic cells: preliminary results

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The use of technology can improve the efficiency of the examinations and analysis process. The Computer-Assisted Sperm Analysis (CASA) brings accuracy to the sperm analysis. But its use is limited due to high costs. The development of a CASA system has two key points: the image capture method and the software. Today, there are various types of camera, from specialized cameras for analyses and tests to smartphone cameras. This study aimed to evaluate a software for bovine sperm cell count, from images taken by smartphone camera under microscopy. Twenty images of bovine semen were made by microscopy from thawed commercial semen doses. The 0.5 mL reed were thawed for 30 seconds in a water bath at 37°C. Semen drops were deposited on slides covered with cover slip for microscopy. The 20 seconds videos were obtained from microscope with a smartphone camera attached on ocular. At 10 seconds of the video, a photo was taken, which was made sperm cell count. The 20 semen samples were analyzed by the technician and by the software for spermatozoa count. For statistical analysis, the results of the counts were subjected to analysis of variance (SAS, 2012) at a significance level of 5%. The average values of the sperm cell counts did not differ and were 407.80 ± 307.21 and 458.80 ± 341.66 by the technician and by the software, respectively ($P > 0.05$). Images were clear and high definition. The smartphone enabled an image with great all image field of the microscope. In relation to the software, it was highly efficient for sperm cell count, being a convenient and easy to use solution. In conclusion, smartphone can be used to records images on microscope for bovine sperm cell count.

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Viability of Soy Lecithin base extender to cooling canine semen

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The high demand for transport of viable cooled semen has increased the number of research in the area. The aim of this study was to compare the efficacy of chilled extenders based on TRIS/egg yolk (G1) and milk powder (G2) versus the soy lecithin (G3) for seven days after semen collection. Were 21 ejaculates (three Border Collies), within the required seminal standards, with average of three years old. After previously collected and examined under optical microscopy (motility, strength, membrane integrity and pathologies), the ejaculate was split into three equal aliquots and each was added, gently homogenizing, the extenders in a proportion of 1/1vol. The samples were stored at 4-5°C throughout the period, and resuspended a daily volume of 75µl per sample and heating in a water bath at 37°C for analysis mentioned above. We used the Tukey or Kruska-Wallis tests to differentiate on treatments. The motility on day 1 was difference ($P<0.05$) in G1 versus the other groups (83.33±5.67% vs 71.43±11.53% and 67.14±15.21% for G1, G2 and G3 respectively). However until the day 4, all groups showed motility above 50% (overall mean: 60.00±18.58%) and fall below 3 to strength in the same day (1-5 scale; overall average: 2.82 ± 0.83). For the sperm pathologies, there was no difference ($P>0.05$) between groups and evaluation days (overall mean: 9.39±17.75% for major and 4.94±2.82% for smaller pathologies; on day 7). Membrane integrity were different ($P<0.05$) in the first day to G1 (93.33±3.11%) versus G2 (85.90±12.82%) and G3 (89.52±9.03), however the parameter remained above 80% until the last day, with G1 (86.40±6.41%) versus G2 (80.07±13.21%) and G3 (81.43±16.81%, $P<0.05$), demonstrating that there was no marked decrease of membrane integrity in all three experimental groups. In conclusion, all the products were effective in maintaining sperm chilled quality until the fourth day of refrigeration.

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Vitrification and *in vitro* culture of domestic cat ovarian tissue: effect of open and closed systems on the follicular funcioning and gene modulation

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Improving vitrification protocols is necessary to apply this technique in wild felines. The aim of the present study was to develop a vitrification protocol for the ovarian tissue from domestic cats as an experimental model, applying the techniques of Solid-Surface Vitrification (SSV – open system) and Ovarian Tissue Cryossystem (OTC – closed system), using ethylene glycol (EG) alone or combined with dymethilsulfoxide (DMSO). Each ovarian pair from five queens was divided into 20 fragments and distributed within the treatments. As controls (fresh and *in vitro* cultured -IVC), four fragments were used: one piece was fixed for routine histology, AgNOR and Ki-67 labelling, and a second one submitted to qRT-PCR. The other two fragments were sent to IVC for seven days. The remaining 16 fragments (four per treatment) were submitted to different vitrification protocols (SSV or OTC) following a two-step concept where the first exposure was performed for 4 min in a vitrification solution 1 (VS1) and the second one for 1 min in a VS2, and all solutions added by 0.1 M trehalose: T1 in SSV: VS1 (20% EG) and VS2 (40% EG); T2 in SSV: VS1 (10% EG + 10% DMSO) and VS2 (20% EG + 20% DMSO); T3 in OTC: VS1 (20% EG) and VS2 (40% EG); T4 in OTC: VS1 (10% EG + 10% DMSO) and VS2 (20% EG + 20% DMSO). After warming, two fragments were processed for histology and for qRT-PCR, while other two were sent to IVC for seven days and subsequently processed for histology and qRT-PCR. Although vitrification with OTC using EG alone presented the highest percentage of normal follicles (56%), no differences among treatments and with control were observed. IVC of vitrified ovarian tissue resulted in a significant decrease in the percentages of normal preantral follicles. Based on Ki67 labelling, it was observed that the percentages of activated follicles were significantly increased when comparing fresh IVC with control tissue. Tissue vitrified in OTC system, showed activation rate significantly higher than that observed for fresh IVC. Independently from non-vitrified or vitrified tissue, cryoprotectant solution and vitrification method, there was a significant increased number of NOR in granulosa cells from primary follicles. Vitrification of ovarian tissue with EG as single intracellular cryoprotectant, especially when applying SSV method, leads to AQP9 down-regulation Immediately after vitrification and cryoprotectant removal, the relative mRNA expression was significantly downregulated for AQP3 (2.5 fold), AQP9 (3.5 fold), and ABCB1 (2.5 fold), only when applying the SSV method and when EG was used as single intracellular cryoprotectant. Only those SSV-EG vitrified-IVC tissue presented up-regulation of ERP29 (2.5 fold) together with the down-regulation of AQP9 (6.7 fold). Expression of AQP3 returned to control levels. Vitrification of feline ovarian tissue can be optimized by using EG combined or not with DMSO. A closed system will not decrease the rates of follicular survival when compared with an open one, but a specific IVC protocol is needed to avoid massive follicular activation.

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Vitrification of Spix's yellow-toothed cavies (*Galea spixii* Wagler, 1831) testicular tissue – Preliminary results

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The Spix's yellow-toothed cavy (*Galea spixii* Wagler, 1831) is a wild rodent that easily adapts to captivity breeding. The species presents a short gestational period and requires low maintenance cost, which makes it a viable alternative as a research model for other hystricognath rodents, especially the endangered ones as the *Galea monasteriensis*. Testicular tissue cryopreservation consists on a practical technique that enables the recovery of genetic potential of valuable animals. The use of this technique, however, is still a challenge since many different cell types are involved in the male gonadal tissue. The objective of this study was stablish a protocol for vitrification of cavies' testicular tissue at comparing the effect of different cryoprotectants on seminiferous tubule morphology. Four adult males were used for the experiment. Their testicles were excised after euthanasia, washed in 70% alcohol, and stored in a Minimum Essential Medium (MEM) for immediate transportation to the laboratory. Testicular parenchyma was sliced into 3 x 3 x 3 mm fragments, with two fresh fragments being readily fixed for histology as the control group. The other fragments were immersed in a MEM-based vitrification solution with fetal bovine serum added of 0.25M sucrose. Samples were divided in four aliquots that were added of dimethyl-sulfoxide (DMSO) or ethylene glycol (EG) at 3M or 6M concentrations. After equilibration during 5 min, testicular tissue was cryopreserved using a solid-surface vitrification (SSV) technique, followed by storage in liquid nitrogen. After approximately two weeks, samples were thawed at 37°C, following the washing in growing concentrations of sucrose for cryoprotectants removal. Tissue was then prepared through histological procedures and the morphology of 30 seminiferous tubules was evaluated per treatment. Nuclei of intratubular cells (spermatogonia and Sertoli cells) were scored as follows: a total absence of nuclei alteration was scored as 0 and the worst score for nuclei morphology was 5. The epithelium (constituted by intratubular cells) was scored from 0 to 5 according to the status of the basement membrane and the presence of gap formation and shrinkage. Data was expressed as means and standard error. Comparison among cryoprotectants at different concentrations was conducted by the Mann-Whitney test ($P < 0.05$). As expected, the control group provided the lowest ($P < 0.05$) scores of 0.6 ± 0.1 for nuclei and 0.9 ± 0.1 for epithelium, highlighting the integrity of seminiferous tubules in fresh testicular tissue. After vitrification and thawing, an increase in the scores for nuclei and epithelium analysis was verified for all the treatments ($P < 0.05$). However, both DMSO and EG at 3 M concentration were more efficient in preserving the nucleus of germ and Sertoli cells, providing scores of 1.0 ± 0.2 and 1.3 ± 0.1 for nuclei analysis ($P < 0.05$), than the same cryoprotectants at 6M concentration that provided scores of $1.8 \pm 0.1$and 1.8 ± 0.4, respectively. Regarding, epithelium analysis, no differences were found among treatments. Scores of 2.3 ± 0.4 for EG 3M, 1.9 ± 0.2 for EG 6M, 2.2 ± 0.3 for DMSO 3M and 2.0 ± 0.1 for DMSO 6M were found. In general, the main alterations found in vitrified testicular tissue were cells detached from the tubular basal membrane and the presence of vacuoles into some cells. It is necessary to emphasize that values of scores found after vitrification of cavies' testicular tissue are similar to that previously reported for other mammal species, thus highlighting the efficiency of the technique. In conclusion, we demonstrated that cavies' testicular tissue could be efficiently cryopreserved at using a solid surface vitrification technique, and we suggest the use of DMSO or EG at a 3M concentration as cryoprotectants. These preliminary data evidenced the applicability of the technique for wild rodents, but further studies focused on the capability of the vitrified tissue in resume the spermatogenesis remains to be conducted. Moreover, we highlight the importance of this technique as a potential tool for the conservation of genetic material derived from valuable males of any species.

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Xenotransplantation of agouti *(Dasyprocta leporina)* ovarian tissue to C57BL/6 Black SCID mice

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The *in vivo* culture of ovarian tissue through xenotransplantation technique is able to promote growth and maturation of oocytes as well as ensuring the preservation, multiplication and conservation of valuable animal germplasm. In this sense, studies using the agouti *Dasyprocta leporine* as experimental models would serve as an initial step for the extrapolation of technique for other endangered hystricognath rodents, as the *D. ruatanica*. This study aims to evaluate the development of agouti ovarian tissue after xenotransplantation to C57BL/6 Black SCID mice. For this purpose, ovaries were obtained from 5 female agoutis and washed in alcohol 70%. The gonads were immediately stored in minimal essential medium plus penicillin and streptomycin for transportation to the laboratory. Each ovarian pair was divided into sixteen fragments, from which one was immediately fixed for histology, constituting the control group. The remaining fragments were randomly destined to the xenotransplantation trial. Five SCID female mice were used as recipients, being initially submitted to the bilateral ovariectomy in order to remove their endogenous hormonal influence. At the same occasion, five agoutis' ovarian fragments (1mm³~) were implanted in the kidney capsule of each recipient. After five days, estrus cycle onset of the recipients was monitored through vaginal wash, that was conducted every 12 h during 40 days. Then, the grafts were removed and submitted to histological procedures for morphological evaluation of ovarian structures The percentage of morphologically normal follicles was compared among groups by Fisher PLSD test ($P<0.05$). Data regarding morphology of the transplants and vaginal washing were expressed through descriptive analysis. Through vaginal washing monitoring, we verified that 80% of the recipient mice (4/5) have returned to ovarian activity at 20.6 ± 8.6 days after surgery, represented as changes in the proportion of cells derived from the vaginal epithelium. These findings prove that the transplanted fragments formed vascularized tissue able to produce hormones in amounts enough to influence changes on the mice vaginal epithelium. In addition, grafts from all recipients were recovered and evaluated. Histological analysis confirmed that grafts presented a parenchyma region compatible to ovarian tissue. In 60% (3/5) recipients, we confirmed the neovascularization due the presence of blood vessels connected to the graft. In two individuals (40%), corpora lutea were identified, possibly indicating the occurrence of ovulation. Regarding the proportion of morphologically normal ovarian follicles, no differences were observed between control (86.7±7.0%) and xenografted group (84.1±3.7%). To the best of our knowledge, this is the first study to demonstrate the xenotransplantation of agouti ovarian tissue to recipient mice. We believe that this promising technique represents a valuable contribution for female germplasm conservation as for agoutis as for other valuable wild rodents.

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Comparison of different selection techniques on sperm capacitation and plasma membrane integrity of ram frozen–thawed sperm

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The success of *in vitro* fertilization and the development of embryos are directly related to sperm selection and quality. This study aimed to compare the effect of different sperm selection techniques on capacitation status and plasma membrane (PM) integrity of ram frozen-thawed sperm. A pool of frozen-thawed sperm from 10 Santa Inês rams was used. The samples were submitted to one of the following techniques: sperm washing, Percoll gradient, mini-Percoll gradient, Swim-up and a control group. At the end of each selection technique, the PM integrity and capacitation status were assessed. The PM integrity was evaluated using acridine orange-propidium iodide combination by computer-assisted sperm analysis with the SCA® system (Sperm Class Analyzer – Microptic Automatic Diagnostic Systems, Barcelona, Spain). Capacitation status was evaluated using chlortetracycline staining and observed under epifluorescence microscopy. Data were analyzed by ANOVA, followed by Tukey test ($P < 0.05$). When analyzing the PM integrity, Swim-up presented greater values ($P < 0.05$) for intact cells (32.1 ± 20.3%) than all treatments, that were not different ($P > 0.05$) among each other (control group: 16.2 ± 17.4; sperm washing: 9.4 ± 10.2; Percoll: 18.1 ± 18.0; mini-Percoll: 10.5 ± 10.1%). Regarding to capacitation status, there was no difference ($P > 0.05$) for noncapacitated cells among all treatments. The capacitated rate was higher ($P < 0.05$) after control group (48.3 ± 10.2%) and sperm washing (47.1 ± 8.4%) than Percoll (34.3 ± 12.9%), mini-Percoll (32.1 ± 10.8%) and Swim-up (30.2 ± 12.2%). On the other hand, Swim-up obtained higher ($P < 0.05$) acrosome reacted cell rates (60.8 ± 14.8%) than the other treatments. Percoll and mini-Percoll were similar ($P > 0.05$; 55.3 ± 14.8 *vs.* 53.9 ± 13.8%) and higher ($P < 0.05$) than sperm washing (40.0 ± 8.9%) and control group (39.2 ± 13.5%). Swim-up allowed better sperm viability after selection, whilst, Percoll protocols were not different than control group. These data suggest these protocols do not infer damages to sperm cells. Although Swim-up obtained better viability, it recovered more acrosome reacted cells. In conclusion, Percoll protocols seems to be an optimal sperm selection method and can be used at sperm preparation for *in vitro* embryo production in ovine frozen-thawed sperm.

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Comparison of extenders for cryopreservation as to integrity of the chromatin of thawed semen of stallions

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Current research has pointed an assessment of the quality of the DNA of stallion sperm, being raw or subjected to biotechnology, such as cooling and freezing, demonstrating that this characteristic relates directly to processes realized (LOVE, 2005). An extender developed in Brazil called Botu-Crio® has been proven superior to others in the ability to preserve the motility of sperm after thawing (PASQUINI, DF, et. al., 2007), however the integrity of the chromatin has not been widely researched. This abstract aims to compare the extenders Botu-Crio®, lactose-EDTA and INRA82, used extenders for stallions semen freezing, relative the ability to protect the damage in equine sperm freezing process as the denaturation of DNA. One ejaculate of 13 different stallions were diluted at a ratio of 1:1 medium (Botu-Sêmen®), divided into three samples and centrifuged (600xg / 10 min). Each samples were resuspended in a extender (INRA 82, Lactose-EDTA and Botu-Crio®) in the concentration of $100x10^6$ spermatozoa/ml, stored in straws of 0.5 ml, and finally frozen using the recommended protocol for each extender: INRA 82 two hours of stabilization at 5°C; Lactose-EDTA without stabilization at 5^oC and Botu-Crio® twenty minutes stabilization at 5^oC. After this stage, all samples were placed in thermic box at 6cm above of liquid nitrogen for 20 min and then immersed and stored in liquid nitrogen container. The samples were thawed in water bath at 46 ° C for 20 seconds, is added 200µl of TNE buffer and immediately after 0.4 ml of acidic detergent solution were added after 30 seconds was added 0.6 ml of Orange Acridine solution containing 0,6µg / mL, the samples were analyzed using the Guava cytometer EasyCyte® flow with excitation 488nm 315mW. The performed statistical methodology was variance analysis with 5% significance. For the results obtained after thawing, statistical differences were not observed ($p = 0.215$) between the extenders studied, the averages of damage in DNA were of 2.04%, 2.48% and 2.36% respectively for Botu-Crio®, Lactose-EDTA and INRA82. The denaturation of the spermatic chromatin is specifically low in stallions, as noted by the authors NAVES, C. S. et. al. (2004) who observed a rate of 3.27%, as well as other species studied: buffalo 1.6% (AX 2002) bulls 0.35% (ROW, JT, 2012), boar 2.82% (Machado et. al., 2003), the human species that presents greater damage to the DNA strand, at a rate of 24.33% (Rocha et. al., 2002). It was observed that the tested extenders no interfere in denaturation of the spermatic chromatin, being important in the future correlate these results with the fertility test and parturition rate. (CEUA/ Pio Décimo College/ Protocol 09/2014).

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Effect of concentration and diluent on equine sperm kinetics parameters evaluated by the SCA®

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Computer assisted sperm analysis (CASA) is an important tool for evaluation of motility, speed, concentration and sperm morphology, because it provides accurate, objective and repeatable semen analysis. The main drawbacks of computerized measuring devices are the extreme need for standardization, optimization and validation of the system before any practical use. This research was carried out in two experimental phases, each using forty four thawed straws of different Mangalarga Marchador stallions. The first experiment evaluated the effect of dilution rate with BotuCrio® to obtain sperm concentrations of 25, 50 and 100 x 10^6/mL under motility and speed assessed by *Sperm Class Analyzer*® (SCA®). The SCA® was set to capture 25 frames/second with a frame rate of 50 Hz. Sperm concentration influenced the results of motility, Medium, Slow, LIN, STR and BCF ($P < 0.05$). Sperm concentration of 25 and 50 x 10^6/mL resulted in a lower percentage of total motility, Medium and Slow compared to 100 x 10^6/mL ($P < 0.05$). Sperm concentration of 100 x 10^6/mL resulted in a higher percentage of progressive motility compared to 50 x 10^6/mL ($P < 0.05$). Sperm concentration of 25 and 50 x 10^6/mL resulted in greater LIN, STR and BCF than 100 x10^6/mL ($P < 0.05$). Concentrations of 100x10^6 /mL resulted in a higher percentage of progressive motility, though the use of 25 and 50 x10^6 /mL resulted in greater LIN, STR and BCF ($P < 0.05$). The second experiment evaluated the effect of diluents BotuCrio®, BotuSemen®, TALP sperm and saline solution using a sperm concentration of 25 and 50x10^6 /mL under sperm kinetics assessed by SCA®. The nature of the diluent significantly influenced the motility, Fast, Medium, VCL, STR, ALH, BCF and Hyperactive independently of the concentration applied ($P < 0.10$). Total motility was higher for sperm diluted in BotuCrio®, TALP sperm and saline solution ($P > 0.10$) and this motility was significantly higher in samples diluted in BotuCrio® compared to BotuSemen® ($P < 0.10$). The use of BotuCrio® resulted in higher percentage of progressive motility, Medium, STR and Hyperactive and higher frequency of BCF ($P < 0.10$) when compared to others diluents tested. The use of BotuCrio® and TALP sperm diluents resulted in greater VCL and Fast sperm compared to BotuSemen® ($P < 0.10$) and Fast sperm was also higher compared to the samples diluted in saline solution ($P < 0.10$). ALH was higher when sperm was diluted in BotuCrio® and saline solution than in BotuSemen® and TALP sperm ($P < 0.10$). The kinetic parameters of motility, Fast, Medium, Slow, VCL, ALH and Hyperactive were increased when the samples were diluted to 50 x 10^6 sperm/mL than to 25 x10^6 sperm/mL independent of diluted used ($P < 0.10$). There was a significant interaction between diluent and sperm concentration on VSL, VAP, LIN and WOB results. Interaction between diluents and sperm concentration was found when the sample was diluted to 25 x 10^6 sperm/mL for VSL, VAP and LIN. At this concentration, it is worth noting the superiority of BotuCrio® and TALP sperm compared to BotuSemen® to preserve the VSL ($P < 0.10$), the superiorness of these diluents compared to BotuSemen® and saline solution to preserve VAP ($P < 0.10$) and their improved performance compared to saline solution to preserve the LIN. In sperm concentration of 25 x 10^6/mL, the use of TALP sperm resulted in a higher percentage of WOB when compared to other diluents ($P < 0.10$). However, the concentration of 50 x 10^6 sperm/mL using TALP sperm as a diluent was better than BotuCrio® and saline solution ($P < 0.10$). It was observed significant interaction between sperm concentration and diluents because VSL, VAP and LIN were higher especially when the samples were diluted in saline solution containing 50 x 10^6 sperm/mL compared to 25 x 10^6/mL ($P < 0, 10$). Based on these results, the optimal concentration for assessment of sperm kinetics by SCA® is between 25 and 50 x 10^6 sperm /mL. For adjustments, the use of BotuCrio® extender, followed by TALP sperm is recommended, and as a third option a dilution with saline solution.

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Effect of ethanolic extract from *Caulerpa filiformis* in human semen

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Marine organisms are invaluable sources of metabolites with diverse pharmacological properties. Among them, the green macroalgae genus *Caulerpa* is a source of chemical compounds from various biological activities; however, there are few reports assessing its contraceptive properties. The aim of this study was to evaluate the effect of ethanolic extract from *Caulerpa filiformis* on human seminal parameters. Algae were collected by hand during low tide in Pucusana bay (Lima, Peru), washed with tap water and dried at 37°C for 12 h. Extraction was performed at room temperature as follows: grained algae was defatted twice with chloroform and the residue was macerated in absolute ethanol for 72 h under constant stirring. Solvent was evaporated overnight at 37°C and the residue was resuspended in SSF at a final concentration of 8 mg/ml. The seminal parameters tested were mortality, motility and sperm chromatin dispersion at 5, 15, 35 and 60 min. Each sample was diluted to a concentration of 10 million sperm per milliliter with 0.9% SSF and then incubated 1:1 (v/v) with the extract at three final concentrations (4mg/ml, 0.4mg/ml, 0.04mg/ml) plus a control. ANOVA analysis showed that above 5 min of exposure treatments did have significant effect ($P<0.05$) on mortality and motility. There were not significant effects on sperm chromatin dispersion in all the times tested. For mortality, the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were 0.4 mg/ml and 4 mg/ml (15-35 min), and 0.04 mg/ml and 0.4 mg/ml (60 min), respectively. The LD50 was 17.35 mg/ml (11.08-57.17 mg/ml), 8.75 mg/ml (7.15-12.00 mg/ml), 6.43 mg/ml (5.53-8.02 mg/ml) for 15 min, 35 min and 60 min, respectively. For motility, a significant decrease ($P<0.05$) was observed at 15 and 35min in 4 mg/ml treatment. At 60min in this concentration, immobilization reached 100%. NOEC and LOEC from 15min to 60min was 0.4 mg/ml and 4 mg/ml, respectively. Two-way ANOVA test showed that time, concentration and their interaction affected the mortality and motility. The latter showed only a dose dependent effect in the 4 mg/ml treatment. In conclusion, the ethanolic extract of *C. filiformis* could reduce significatively the sperm motility at 4 mg/ml since 15 min of exposure without being genotoxic or cytotoxic for human spermatozoa. These results suggest the presence of compounds in this extract with promising contraceptive properties.

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Effect of storage temperatures of epididymis from slaughter bulls on sperm quality and freezability

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The objective of this research was to evaluate the sperm quality post-thawing of sperm from cauda epididymis of bulls maintained at 5°C or 20°C during 6 hs. Eight pairs of testicles were collected at slaughterhouse and stored tightly in plastic bags with Lactate Ringer for 6 hours. The gametes were recovered by retrograde flow of the cauda epididymis and sperm concentration and viability were assessed. Spermatozoa were stored in straws after adjusting doses of 50 x 10^6 sperm / 0.25ml with the diluent AndroMed® to 20%. Thereafter straws were placed on a floating ramp 4 cm above liquid nitrogen (LN) and kept 10 min in LN vapors after which the straws were submerged into LN. Seven days after, the samples were thawed (37°C/60 sec) and evaluated for individual motility (IM), vitality (eosin/nigrosin stain) (V), total abnormalities (TA) and membrane integrity (HOS Test). The CASA system SpermVision™ version 1.01 (Minitube, Verona, WI, USA) was used to define total motility (TM), individual progressive motility (IPM), local motility (LM), immobile sperm (IS) and TA. It was used a completely randomized design and data were processed with software SPSS version 22.0. Results were expressed by the mean and standard deviation. The normality of the data was checked with the Kolmogorov-Smirnov test, and Levene's test was used to define if the groups have equal variance. The temperature effects in both groups were evaluated by ANOVA. The values of IM determined with the optical microscope pre-freezing were better ($P<0.05$) for 5°C temperature than 20°C (respectively, 84.2 ± 1,54 vs. 67,7 ± 7,04%). The results of post-thawing sperm quality were better ($P<0.05$) for 5 °C than for 20°C for IM (33.4 ± 2.42 vs. 22.8 ± 2.81%), VE (63.0 ± 2.61 vs 52.9 ± 2.96%) and TA (31.6 ± 1.45 vs 33.8 ± 2.85%), respectively. However, in HOS-Test values were better for 20°C (40.4±3.26 vs. 31.3±1.99 vs%) than 5°C temperature. The results of motiliy quality determined by CASA analysis produced some controversial results. There were no significant differences ($P>0.05$) for any variable analyzed: TM, IMP, LM, IS and AT. In conclusion, freezabilty of sperm from the cauda epididymis of testicles maintained at 5°C after slaughter was the better option for preserve these gametes.

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Effect of two collection methods and two extenders on the freezability of semen of native bulls in Ecuador´s Andean highlands

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The objective of this study was to evaluate two methods of semen collection and two extenders on the freezability sperm of native bulls in Ecuador's Andean highlands. For this purpose, semen of three adult bulls of Creole type were collected using two collection methods: Artificial Vagina (AV) and Electroejaculation (EE); collections were performed twice a week for five weeks for each method and for each bull, obtaining a total of 30 ejaculates for VA and 30 ejaculates for EE. The ejaculates of each bull were evaluated, immediately split into two parts and diluted in Triladyl® and AndroMed®, respectively to obtain a concentration of $80x10^6$ spermatozoa/ml, enough to fill 0.25 ml straws with $20x10^6$ spermatozoa. The packed filled straws were stored in a refrigerator reaching 4 °C in one hour. At this time, the straws were kept at the same temperature for 2 hs. Thereafter straws were placed on a floating ramp 4 cm above liquid nitrogen (LN) and kept 10 min in LN vapors after which the straws were submerged intoLN. The samples were thawed (37°C/60 sec) before detailed analysis. Measured variables under the microscope were individual motility (IM), vitality (eosin/nigrosin stain) (V), total anomalies (TA), tail anomalies (TaA), and membrane integrity (HOS Test). The CASA system SpermVision™ version 1.01 (Minitube, Verona, WI, USA) was used to define total motility (TM), individual progressive motility (IPM), local motility (LM) and immobile sperm (IS). It was used a completely randomized design with factorial arrangement 2x2 and the SPSS *24.0 version* software was used for the statistical processing. Results of each group were expressed by the mean and standard error; the normality of the groups was checked with the Kolmogorov-Smirnov test, and Levene's test was used to define if the groups had equal variance. The effects of methods of collection, extender, and block (bull) were evaluated by ANOVA. Results revealed that there was ($P<0.05$) interaction between method of collection and the type of extender only for V. The values of the variables determined with the optical microscope were better ($P<0.05$) for the EE method than AV. The EE method presented efficient ($P<0.05$) results, for IM (51.7±1.90 vs. 43.3±1.40 %), TA (16.2±0.93 vs. 22.3±0.94 %), TaA (11.9±0,80 vs. 14.0±0.58 %) compared with VA method. The Triladyl® extender presented superior ($P<0.05$) results, for VE (60.2±1.47 vs 53,4±1,69 %) and HOS Test (22.4±0.90 vs. 17.3±0.75 %) compared with AndroMed®, respectively. However, in the CASA analysis the revealed results were controversial ($P<0.05$), there was interaction between factors for TM, LM and IS. The TM (53.4±1.66 vs 41.7±2.13 %) was higher ($P<0.05$) in Triladyl® compared with AndroMed® extender. On the other hand, LM (16,1±1,27 vs. 30.8±1.32 %), and IS (58,5±2.23 vs. 47.3±1.70 %) results were more efficient with AndroMed® ($P<0.05$) than Triladyl®, respectively. In the factor analysis method of collection, no difference ($P>0.05$) was found in any analyzed variable. With these controversial results, we can conclude that the semen collected with EE and diluted with Triladyl® showed some advantages in sperm quality and would be a better option to freeze bull sperm with local breeds.

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Effects of human Chorionic Gonadotropin (hCG) in proteome of lane snapper (*Lutjanus synagris*, Perciformes) sperm

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Marine fish farming is an alternative to prevent decrease in production of marine food. In the last decades, the number of domesticated marine species had grown rapidly and a mandatory requirement for such activity is the control of reproductive processes in captivity and production of high quality eggs and sperm. The lane snapper, *Lutjanus synagris*, figures among the marine fish species with highly farming potential because of its greater adaptation to domestication. The objective of the proposed study is to evaluate the protein profile in sperm from lane snapper males subjected to hormonal therapy with human Chorionic Gonadotropin (hCG). Nine sexually mature males were used in this study, being 5 controls (186 ± 22.5 g; without hormone treatment) and 4 males (186.7 ± 29.6 g) treated with hCG. Each male was injected with hCG (500UI/Kg of body weight) before sperm collection. Sperm were collected through abdominal pressure and immediately after the collection, an aliquot of semen was obtained for evaluation of the motile percentage of spermatozoa (under light microscope), sperm concentration (on Newbauer chamber, 1:4000 formal saline) and for the Sperm Chromatin Dispersion (SCD) test which detects the chromatin fragmentation. The remaining semen samples were saved and treated with a protease inhibitor. Protein extraction was conducted with Triton X-100 and manual maceration and protein concentration was measured according to Bradford's methodology. Aliquots of 15µg proteins were loaded onto 12.5% polyacrylamide gel, for 1D SDS-PAGE. Number and intensity in the 1D gels were analyzed by *Quantity One* software v.4.6.3 (BioRad, USA). Bands were digested with trypsin and peptides were identified by tandem mass spectrometry (ESI/QUAD-TOF). Peptides searches were made in MASCOT database and tools of bioinformatics. We used Strap software for gene ontology annotation. Data were subjected to analysis of variance. Collected semen volumes and chromatin fragmentation for controls (70 ± 36.3 µL and 81.8 ± 6.25%) and hormone induced males (99.5 ± 45.2 µL and 91.3 ± 3.49%) were not different ($p < 0.05$). However, sperm concentration, sperm motility and protein concentration were higher ($p < 0.05$) for the hCG-treated males ($2.56 \pm 0.12 \times 10^{10}$ spz/mL; 85 % and 6.9 ± 0.8 $mgmL^{-1}$) than for the non-treated individuals (0.53 ± 0.14 x 1010 spz/mL; 62 % and 3.4 ± 0.08 $mgmL^{-1}$). It was identified 40 different bands in *L. synagris* sperm. Bands with greatest relative intensity were identified as Creatine Kinase and Tubulin that have a molecular weight of 43 and 63 kDa, respectively, in both analyzed groups. The intensity of the bands identified as Creatine Kinase (43 kDa) accounted for 58 and 34% of all bands identified in the gels from control and treated males, respectively ($p < 0.05$). Creatine kinase catalyzes the transfer of phosphate between ATP and various phosphogens playing a central role in energy transduction in tissues with large, fluctuating energy demands, such as spermatozoa. Moreover, control males expressed eight unique bands (32, 47, 61, 75, 79, 116, 124, 136 kDa), which were identified as tyrosine 3-monooxygenase/tryptophan, 5-monooxygenase activation protein beta polypeptide-like, citrate synthase mitochondrial, glutamate dehydrogenase mitochondria, Wap65-2 precursor; stress induced phosphoprotein-like ubiquitin carboxyl-terminal hydrolase 5 and MAGUK p55 subfamily member 7. Hormone-treated animals presented two exclusive bands (172 and 114 kDa), identified as armadillo repeat-containing protein 4, which is involved in the flagellum beat frequency regulation, Endoplasmin one chaperone protein involved in processing and transport of other proteins and Glutamine-rich protein 2 that interacts with Histone methyltransferase (MLL4), that, in turn, methylates 'Lys-4' of histone H3 in epigenetic transcription. The most prominent molecular functions linked to the identified proteins were binding and catalytic activity, followed by structural molecule activity. The two main biological processes of the lane snapper sperm proteins identified here were characterized as cellular process and regulation. Concerning cellular component, proteins were mainly cytoplasmic, followed by extracellular and cytoskeleton. This is the first description of the sperm protein pattern in *L. synagris*. The use of hCG signiticantly affected semen parameters as well as protein expression in the gametes of that species.

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Effects of reduced glutathione on the free cysteine radicals and reactive oxygen species levels in boar sperm during the *in vitro* capacitation

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Nowadays, with the advent of assisted reproductive technologies through of *in vitro* studies a large amount of information has been gathered on how the sperm achieves the capacitation through a sequence of biochemical modifications that lead to the establishment of fully fertilizing ability. However, little is known about the possible effects of antioxidants such as reduced glutathione (GSH) on these cellular changes during sperm capacitation.Thus, the aim of this study was to evaluate the effects of GSH on the putative changes in free cysteine radicals (FCR) and reactive oxygen species (ROS) levels in the boar sperm of cooled semen during the achievement of a standard *in vitro* capacitation (IVC). Four treatments (control and three different GSH concentrations: 1, 2 and 5 mM) for each assay (n=6) were analyzed after the incubation for 4h at 38.5ºC (in a 5% CO_2 atmosphere) of sperm in an IVC activation medium (CM) containing $NAHCO_3$ and bovine serum albumin (BSA). The incubation increased ($P<0.05$) the intracellular levels of FCR in both head (from 3.0 ± 0.3 nmol/µg protein at 0h to 17.4 ± 1.7 after 4h of incubation) and tail (from 3.4 ± 0.4 nmol/µg protein at 0h to 8.4 ± 1.0 nmol/µg protein after 4h of incubation). Similarly, an increase ($P<0.05$) in the rate of sperm with high intracellular levels of both peroxides (from 2.4 ± 0.7% at 0h to 18.6 ± 2.0% after 4h of incubation) and superoxides (from 11.6 ± 1.7% at 0h to 17.5 ± 1.9% after 4h of incubation) was observed. These increases were concomitant with capacitation-like changes of parameters like sperm motility, membrane fluidity, mitochondrial membrane potential and tyrosine phosphorylation levels. The addition of GSH at the CM prevented the majority of these changes in a dose dependent manner, reaching a maximal effect at concentrations of 5 mM. Our results seem to indicate that IVC in boar sperm is related with an increase of both overall disrupted disulfide bonds and intracellular ROS levels which can be prevented by the addition of GSH to the medium.

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Efficacy of glutathione addition in egg yolk and soy lecithin based extenders of cryopreservation on bovine sperm viability

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With the advances in animal reproduction biotechnology, the use of cryopreserved semen in artificial insemination stands out as an important tool for the genetic improvement of various animal species. However, the genetic impact on the artificial insemination industry is limited by the cryopreservation process, causing physical and chemical changes in the membranes of spermatozoa, leading also to the production of reactive oxygen species (ROS). In order to reduce the oxidative damage caused by high concentrations of ROS, antioxidants have been added in cryopreservation extenders of bovine semen. The present study aimed to evaluate the effect of **g**lutathione addition in egg yolk (Bovimix®; Nutricell, Campinas, Brazil) and soy lecithin based extenders (AndroMed®; Minitube, Hauptstrasse, Germany) of cryopreservation, on post-thaw viability of bovine semen. Five Holstein bulls were used, being submitted to six semen collections. The ejaculates were split into two portions, diluted to a concentration of $40x10^6$ spermatozoa/mL, with egg yolk and soy lecithin-based extenders, Bovimix® and AndroMed®, respectively. Each portion was fractioned in three 15 mL centrifuge tubes, resulting in 6 treatments: 1. Control Bovimix® (BC), without adding antioxidants; 2. Bovimix® 1.5 mM glutathione (B1.5); 3. Bovimix® 2.5 mM glutathione (B2.5); 4. Control AndroMed® (AC), without adding antioxidants; 5. AndroMed® 1.5 mM glutathione (A1.5); 6. AndroMed® 2.5 mM glutathione (A2.5). After homogenization, the semen was packaged in 0.5 mL straws, arranged horizontally on a screened steel tray and maintained at 5° C for five hours. After the refrigeration period, the samples were kept for 20 minutes in nitrogen vapor, 3 cm above the nitrogen level. After frozen, they were stored in a nitrogen container. For analysis, the straws were thawed at 37 °C for 30 seconds and computer assisted sperm analysis (CASA) and analysis of sperm membrane integrity were evaluated. The percentage of sperm membrane integrity were evaluated by immunofluorescence microscopy with fluorescent probes (propidium iodide and carboxyfluorescein). Sperm variables assessed by CASA in the present study were total motility (MT-%), progressive motility (MP-%), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), average path velocity (VAP-µm/s), amplitude of lateral head displacement (ALH-µm), beat cross frequency (BCF-Hz), straightness (STR-%) and linearity (LIN-%). The data were analyzed using ANOVA and means comparison were analyzed by Tukey test at 5% of significance. There was no statistical difference in MT, MP, VCL, VSL, ALH, BCF and STR between treatments. However, the variable VAP was higher in all treatments in which the exterder AndroMed® were used (AC-68.42, A1.5-68.92, A2.5-65.44 x BC-56.10, B1.5-56.45, B2.5-56.57). The variable LIN was higher in all treatments in which Bovimix® was used (BC-51.00, B1.5-50.80, B2.5-49.73 x AC-42.96, A1.5-44.43, A2.5-43.50). There was no statistical difference in the percentage of sperm membrane integrity between treatments. In conclusion, both treatments were efficient to maintaining post-thaw viability of semen. However, glutathione didn't improve the sperm viability of the post-thaw bovine semen.

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Evaluation of reproductive potential of young and adult Blonel bulls

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Blonel was developed to be a new race for meat production in tropical countries. Composed by 5/8 Blonde and 3/8 Nellore, Blonel was developed to unit Zebu rusticity to good reproductive characteristics of the Blonde race and is the newest bovine race recognized and registered by the Ministry of Agriculture, Livestock and Supply (MAPA) in Brazil. This study aimed to evaluate the reproductive performance of Blonel bulls of different ages by andrological examination. The experiment was conducted in Três Furnas Farm in Buriti Alegre, Goiás. For this survey, 14 animals aged 24 to 63 months were subjected to andrological examination during August and September of 2012. The andrological examinations were conducted by the same veterinarian. Scrotal circumferences were determined in cm. After the collection, were conducted the immediate analyzes of semen: gross motility, motility, vigor and sperm concentration. From a drop of semen, were made a smear microscopy slide for evaluation. Subsequently, in the laboratory, this slide was stained (Gram stain) for conducting the mediate analysis of sperm cells (sperm morphology) under microscopy, when were evaluated all types of defects and their frequencies. The results of these evaluations mentioned above are part of the andrological exam report. For statistical analysis, results of scrotal circumference, semen volume, turbulence, motility, FPM, concentration, total major defects and total minor defects evaluations were submitted to ANOVA at 5% of significance level. The results of this study did not differ between groups ($p>0.05$) and were: 30.14 ± 1.34 and 28.93 ± 1.69 cm for scrotal circumference, 5.50 ± 0.00 and 4.64 ± 0.80 ml for semen volume, 2.71 ± 0.49 and 2.57 ± 0.53% for turbulence, 54.28 ± 6.07 and 67.86 ± 9.51% for motility, 3.57 ± 0.79 and 3.28 ± 0.75 for FPM, 657.14 ± 97.59 and 678.57 ± 138.01% for concentration, 7.57 ± 1.27 and 5.71 ± 2.43% for total major defects, 5.43 ± 2.76 and 2.29 ± 1.60% for total minor defects for young and adult Blonel bulls, respectively. Results observed were just below the recommended by CBRA, except for total major and minor defects. Among total defects were observed no: acrosome defects, proximal cytoplasmic droplet, sperm underdeveloped, curled tail to the head, abnormal small, abnormal color, abnormal contour, pouch formation, teratological forms and pathological middle piece. This study indicates that young and adult Blonel bulls shows similar reproductive potential and both has to be more studied.

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Fertilization rates of bovine frozen-thawed sperm cells capacitated with Binder of Sperm Protein-1 (BSP1) and/or Heparin

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Binder of Sperm Protein 1 (BSP1) and heparin induces sperm capacitation by removing cholesterol and phospholipids from the sperm membrane. Thus, the present study was conducted to evaluate the effects of BSP1 and/or heparin on in vitro fertilization (IVF) rates of bovine frozen-thawed sperm. The aim of this study was to evaluate the fertilization rates of bovine sperm cells capacitated with heparin and/or BSP1. Cumulus-oocyte complexes (COCs, n = 1138) obtained from slaughterhouse ovaries were *in vitro*-matured in modified TCM-199 for 24 h in a controlled atmosphere, as described (Rodriguez-Villamil et al, 2016). Then, COCs were selected and randomly allocated into four groups to be *in vitro*-fertilized in the presence or not of different capacitating agents (CA), as follows:: COCs fertilized in Fert-TALP medium either without any CA (Control group), or with 10 UI/mL heparin (Group 1), 40 µg/mL BPS1 (Group 2), or 10 UI/mL heparin + 40 µg/mL BSP1 (Group 3). Tested commercial frozen-thawed semen from different bulls was used for the IVF. BSP1 was purified from bovine seminal vesicle fluid by affinity chromatography. Zygotes were *in vitro*-cultured in mSOF medium for 7 days at 38.8°C in a controlled atmosphere. Cleavage and embryo development were evaluated on Days 2 and 7 after fertilization (Day 0). Data for cleavage and blastocyst rates were transformed and analyzed by ANOVA ($P<0.05$). Cleavage and blastocyst rates were lower ($P<0.001$) in the Control group (64.7±2.0% and 21.8±3.9%, respectively) than heparin (77.0±2.2% and 40.0±1.5%, respectively) or BSP1 (79.1±5.3% and 41.7±3.1%, respectively) treatments ($P<0.001$). However, the combination heparin + BSP1 (Group 3) had similar cleavage (76.4±2.8%) and blastocyst rates (37.7±5.7%) as compared to heparin or BSP1 alone ($P=0.09$). Results were contrary to the hypothesis that both CAs could act synergistically to promote *in vitro* ejaculated sperm capacitation, at least under the conditions of our study. In conclusion, BSP1 and/or heparin had similar capacitating effects on frozen-thawed bovine sperm, allowing a proper *in vitro* embryo development following IVF procedures.

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In vitro sperm thermoresistance test on sperm from Aberdeen Angus bulls with different field fertility

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Conventional semen analyzes (motility, vigor and abnormalities) have noted associations with bulls' fertility rates. Nevertheless, some special cases of male fertility problems are not explained by these exams. Although semen evaluation has been advanced with Computer-Assisted Sperm Analysis (CASA), fluorescence microscopy and flow cytometer techniques, some cases of male infertility still demand other analyzes. Thus, the present study aimed to investigate if thermoresistance test (TRT) associated with CASA and fluorescent microscopy techniques is related with field fertility. For this, a total of 18 batches of semen from six Aberdeen Angus bulls were used. Bulls presented different fertility rates (P=0.007) evaluated previously: three presented high fertility rates (HFR; 54.3±1.0%) and three presented low fertility rates (LFR; 41.5±2.3%) based on breeding predicting value analysis. Four straws from each batch were thawed (37°C/30s) and analyzed in duplicate (two in a first replicate of analyzes and two in a second replicate) for motility characteristics by CASA using Sperm Class Analyzer (SCA, Microptics, Spain), abnormalities by differential interference contrast microscope (Nikon 80i, Japan) and plasma and acrosome membranes integrity (PMI and AMI, respectively) and high mitochondrial membrane potential (HMMP) by fluorescent probes (Hoescht 33342, propidium iodide, JC-1 and FITC-PSA). Besides, an aliquot of 150µL of semen was submitted to TRT (temperature=45°C, during 1 hour). After TRT, semen was analyzed by the same parameters. For statistical analyzes, two groups were considered (HFR x LFR) and the data was evaluated by variance analyzes by MIXED procedure of SAS software (SAS inc, 2004). Significance difference was considered when P<0.05. Data from TRT were previously transformed in a difference between before and after the test and then submitted to variance analyzes. Results from post-thawed semen analyzes showed no difference between bulls of HFR and LFR, except to major defects (P=0.006; HFR=18.71±1.99%; LFR=25.05±0.89%), VCL (curvilinear velocity; P<0.0001; HFR=145.79±2.59 µm/s; LFR=172.79±3.69 µm/s), VAP (average path velocity; P=0.001; HFR=97.45±1.53 µm/s; LFR=105.39±1.58 µm/s), LIN (linearity coefficient; P=0.01; H=53.71±1.60%; LFR=48.71±1.02%), WOB (wobble coefficient; P<0.0001; HFR=66.02±0.98%; LFR=60.37±0.79%) and ALH (amplitude of lateral head displacement; P<0.0001; HFR=2.66±0.04 µm; LFR=3.41±0.12 µm). In concern to the difference between before and after TRT, groups are different in almost all characteristics: major (P=0.001; HFR=-2.03±1.37; LFR=-12±2.43) and total defects (P=0.001; HFR=-0.74±1.75; LFR=-11.26±2.47); total motility (P=0.0005; HFR=15.76±2.18; LFR=37.11±4.98), progressive motility (P=0.003; HFR=11.60±1.88; LFR=29±5.07), rapid cells (P=0.0009; HFR=16.81±2.32; LFR=37.92±5.15), VCL (P=0.004; HFR=15.51±4.99; LFR=51.98±10.72), VSL (straight-line velocity; P=0.01; HFR=13.58±3.04; LFR=33.69±6.67), VAP (P=0.004; HFR=14.92±3.09; LFR=37.20±6.50) and BCF (beat cross-frequency; P=0.03; HFR=2.94±0.59; LFR=7.34±1.99); and quantity of PMI cells (P=0.02; HFR=11.43±1.65; LFR=13.47±2.59), AMI cells (P=0.007; HFR=11.63±2.35; LFR=22.55±2.96) and sperm with plasma and acrosome membrane integrity and high potential of mitochondrial membrane (PIAIH cells; P=0.0009; HFR=15.54±1.69; LFR=24.33±1.72). The greater the difference in TRT, the greater is the effect of the test on semen quality. Thus, HFR group presented increased resistance to TRT compared to LFR. TRT consists in elevate the temperature and, consequently, cellular metabolism. So LFR group seems to be more sensitive to this challenge than HFR group. Besides, LFR post-thawed semen evaluation suggested that sperm are more active, once this presented increased values of VCL, VAP and ALH. High activity of these sperm could influence the sensibility to TRT. Hence, although there were few differences between the batches in post-thawed evaluations, semen from high fertility bulls presented increased resistance to TRT compared to semen from low fertility bulls.

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Lack of *Bauhinia forficata* effect on sperm motility and redox status of diabetic mouse

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Diabetes mellitus type 2 (T2DM), characterized by insulin resistance and/or relative insulin production deficiency, has been shown to cause male infertility. Hyperglycemia is known to directly induce reactive oxygen species (ROS) production, resulting in oxidative stress. *Bauhinia forficata* Link (*Bf* L.) is a powerful source of natural antioxidants with hypoglycemic and antidiabetic activities. Due to these effects, *Bf* L has been suggested as an alternative treatment to minimize T2DM complications associated to oxidative stress. Therefore, the present study investigated *Bf* L effects on the spermatozoa function of T2DM mice. For that, 16 C57Bl/6J mice with streptozotocin (STZ)-high fat diet (HFD)-induced T2DM were randomly distributed on diabetes control group (T2DM-C) and diabetic group fed with aqueous extract of Bf L. fresh leaves decoction (Dec) (T2DM-B) for 6 weeks. After treatment, animals with glycaemia greater than 230 mg/dL were considered diabetic and epididymis cauda sperm recovered for analysis. The data was analyzed by t-test using MiniTab 17 software. Data are expressed as mean ± SEM. Epididymal sperm motility, as evaluated by CASA, was not affected by bauhinia treatment in any parameters analyzed. There was no effect of *Bf* L. on glutathione peroxidase (28.2 ± 5.1 and 25.3 ± 4.4 for T2DM-C and T2DM-B group, respectively) and superoxide dismutase (59.8 ± 18.3 and 55.2 ± 11.8 for T2DM-C and T2DM-B group, respectively) sperm activity. Lipid peroxidation as assessed by thiobarbituric acid reactive substances (TBARS) was not different between T2DM-C (163.6 ± 29.2) and T2DM-B (137.3 ± 28.8) mice sperm cells. ROS evaluation by flow cytometry using CelRox probe showed that ROS positive sperm in T2DM-C (4.40 ± 1.9) was not different from those observed in T2DM-B mice sperm (9.80 ± 2.9). Similarly, sperm high mitochondrial potential evaluated by flow cytometry using JC-1 probe was similar between T2DM-C (10.86 ± 3.4) and T2DM-B (19.88 ± 3.7) mice. In conclusion, *Bauhinia forficata* Link did not affect function and redox status of spermatozoa from STZ-HDF-induced diabetic mouse.
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New multicolor protocol to assessment dog spermatozoa by flow cytometer

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The use of flow cytometry in sperm evaluation became essential as they may predict fertility, as many sperm parameters are individually evaluated. Thus, aim of this study was to evaluate the efficiency of the association of Hoechst 33342 (14533; Sigma Aldrich - H342), propidium iodide (P4170; Sigma Aldrich - PI), fluorescein isothiocyanate-conjugated *Pisum sativum* agglutinin (L0770; Sigma Aldrich - FITC-PSA) and MitoStatus Red (564697; BD Pharmigen) to assessment plasma and acrosomal membranes integrity and mitochondrial function by flow cytometer. Five ejaculates from 5 stud dogs were used. Semen was diluted in TALP sperm medium to a final concentration of $2x10^6$ spermatozoa/mL. Diluted semen was split into two aliquots. One of them was maintained (fresh semen) and the other was submitted to flash frozen in liquid nitrogen and thawed, in six continuous cycles, to damage plasmatic and acrosomal membranes, and to perturb the mitochondrial function. Fixed ratios of fresh semen:flash frozen semen were prepared to perform three treatments as following: 0:100 (T0), 50:50 (T50), and 100:0 (T100). Then, in 200 µL for each group was added 1.5 µM of PI, 7.0 µM of H342, 1.0 ng of FITC-PSA and 20 nM of MitoStatus Red. Samples were incubated for 15 minutes at 37°C. For flow cytometry analysis, samples were run on a BD LSRFortessa flow cytometer (Becton Dickinson, Mountain View, CA, USA) equipped with 488 nm blue laser, one 640-nm red laser and 405 nm violet laser. For the study, excitation was performed at 488 nm and the emission filters used were 695/40 nm (IP), 530/30 nm (FITC-PSA), for excitation in 640 nm, the emission filter was 660/20 nm (MitoStatus Red) and for excitation in 405 nm the emission filter was 450/50 nm (H342). At least 10,000 cells per sample were analyzed and data were stored in list mode files. Statistical analysis was performed using GraphPad Prism 5. Linear regression was carried out to determine the ratio between treatments. Regressions plots allowed simple visualization of the distributions of data points around the line of equality (slope=1.0) by showing how close the slope of the regression line of the data is to the line of equality. Linear regression indicated a high degree of correlation between PI negative and plasmatic membrane integrity (R^2=0,957), FITC-PSA negative and acrosome integrity (R^2=0,998) and MitoStatus Red positive and cells with high potential mitochondrial (R^2= 0,931). In conclusion, this protocol was consistent, applicable and had a reliable measure of plasma and acrosomal membranes integrity and mitochondrial function analyzed by flow cytometer.

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Partial sperm proteome of New Zealand White rabbits

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The quantification and identification of proteins expressed in cells and tissues could provide information about its functions. Information on the sperm proteome of rabbit is crucial for the understanding how these proteins regulate sperm attributes. The present study was conducted to identify the main proteins of rabbit sperm. Semen was collected from 18 New Zeland male rabbits raised at Federal University of Ceará, Fortaleza, Brazil (3°43′6″S 38°32′34″W). An artificial vagina made by tempered glass (Ghislandi & Ghislandi, Covo, Bg, Italia) and a dummy female was used to collect semen samples. Two ejaculates from each animal were harvested, with an interval of 15 minutes between collections, but only the second sample was used. Semen samples had at least 70% motile sperm. Sperm were separated from seminal plasma by centrifugation. Sperm protein were extracted with Triton X, precipitated with acetone and resuspended with a buffer containing Urea 7M, Thiourea 2M, 2% CHAPS and 10 m MDTT. Seven pools of two animals (a total of 14 ones) were made to obtain the necessary amount of protein for proteomic study. Sperm protein (30µg) were separated in 15% polyacrylamide gels and stained with Commassie blue (R-250) overnight. Gel was scanned and analyzed using Quantity One (Bio Rad, USA). The average of sperm protein concentration was 3.6 ± 1.76µg/µl. There were found a minimum of 18 bands and a maximum of 24 (average 21.25±2.6) weighing between 12 and 225kDa in the pools analyzed. A total of 5 bands (90kDa, 58kDa, 32 kDa, 31 kDa and 29 kDa) were identified by mass spectrometry, which represented 46.5% of all bands intensity. There were identified FAM 115-like protein, Mutant beta-actin, Annexin 5, Binder of sperm 1(BSP1) precursor and Acrosin precursor. An in silico analysis was performed to evaluate and discuss these proteins and their interactions. Based on STRING platform we confirmed Annexin 5 interacts with other proteins related to DNA repair (Ubiquitin B) and sperm membrane functionality (Cystic fibrosis transmembrane). FAM 115-like protein interacts with Transglutaminase 4, which plays role in the formation of seminal coagulum and is required to stabilize the copulatory plug in mammals. BSP1 potentially interacts with Oviduct-specific protein precursor and Acrosin interacts with Zona Pellucida glycoprotein 2 (ZP2), which mediates species- specific sperm binding. Further studies are being performed to identify more sperm rabbit proteins and their interactions to better understand sperm cells dynamics.

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Plasma membrane and acrosomal status in stallion sperm during epididymal transit

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Morphological and biochemical changes during epididymal transit are required for development of the fertile spermatozoa. The plasma membrane of the maturing sperm cell is a target for a variety of post-testicular modifications. The remodeling of the molecular structure of the plasma membrane during sperm maturation in epididymis leads to significant consequences for intracellular events, such as the regulation membrane permeability and functionality. This study was design to assess the changes in stallion sperm plasma membrane and acrosome status during epididymal transit. Eighteen epididymides from nine adult (3-4 yr old) stallions were subdivided into 10 regions (E0–E1, proximal caput; E2, middle caput; E3–E4, distal caput; E5–E6, corpus; E7–E9, cauda). Epididymal sperm were obtained by microperfusion with sterile phosphate saline buffer (pH 7.2). Samples were diluted in modified TALP medium to a final concentration of 5×10^6 sperm/mL. Aliquots of 200µL of sperm suspension of each segment from each animal were mixed with 7µM Hoechst 33342 (Sigma), 1.5µM Propidium Iodide (Sigma), 1 ng FITC-PSA (Sigma) and then incubated at 37°C for 15 min in the dark. A total of 10000 gated-events were analyzed per sample by flow cytometry (BD LSRFortessa). The green fluorescence (FL1) was collected through a 580-nm bandpass filter and the red fluorescence (FL3) through a 635-nm bandpass filter. The mean channel fluorescence was determined for both red and green. Statistical analyses were performed using One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with a significance level of $P \leq 0.05$. Data are presented as means ± standard error (SEM). The percentage of intact plasma membrane and acrosome (PMA) was significant higher in E0 (73.2 ± 2.3) and E9 (74.4 ± 4.1) compared to E5 (58.9 ± 1.8). Similarly, the segment E8 (78.4 ± 3.5) showed higher PMA integrity in comparison with E3 (64.0 ± 2.3), E4 (61.9 ± 2.3) and E5 ($P \leq 0.05$). No difference was observed in PMA integrity among other epididymal regions ($P \geq 0.05$). Considering the caput (E0-E4), corpus (E5-E6) and cauda (E7-E9) regions, epididymal cauda (74.6 ± 2.0) showed higher percentage of PMA integrity compared to caput (68.1 ± 1.3) and corpus epididymal sperm (62.8 ± 2.4) ($P \leq 0.05$). During epididymal maturation, an important structural change in spermatozoa is the increased adherence of the 'inner' acrosome membrane to the subjacent membranes (nucleus) and the reduction in dimensions of the acrosome. Probably, the lower percentages of sperm with intact PMA in caput and corpus epididymal sperm is due to changes in the permeability and acrosome structure in immature epididymal sperm, which makes it more fragile and susceptible to damage, since the acrosome is not firmly attached to the underlying structures. In conclusion, the population with intact PMA increases along epididymal maturation. The lower population of sperm with intact PMA in caput and corpus epididymal sperm may be associated with the immaturity of equine epididymal sperm, as permeability of plasma membrane and re-structure of acrosome.

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Purification of BSP5 from bull seminal plasma by affinity chromatography

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Secretions from the testes, epididymis, seminal vesicles and other acessory glands contribute to the complex mixture of fluid and proteins that comprise seminal plasma (Suarez et al., 2006). In bovine species, three proteins BSP1, BSP3 e BSP5 from the family of Binder of SPerm proteins have been purified and characterized. Binder of SPerm protein 5 is a protein involved in sperm capacitation that is known to have the longest N-terminal domain and it is the most glycosylated and active member of BSP's (Manjunath et al., 2015). In this regard, the study was conducted to purify the BSP5. For this experiment, semen was obtained by eletroejaculation from twenty-one adult, Guzerá bulls. Samples were centrifuged (700 x g, 4°C, 15 min) to separate sperm from seminal plasma, and the seminal plasma was re-centrifuged (5000 x g, 4°C, 60 min) to confirm absence of sperm. Proteins of seminal plasma (3 mg) were loaded into a 1 ml Hitrap Heparin HP column attached to an Äkta Prime Plus Chromatographic System (GE Healthcare, USA). The heparin binding protein (HBP) fractions were pooled, desalted and concentrated using the Amicon MWCO 10 kDa filters (Sigma – Aldrich, USA). Seminal plasma and other fractions obtained by the heparin chromatography were subjected to SDS-PAGE (12,5%). All gels were stained in a 2% colloidal solution with Coomasie blue R-250 (Bio-Rad, USA). Whole seminal plasma and HBP obtained by heparin chromatography were evaluated by Western blots. In brief, 20 µg SP and HBP were separated in 12% acrylamide gradient precast gels and then transferred (at 90 mA for 80 min) to PVDF membrane (GE Healthcare). The membranes were blocked overnight at 4°C with 30 ml of PBS-T containing milk, followed by 1-hour incubation with antibody against bovine BSP5 AB Anti-BSP5(1:6,000). PVDF membrane were washed again three times in PBS-T followed by 1-hour incubation with a secoundary antibody (1:5,000 Dnk pAb to Rb IgG; abcam or Donkey anti-rabbit) and rinsed twice with Tris-HCl (50mM). The immunoreaction was visualized after exposing membranes to BCIP/NBT alcaline phosphatase substrate. Based on the chromatographic profiles, heparin-binding proteins represented 12,18% of seminal plasma proteins from *Bos indicus* bulls. One – dimensional SDS-PAGE confirmed the presence of BSP's proteins in seminal plasma and heparin- binding protein fractions.Western blot using antibodies against BSP confirmed the presence of a 30-kDa band in the HBP fraction. Therefore, this 30-kDa protein known as BSP5 binds to heparin and the fractions were separated into two peaks when passed through the heparin column. This study is a partial purification of BSP5 from bovine seminal plasma, moreover other columns might be needed to completely purify the protein. Binder of SPerm proteins comprise approximately 50% of all proteins of the accessory sex gland fluid and seminal plasma of *Bos indicus* bulls (Kelly et al., 2006). Binder of SPerm 5 (BSP5) is a multifunctional protein primarily involved in sperm capacitation that contains two similar and highly conserved fibronectin type II. Thus, that process of purification and it uses in functional tests allows to understand the mechanisms by which BSP's modulate sperm capacitation.

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Reduced fertility of cryopreserved semen can be associated with decreased mitochondrial activity

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Mitochondrial activity appears to be an important characteristic associated with sperm quality and fertilization capacity. The objective of this study was to determine the influence of sperm mitochondrial activity with fertility rate in different batches from high, medium and low fertility and its relationship with other semen quality parameters. Samples were classified in high fertility (HF), above 56% of pregnancy rate, medium fertility (MF) between 45 and 55% pregnancy rate and low fertility (LF) below 44% pregnancy rate. This study included sixteen straws (n=16) of different bulls, nine (n=9) with high fertility (HF) and seven (n=7) of medium fertility (MF), that were used in a timed-AI program, under the same standard management conditions and as minimum 100 AI per batches. No batch showed low fertility. Pregnancy diagnosis was made with a B-mode sonogram 30-45 days after insemination. Two semen straws from the same batch were thawed at 37ºC for 30 seconds in water bath and were assessed by conventional sperm analyses (motility, vigor, concentration and morphology), computer-assisted sperm analysis (total and progressive motility) by Sperm Class Analyzer software (SCA, Microptics, Barcelona, Spain) and flow cytometric analyses to estimate the percentage of cells with plasma membrane integrity (PMI), acrosomal integrity with intact plasma membrane (AIMI), high mitochondrial membrane potential with intact plasma membrane (MIHP), proportion of sperm with a ratio of high: low mitochondrial potential (HP/LP) and mitochondrial ROS generation (mROS). Samples for flow cytometry analyses were diluted (5×10^6 spermatozoa/mL) in a Tyrode's medium (TALP). For simultaneous assessment of AIMI, a sample of 150 µL of semen diluted was incubated per 8 min at 37 °C, in the dark, with 1 µL peanut agglutinin conjugated with fluorescein isothiocyanate (FITC-PNA; L-7381 Sigma Chemical Co.), 1 µL of propidium iodide (PI, 0.5 mg/mL; P4170, Sigma) and 1 µL of Syto-59 (750 mM; S11341-Thermo Fisher). For simultaneous assessment of MIHP and HP/LP, a sample of semen diluted (150 µL) was incubated per 8 min at 37°C in the dark with 1 µL of 5,5′,6,6′tetracloro 1,1′,3,3′tetraetilbenzimidazolilcarbocianin iodide (JC-1, 153 µM, Life Technologies), 1 µL of PI (0.5 mg/mL) and 1 µL of Syto-59 (750 mM). For mitochondrial ROS generation samples were incubated 1 µL Mitosox red (2 µM, MSR-M36008, Thermo Fisher), 2 µL de SYTO-59® and 1 µL de YOPRO (25 nM, Y3603, Thermo Fisher) per 30 min at 37 °C in the dark. Flow cytometry assessments were conducted using a conventional flow cytometer Accuri™ C6 (BD Pharmingen™). Data were analyzed with R software (version 3.3.1.) by analysis of variance. Preliminary results were expressed as mean and standard error of mean (s.e.m.). The pregnancy rates (%) were 62.55±5.36% for HF and 51.85±3.23% for MF ($p<0.05$). As regards to conventional sperm analysis, no differences were observed between groups of HF and MF. There were no differences ($P>0.05$) observed to total motility between HF (66.91±8.8%) and MF (68.7±17.57%), or for progressive motility (HF= 49.95±12.5%; MF = 46.5±16.5%). Flow cytometric tests had not statistical differences ($P>0.05$) between HF and MF, to PMI (49.14±10.02 and 44.94±9.5%) and AIMI (48.76±9.9 and 43.60±8.1%) respectively, however, numerically MIHP was higher for HF (23.83±12.98%) than MF (16.88±9.85%) and the HL/PL proportion decreased to MF (1.86±1.58%) when compared to HF (2.38±1.64%). The median fluorescence intensity of MSR were 451.50±343.88% to HF and 540.57±223.38 to MF ($P>0.05$). The decrease of MIHP and HP/LP ratio, together with and the increase of mROS in the group of MF, with no decrease in sperm motility, suggested that the decrease mitochondrial activity could be affecting the fertilization capacity. In conclusion, the sperm mitochondrial activity could be influencing the sperm fertilization. Then, analyses of sperm mitochondrial functions may be useful characteristics to consider predicting bull fertility; however, further study should be conducted to test this hypothesis.

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Semen quality in young men from Belo Horizonte metropolitan area: a matter of concern?

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After the publication of a broad revision by Carlsen et al (1992), the human fertility that in recent decades is showing a sharp decline in many different countries, has been a recurrent issue of debate. Particularly in Brazil, in the last 50 years the total fertility rate (the number of offspring born per mating pair) has drastically decreased from 6 to 1.86 children, which is below the replacement rate (2.1) of the population. Despite the great influence of socio-economic factors, it has been shown in some countries that biological factors also play an important role in the decline of men fertility. Accordingly, several biological factors such as reproductive tract diseases, diet, alcohol and tobacco consumption and exposure to environmental contaminants, among others, can be linked to the falling of sperm quality. In Brazil, in spite of the apparent decline in fertility, there are no detailed and comprehensive reports investigating the decrease of sperm quality and their eventual causes. Our main objective was to evaluate the semen quality of young men from Belo Horizonte metropolitan area. Men aged between 18 and 23 year-old from several universities of Belo Horizonte and its metropolitan region were invited to participate as volunteers through posters, flyers, electronic media, social networking, radio and television. The inclusion criteria were place of residence in the Belo Horizonte metropolitan area, and both the man and his mother being born and raised in Brazil. The participation rate among men was 60%. Semen analyses of 235 young men were performed according to the WHO (2010) guidelines. In brief, semen samples were collected by masturbation in the privacy of a room and kept at 37°C. Semen volume was estimated by weighing the collection tube with the semen sample and subtracting the predetermined weight of the empty tube, assuming that 1 mL semen =1 g. For sperm motility assessment, 10 µL of well-mixed semen was placed on a glass slide kept at 37°C and covered with a 22×22 mm coverslip. The preparation was placed on the heated stage of a microscope at 37°C and immediately examined at ×400 magnification. The sperm were classified as progressively motile, locally motile or immotile. For the assessment of the sperm concentration, the samples were diluted in a solution of 0.6 mol/l NaHCO3 and 0.4% (v/v) formaldehyde in distilled water. The sperm concentration was subsequently assessed using a Neubauer chamber. Regarding sperm concentration, 27% and 21% of men evaluated had a sperm concentration below 20 x 10^6/mL and 15 x 10^6/mL, respectively; and 49% below 40 x 10^6/mL, a cutoff for subfecundity. Furthermore, 16% and 13% of them showed total sperm count and total motile spermatozoa in the ejaculate below 39 million/mL and 15.6 million/mL, respectively. In addition, nearly 8% of men had semen volume and percentage of progressive motile spermatozoa (AB) and of motile spermatozoa (AB + C) below the suggested values by WHO (2010). Overall, 32% from analyzed men had at least one seminal parameter below the reference values. Median of some sperm parameters were comparable to those seen in young men in Denmark, Finland, and Japan. However, young Brazilian men have showed lower median sperm concentration (40 x 10^6/mL) compared to data from the aforementioned countries (48 x 10^6/mL, 50 x 10^6/mL and 59 x 10^6/mL, respectively). To our knowledge, this is a pioneer study in Brazil concerning young men fertility. In association with the questionnaires data, hormonal and endocrine disrupter measurements, we expect to provide more comprehensive information regarding men fertility, allowing us, for instance, to evaluate the eventual influence of lifestyle, diet, and endocrine disruptors on semen quality.

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Seminal plasma proteins after testicular insulation in Brangus bulls

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Seminal plasma is comprised of secretions originating from the accessory sex glands, epididymis, and the testis, that contribute to reproductive performance and helps to prevent premature capacitation and to protect sperm against oxidative stress for normal spermatogenesis. Detection of changes in these mechanisms will serve to determine possible causes of the decrease in the seminal production. The objectives of the present study were to identify seminal plasma proteins using 2D-GE and to determine the correlation between individual proteins and semen characteristics among insulated and non-insulated Brangus bulls. We evaluated seminal plasma proteome using 2D-GE among insulated and non-insulated Brangus bulls**.** All procedures were approved by the Ethical Committee for Care and Use of Experimental Animals (Project 26250, CEUA/UFRGS). Nine Brangus bulls were randomly allocated into two groups: scrotal insulated bulls for 72 hours (Insulated Group; n=6) and non-insulated bulls (Control Group; n=3). Semen collections were performed using eletroejaculator on the day of insulation (D0) and weekly for the following 13 weeks (W1 to W13). In order to verify the effect of insulation on seminal plasma protein composition, samples were analyzed by 2D SDS-PAGE on D0, W4 and W13. Semen samples were centrifuged at 700 × g for 10 min (at 4°C) to separate seminal plasma from sperm. Then, the supernatant was centrifuged at 10,000 × g for 60 min, at 4°C, divided into aliquots and kept at −80 °C. Total protein concentration was determinate using a BCA kit (Pierce) using BSA as standard. 2D SDS-PAGE was carried out as described by Moura *et al.* [Journal of Andrology, 27:201-211, 2006; Journal of Proteomics, 73:2006-2020, 2010] and images were analyzed using PDQuest software. Seminal analyses and optical density spots were normalized and statistically compared between groups (at insulation, post-insulation and recovery) by means of Student's t-test or ANOVA followed by Tukey test, assuming significance when $P<0.05$. Overall, sperm motility was greater in the control group than in the insulation group (76.7 vs. 52.0%, respectively; $P<0.05$). The proportion of total sperm defects in insulated group was higher than control group from W2 to W7 ($P<0.05$). PDQuest software in 30 seminal plasma gels constructed within the 3–10 pH range detected an average of 65±28 spots. No difference in the number of spots was observed between groups tested: D0 61±27, W4 72±18 and W13 52±18 spots. The analysis of 2D gel by PDQuest software allowed the quantification and identification of protein spots differently present at D0, 4, and 13 weeks after insulation when sperm parameters were normalized. We observed ten spots that were differentially expressed, where eight had a higher optical density in post insulation group and two where more abundant in recovery group. This variation in sperm function after insulation could be explained by variation in the protein composition of seminal plasma. This study confirms that multiple proteins collected from Brangus bulls are associated with semen parameter after scrotal insulation. We conclude that interactions among several proteins explain a significant proportion of the variation during scrotal heat stress induction in Brangus bulls. These findings emphasize that further studies at the molecular level, e.g. using tandem mass spectrometry, are needed to characterize and to understand different semen handling procedures in bovine.

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Strategy for purification of Binder of Sperm proteins from ram seminal plasma

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The seminal plasma is considered a heterogeneous mixture of components that affect sperm functions *in vivo* and *in vitro*. Numerous studies in different species of mammals show the presence of seminal plasma proteins directly linked to fertility. Thus, the objective of the present study was to develop a protocol for purification of two proteins of the Binder Sperm protein (BSP) family, named Ram Seminal Vesicle Proteins with 14 kDa (RSVP14) and 22 kDa (RSVP22). Seminal samples were collected from 16 adult Morada Nova rams by eletroejaculation. Andrological evaluation confirmed that all animals were healthy and reproductively sound. Seminal plasma was separated from sperm by two centrifugations (700g 4ºC 15 min and 5000g 4ºC 60 min). A pool was made with the seminal plasma from all 16 animals and stored at -20°C. Protein concentration was determined by the Bradford method. Fast Protein Liquid Chromatography (FPLC) was used to separate proteins (10 mg total) from the seminal plasma samples using a Gelatin-Sepharose™ column and binding buffer consisting of 40mM Tris and 2mM $CaCl_2$, pH 7.0. In addition, elution buffer comprised of 40mM Tris, 8M Urea and 2mM $CaCl_2$, pH 7.0 was also used. Proteins were eluted in three chromatographic peaks. The first two peaks contained proteins with low affinity for gelatin and the third peak included proteins with high affinity for gelatin. This last group of proteins probably contains fibronectin type II domains in their structure. All three peaks of the eluted content were concentrated using a 10kDa filter (MWCO Vivaspin 10,000 Da, GE) and the material retained by the filter was quantified by Bradford's method. An aliquot of 20 µg was removed from each peak and subjected to 12.5% SDS-PAGE. To confirm the presence of the BSP proteins in the seminal plasma samples, Western blots were carried out using anti-ram BSP as primary antibody (1: 6,000) and Dnk to pAb Rb IgG anti-rabbit (1: 5,000) as secondary antibody (Abcam, Cambridge, UK). The molecular weights of the proteins separated by electrophoresis were confirmed using Quantity One software (Bio-Rad Laboratories, USA). The bands detected in the gelatin affinity peak were between 12 and 52kDa. Immunodetection by Western Blot showed specific staining for BSPs in all peaks analyzed, with higher staining in the gelatin affinity peak. The gelatin affinity peak was subjected to affinity chromatography on HiTrap ™ Heparin HP column with binding buffer consisting of Tris 40 mM and $ClCa_2$ 2mM, pH 7.0 and elution buffer consisting of Tris 40 mM, 2 mM $CaCl_2$ and $NaCl_2$ 1mM, pH 7.0. Two chromatographic peaks were well formed and defined. The first peak represented the non-ligated fraction with a maximum amplitude of 120 mAu; and the second peak representing the heparin affinity with 80 mAu for maximum amplitude. The two peaks were centrifuged twice at 5000g for 60 min at 4°C using the 10kDa filter used before. All the material retained on the filter was again quantified by Bradford's method. The proteins in the two peaks were detected by SDS-PAGE and Western Blots as described above. By Quantity One analyses, the first peak showed 5 bands of 14, 15, 22, 24 and 40 kDa, while the second peak showed 6 bands of 12, 16, 21, 22, 170 and 198 kDa. The Western blot testing confirmed the presence of BSP proteins of 14 kDa for the first peak and 22-kDa in the both peaks Therefore, it was observed in the present study that quantitative technique using two affinity columns identified two proteins of interest, but RSVP´s couldn't be completely isolated. Soon, it is necessary to use a third chromatographic column to separate completely the two protein bands and use them in functional tests.

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Testis-specific isoform of Na, K-ATPase: a novel potential fertility marker for dairy bulls

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Testis-specific isoform of Na, K-ATPase (ATP1A4) regulates motility and capacitation in bovine sperm (Thundathil et al., 2006). Objectives were to evaluate: 1) differences among bulls in the content of ATP1A4; 2) effects of sperm cryopreservation on relative content of ATP1A4; and 3) associations between the content of ATP1A4 and post-thaw sperm quality. Semen was collected from five mature Holstein bulls (three ejaculates per bull, 7-d intervals), all with acceptable semen quality (fresh and frozen-thawed). An aliquot of each fresh ejaculate was washed in Percoll™, re-suspended in TALP and evaluated (flow cytometry) to determine sperm viability (LIVE/DEAD®; Life Technologies) and the relative content (median fluorescence intensity in live cells) of ATP1A4. Sperm kinematic parameters (CASA; Sperm Vision; Minitube), acrosomal status (FITC-PSA), and sperm morphology (eosin-nigrosin staining) were evaluated. Semen was extended in Triladyl (20% egg yolk; final concentration, 50×10^6 sperm/mL), chilled (5 °C), loaded into 0.5-mL straws, and cryopreserved using the standard bovine program (Ice Cube 14 S-B; Minitube). After thawing (37 °C for 30 s), semen samples were washed in Percoll™ and evaluated as described above. Data from fresh and frozen-thawed samples were analyzed using two-way ANOVA, with bull and sample as main effects, and Tukey's HSD test for multiple comparisons. In addition, Pearson correlations (two-tail) were determined. Data are expressed as non-transformed means ± SEM and results were considered significant when $P < 0.05$. Sperm kinetics (CASA), morphologically normal sperm, and sperm with an intact acrosome did not differ significantly among bulls for either fresh or frozen-thawed samples. However, viability of frozen-thawed samples differed among bulls (range 23.2 ± 0.7 to 42.3 ± 1.7%, $P < 0.0001$). Similarly, relative content of ATP1A4 for fresh (713.3 ± 26.0 to 1043.0 ± 49.8, $P < 0.001$) and frozen-thawed sperm (604.3 ± 4.1 to 829.0 ± 4.7, $P < 0.0001$) differed among bulls. Compared to fresh sperm, frozen-thawed sperm had reduced kinematic parameters ($P < 0.0001$ for all except ALH), and it had fewer intact acrosomes (92.3 ± 0.8 vs 84.4 ± 0.9%, $P < 0.0001$) and a lower relative content of ATP1A4 (867.9 ± 31.4 vs 709.4 ± 21.1, $P < 0.0001$). Furthermore, relative contents of ATP1A4 in fresh and frozen-thawed sperm were correlated ($r = 0.702$, $P = 0.004$), although there were no significant correlations between ATP1A4 content and any other sperm parameter. The reduction in the relative content of ATP1A4 on thawed sperm could be a consequence of capacitation-like changes after sperm cryopreservation (Medeiros et al., 2002), including cholesterol efflux of sperm membrane (Rajoriya et al., 2013), inducing an alteration in the ATP1A4 orientation (Hickey and Buhr, 2011) as cholesterol is the lipid-binding site of β subunit ectodomain of ATP1A4 (Morth et al., 2011). In conclusion, content of ATP1A4 differed significantly among dairy bulls and cryopreservation procedures reduced the relative content of ATP1A4. Considering its role in sperm function, since ATP1A4 null mice are sterile (Jimenez et al., 2011); the significant variation among dairy bulls, and lack of association with classical sperm parameters, we inferred that ATP1A4 has potential as a novel marker for sperm quality and/or fertility.

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The action of L-arg/no in the in vitro bovine sperm capacitation does not occur via cGMP signaling pathway

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Nitric oxide (NO) participates in intra- and intercellular signaling and it is formed by a catalytic reaction from L-arginine (L-arg). In sperm, NO has been related to improvement in motility and capacitation, which favors the oocyte fertilization. Its biological effects can be induced by cyclic guanosine monophosphate (cGMP). It's not clear if there is any relationship between NO, increased level of sperm cGMP and sperm capacitation in bovines so far. Thus, the aim of this study is to investigate whether sperm capacitation is related to the L-arg/NO/cGMP signaling pathway. Two experiments were performed as follows: 1) Evaluation of sperm capacitation; and 2) Measurement of the concentration of cGMP in sperm after capacitation. Cryopreserved sperm from three Nellore bulls (*Bos indicus*) were used with four replicates for each bull (n=12) for experiment 1 with *in vitro* capacitation induced in 200 µL of capacitating medium supplemented with 20 µg/mL heparin (control) plus treatments, as follows: L-arg) 1mM L-arg (precursor of NO); PTIO) 1mM PTIO (chelator of NO); L-arg+PTIO) 1 mM L-arg and 1mM PTIO. Experiment 2 was carried out with three replicates for each bull (n=9) and one more group was included for treatment: Br-cGMP+PTIO) 5mM 8-Br-cGMP (cGMP analogue) and 1mM PTIO. Sperm capacitation was evaluated by fluorescent labeling of chlortetracycline (CTC), and the measurement of the concentration of cGMP was performed by ELISA. Data were subjected to analysis of variance (ANOVA) and means compared by Tukey test at 5% probability. In experiment 1, L-arg had a higher percentage ($P<0.05$) of capacitated sperm (69.82 ± 3.42) compared to control, PTIO and L-arg+PTIO (54.36 ± 3.38, 45.45 ± 2.70 and 52.93 ± 2.47, respectively) and PTIO had the lower percentage compared to the other groups ($P<0.05$). Opposite results were found for non-capacitated sperm when PTIO had a higher percentage (21.65 ± 4.14, $P<0.05$) and L-arg had the lowest percentage (6.67 ± 1.68, $P<0.05$) compared to control and L-arg+PTIO (14.84 ± 2.36 and 16.75 ± 2.30). L-arg had a lower percentage of reacted acrosome (23.49 ± 3.51, $P<0.05$) compared to control, PTIO and L-arg+PTIO (30.79 ± 3.91, 32.90 ± 3.39 and 30.07 ± 2.37, respectively). In experiment 2, the concentration of cGMP intrasperm was higher ($P<0.05$) in Br-cGMP+PTIO (4.37 ± 1.06) compared to control, L-arg, PTIO and L-arg+PTIO (1.09 ± 0.58, 1.52 ± 0.51, 1.25 ± 0.34 and 1.34 ± 0.67, respectively). There was no difference in the concentration of cGMP between control, L-arg, PTIO and L-arg+PTIO ($P>0.05$). These results suggest that during *in vitro* sperm capacitation: 1) the increase of NO by the addition of L-arg improved sperm capacitation and 2) the action of L-arg/NO in the *in vitro* bovine sperm capacitation does not occur via cGMP signaling pathway.

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The major proteome of *Bubalus bubalis* seminal plasma

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The seminal plasma is a complex fluid that serves as a carrier for the spermatozoa in the female genital tract and its constituents are relevant for sperm functions. Composition of seminal fluid has been extensively investigated in several species because of its potential applications for diagnosis and treatment of infertility and markers of sperm resistance to freezing. Thus, the present study was conducted to describe the main proteins expressed in the buffalo seminal plasma. Four ejaculates of each bull were collected with artificial vagina from six adult buffalo bulls (Murrah breed) with acceptable semen attributes (motility ≥ 70%, morphological abnormalities ≤ 30%). The seminal plasma was harvested from semen samples by centrifugations at 4°C (700xg for 15 min. followed by 10.000 xg for 1 h) and total protein was estimated by Bradford's method. Seminal plasma proteins were subjected to electrofocusing (18 cm, pH 3-10NL) followed by SDS-PAGE. Gels were then stained with Coomassie blue and analyzed using PDquest software (Bio-Rad, USA). Proteins were identified by tandem mass spectrometry (ESI/QUAD-TOF) and data sets were analyzed using bioinformatics tools. We used Strap software for gene ontology annotation and *in silico* protein-protein interaction networks were evaluated by String, v. 10.0 (http://string-db.org/). An average of 360 protein spots was identified in the 2-D maps of buffalo seminal plasma, 171 spots were identified by mass spectrometry, corresponding to 67 different proteins. Secretoglobin accounted for 12,2% of all spots identify in the gels. Clusterin and albumin comprised the second and third most abundant constituents. Other proteins identified in *Bubalus bubalis* seminal plasma include spermadhesin, zinc-alpha2-glycoprotein, alpha-2-macroglobulin, epididymal secretory glutathione peroxidase, epididymal secretory protein E1, epididymal-specific lipocalin-5, superoxide dismutase, beta-hexosaminidase, double-headed protease inhibitor, leucine-rich alpha-2-glycoprotein, melanotransferrin, BSP30 kDa, serotransferrin, seminal plasma protein A3, among others. The most prominent molecular functions linked to the identified proteins were binding and catalytic activity, followed by antioxidant activity. The two main biological processes of the buffalo seminal plasma proteins were characterized as cellular process and regulation. With regards to cellular component, proteins were mainly extracellular, followed by cytoplasm and other intracellular organelles. Based on network analysis, the most abundant seminal plasma constituents (secretoglobin, spermadhesin, albumin, clusterin and zinc-alpha2-glycoprotein) have a diverse array of interactions, including protease and protease inhibitors, enzymes and growth factors. In conclusion, the present work describes, for the first time, the major seminal plasma proteome of adult the buffalo. Knowledge of physiological characteristics of each species allows the improvement of reproductive technologies and efficiency of animal production.

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Volume and testicular weight correlation with sperm quality of domestic cats

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The study of reproductive function of domestic cats are important, particularly because its potential as a model for wildlife. Although the morphological characteristics of cats testicles has been described, the comparison between the volume or testicular weight with sperm quality is scarce. The aim was to compare the testicular volume and weight with the characteristics of the sperm collected from the epididymis tail of cats. Twenty two adult cats were used, with testicles in the scrotum, mongrel, semi-domiciled, in good nutritional status, body weight between 2 and 6 kg. The testes were measured for length (L), width (W) and height (H) individually using stainless steel digital caliper (Kobalt®, model 53247, North Carolina, USA). To calculate the testicular volume of each cat was applied the formula: testicular volume (VT) = L x W x H x 0.5236. The animals underwent elective orchiectomy and, after surgical resection, the testes were weighed in analytical precision scale (Bel Engineering®, Piracicaba / SP, Brazil) getting the results in grams (g). The values of the left and right testes were added and was obtained a mean weight and volume for each cat. The collection of spermatozoa was performed by compressing the vas deferens and the epididymis tail in a Petri dish containing 100 mL of 0.9% NaCl solution for each epididymis. Total motility, progressive motility, sperm concentration, VAP, VSL and VCL were obtained by computer analysis (CASA, Hamilton-Thorne® IVOSII, Beverly, USA), sperm morphology was evaluated in smear stained by the modified Karras. Data were analyzed using Pearson correlation test by BioEstat 5.3 program, with a 1% significance level. The overall mean (± standard deviation) of the testicular volume was 1295.5 (± 596.4) mm³, the average weight was 1.94 (± 0.4) g, the average sperm motility was 67.7 (± 13.5)% progressive motility 37.5 (± 11.4)%, VAP 168.4 (± 75.2) µm/s, VCL 168.4 (± 75.2) µm/s, VSL 100, 8 (± 35.4) µm/s and concentration was 41.2 (± 22.9) x106/ml. There was a moderate correlation ($r > 0.5$) between testicular volume and sperm concentration ($r = 0.60$; $p = 0.002$), the other correlations with volume and average weight were low ($r < 0.5$). Interestingly, there was a low correlation between the weight and testicular sperm concentration ($r = 0.42$; $p = 0.046$) as well as between the weight and testicular volume ($r = 0.42$; $p = 0.532$), so the heavier testicles have not always higher volume, higher sperm concentration or sperm with better quality. We concluded that the volume and weight of the testicles did not influence the quality of the harvested sperm from epididymis tail of cats, however, testicular volume influenced the obtained sperm concentration

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Bovine Induced Pluripotent Stem Cells Attachment and Proliferation in Synthetic, PVA-Based Hydrogels

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Introduction: Tissue engineering is a growing field in biomedical sciences that seeks to combine cells and matrices in an appropriate culture environment, so that cells proliferate and differentiate to repair or regenerate a damaged tissue or organ. Several combinations of stem cells and substrates have been tested with reasonable success in terms of cell adherence and proliferation. However, the therapeutic potential of stem cells depends largely on their capacity to be created and maintained under strict controlled conditions, without any undefined component in culture which excludes the use of animal derived substrates. Induced pluripotent stem cells (iPSCs) represent an important source of undifferentiated embryonic-like stem cells, that are usually derived and maintained on a substrate of mouse embryonic fibroblasts (MEFs) used as feeder cells. Culture of iPSCs under feeder-free conditions is a critical step to make them a clinically suitable cell source for regenerative medicine. In addition, the ability of stem cells to interact, adhere and replicate in culture is paramount for their use in cell-based therapies. In the present study, PVA-based hydrogels were used as substrates for bovine iPSCs adherence, proliferation and generation of new primary colonies. Material & Methods: Hydrogels were prepared with the water-soluble synthetic polymer polyvinyl alcohol (PVA; 10% and 20% w/v) dissolved in deionized water at 40º.C. 1, 2, 3, 4-butanetetracarboxylic acid (BTCA; 1% w/v) was added to the solution as a cross-link agent and hydrochloric acid (HCl; 1% w/v) as a catalyst. The reaction mixture was kept under magnetic agitation for 10 minutes at 40º.C. Hydrogels were obtained after solvent evaporation and placed on the bottom of 4-well dishes prior to the experiments. Mouse embryonic fibroblasts were isolated from day 13–14 crossbred fetuses. Cells were used to assess a putative cytotoxicity of the hydrogels prior to their use as substrates for biPSCs culture. BiPSCs were obtained from the 2, 4 biPS cell line at 22 passages. Trypsin isolated biPSCs were placed onto the surface of the pre-equilibrated PVA- based hydrogels (150 x 10^5 cells/well). Cell adherence to the substrates was assessed as the dishes were gently swindled 24 h later. The attached cells were left in culture to generate new biPS colonies for up to five days in a humidified atmosphere of 5% CO_2 in air at 38.5^{o}C. Medium was changed every 48 hrs and cultures were examined with Inverse phase-contrast microscope. At day-5 the hydrogels were fixed and stained with hematoxilin and eosin for optical microscopy analysis. Experiments were made in triplicate and repeated three to four times. Results: Results show that the two hydrogel formulations are not cytotoxic to MEFs and biPSCs. Both cell types attached to the substrates and proliferated over the culture period. MEF cells interaction with the hydrogel were mediated by cell extensions that twisted around the substrate fibers suggesting cell migration on the hydrogels. Cell extensions from a single cell appeared to interact with more than one substrate fibers. Isolated biPSCs attached to the hydrogels within 24 hrs of seeding them onto the hydrogels. They formed primary colonies that expanded over the 5-days culture period indicating good cell-substrate interaction. Comparisons of the two hydrogel formulations show that the 10% PVA-based substrate allowed superior biPSC adhesion and proliferation rates. Conclusion: PVA-based hydrogels are not cytotoxic for somatic and pluripotent stem cells. They allow biPS cell attachment and proliferation, which make them an interesting chemically defined substrate to be tested for the derivation and maintenance of clinical grade human iPSCs.

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Caracterization of canine amniotic membrane stem cells in diferents gestational stages

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Mesenchymal stem cells (MSCs) are undifferentiated cells that have the ability of immunomodulation and tissue renovation. Based on their properties that are ideal for cell therapy, the aim of this study was to characterize the stem cells of canine amniotic membrane (AMSCs) in different gestational stages. Twenty canine fetus placenta (mixed breed) at 20 to 30 days, 35 to 45 days and 50-60 days of gestation were obtained from dogs during neutering campaigns (Pirassununga-SP). The amniotic membrane samples were processed, cell were cultured, and frozen for cell characterization through the cell viability, grow curve, colony forming (UFC), and *in vitro* differentiation into adipogenic, chondrogenic and osteogenic linages. The results showed that all different gestational stages cells were fibroblast morphology and adherence to the cell culture plate. The cell viability test there were an average of the 83.3% at 20-30 days, 80.1% at 35-45% and 75.22% at 50-60 days of life cells after frozen. The grow curve cells showed moderate growth for the first two passages to confluency of the 80% after 144hours. The colony forming it was used $5x10^4$ cells at the third passage at 20-30 days of gestation without colony formation. However, all the cells had the capacity to differentiate into the three linages after 21 days of culture. Based on these results, we can conclude that AMSCs have characteristics compatible with MSCs, which can be confirmed after testing immunophenotyping and gene expression.

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Generation of porcine induced pluripotent stem cells (piPS) in different culture conditions

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The discovery that somatic cells can be reprogrammed into a pluripotent state with exogenous expression of four transcription factors (induced pluripotent stem cells or iPS) has changed the future of stem cell research, biotechnology and cell therapy. There is a great interest in deriving iPS cells from domestic animals such as pigs. Reprogramming of pig somatic cells into iPS cells provide an advance in the field of regenerative medicine by representing an ideal large animal model for the preclinical therapies, however a reproducible protocol is still not reported. Therefore, the aim of this study was to generate porcine induced pluripotent stem (piPS) cell lines testing different culture conductions during reprogramming period. Fibroblasts used in this study were derived from the skin of stillbirth piglets. Briefly, skin tissue was sliced into 1–2mm^2 explants, plated on a 60mm culture dish and incubated in 5% CO_2 at 38,5°C. Fibroblasts were cultured in Iscove's Modified Dulbecco's Media (IMDM; Invitrogen), 10% fetal bovine serum (FBS; Invitrogen), penicillin/streptomycin (100 U·ml−1 penicillin and 100 µg·ml−1 streptomycin; Sigma). The fibroblasts were transduced with lentiviral polycistronic excisable vector containing the murine or human cDNA of OCT4, SOX2, c-MYC and KLF4 transcription factors (OSMK, STEMCCA vector, Sommer et al. 2009). Five to six days after transduction the cells were replated onto mouse inactivated fibroblast feeder layers (MEFs) and further cultured in iPS medium: KnockOut DMEM/F-12 medium (Invitrogen, USA) with 20% KnockOut Serum Replacement (KSR; Cat# 10828028, Invitrogen, USA), 0.5 mmol L−1 L-glutamine, 1% NEAA, 0.1 mmol L−1 β-mercaptoethanol, 1% penicillin/Streptomycin. During reprogramming period, the cells transduced with murine or human OSMK were further supplemented with the conditions: 1) 4 ng mL−1 basic Fibroblast Growth Factor (bFGF, Cat # 13256029, Invitrogen, Carlsbad, Califórnia, EUA) and 4ng ml-1 Leukemia inhibitory factor (LIF; Cat # ESG1107, Millipore, Alemanha), 2) only bFGF or 3) only LIF. The results of piPS cell colony derivation showed that both murine or human OKSM efficiently derived the first colonies at day 12 post-transduction. All of the piPS cells generally grew in colonies with a well-defined border. Most piPS cells exhibited a round or oval shape similar to the morphology of mouse iPS cells. piPS lineages were replated at 3-4 days when in first passage and approximately at every 7 days in subsequent passages. When mouse OKSM was used for reprogramming, efficiency rates of colonies formation were 56.27% overall and when different reprogramming supplementation was analyzed rates were 54.6% for bFGF group, 54.2% for LIF + bFGF and 60% for LIF group. The percentage of alkaline phosphatase (AP)- positive colonies detection after reprogramming with murine OSMK was 95.81% for bFGF group, 94.78% for bFGF+ LIF and 58% for LIF groups, and these same colonies were OCT4 positives. When human OSMK was used, reprogramming rates were 52.7% in total and 53.3 % for bFGF group, 52.6% for LIF+bFGF group and 52.17% for LIF group. Interestantly, piPS cell lines generated with human OSKM were not positive for AP or OCT4. Overall, our preliminary results showed that somatic cells derived from swine skin can be reprogrammed to a pluripotent state with murine OSMK, when cultured with LIF, bFGF or both. Human OSMK were not able to efficiently reprogram swine cells. Best results were observed when reprogramming was performed with both LIF and bFGF or only bFGF.

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In vitro induced differentiation and reprogramming into pluripotency of mesenchymal cells and fetal fibroblasts in cattle

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In vitro induced pluripotent cells (iPS) have been produced from different cell types in humans and mice, however, generation of these cells in other animal models is yet not well established and reproducible. The generation of iPS cells from large animals is highly desirable for the establishment of biomedical models and also may contribute with different reproductive technologies. For example, less differentiated cells have been reported to be more easily reprogrammed through nuclear transfer of somatic cells allowing the generation of genetically superior individuals with a higher efficiency. The objective of this study was to characterize two types of differentiated cells - adult mesenchymal derived from adipose tissue (AdMSC) and fetal fibroblasts (FF) regarding their potential of *in vitro* differentiation and reprogramming. Cell lineages were assessed for doubling time (DT), *in vitro* differentiation into mesodermal lineages and induction of pluripotency in different conditions. The DT was performed in triplicate in 6 wells plates, cell passaging was performed enzymatically (Tryple Express, Gibco) at every 48 hours, the number of cells counted by Neubauer chamber and calculated with the formula DT= (T -T0) log 2/(log N -log N0) where (T - T0) = time (hours) of incubation between passages, N0 = initial number of cells plated, and N = number of cells recovered after tripsinization. The induction into mesodermal lineages (adipocytes, chondrocytes and osteocytes) were performed using two different commercial protocols GIBCO life Technologies – StemPro e R&D SYSTEMS StemXVivo™ and the results analyzed by cytological morphology and specific staining (Sudan Black, Alcian Blue and Alizarin Red). FFs and AdMSCs were submitted to reprogramming trough transduction with human or murine transcription factors OCT4, SOX2, c-MYC and KLF4 (OSMK, STEMCCA vector) and cultured in KnockOut DMEM F-12, 1% MEM non-essential Amino Acids, 20% KnockOut Serum Replacement (KSR), 1% penicillin/streptomycin, 0,5 mmol L-glutamine and 0,1 mmol 2-mercaptoethanol, in different supplementation conditions: 10 µg/mL bFGF (Peprotech), 1:1000 µL LIF (Millipore), or both. FFs showed a decreased DT when compared to AdMSC, 22.76 h and 26.17 h respectively. Both lineages differentiated into mesodermal lineages, however FFs showed improved morphological changes. iPS cells were generated from FFs reprogrammed with murine pluripotency factors but not human factors in the different conditions during reprogramming. Preliminary analysis shows that the obtained iPS cells are positive for the detection of alkaline phosphatase and immunocytochemistry for Nanog, Oct4 and Sox2 proteins. Interestingly, mesenchymal cells did not generate iPS cells in any of the conditions tested, suggesting that cellular lineages with improved DT and differentiation capacity of FFs cells may be more amenable to *in vitro* reprogramming into pluripotency.

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Viability of canine spermatogonial stem cell (SSCs) by flow cytometry after cryopreservation *in vitro*

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The establishment of spermatogonial stem cells (SSCs) cryopreservation protocols have been described in many domestic animals, including to dogs. This study aims to subjugate these cells biotechnology the transplantation of germ cells, the genetics storage preservation and viability, especially of valuable animal, and comprehend the spermatogenesis' process. The aim of this study is perform several cryopreservation protocols in order to rate viability and survival indexes of the canine spermatogonial stem cells after cryopreservation. SSCs were obtained from testis of prepubertal and adults (3-6 month-old, 12 month-old or more) processed enzymatic digestion and by percoll's gradient density. After this process, the cells were cultured in DMEM F12 (Cat #BR-30004-05, LGC, Cotia, Brazil), supplemented with 10% of Fetal Bovine Serum (FBS, Cat#12657029, Gilbco, Invitrogen, Carlsbad, California, USA), 6mM de L-Glutamine (Cat#25030-081, Invitrogen, Carlsbad, California, USA), 0,5mM of Pyruvate (11360-070, Thermo scientific, Carlsbad, California, USA), 10.000 U/ml of Penicillin-Streptomycin (Cat#15140-122, Thermo scientific, Carlsbad, California, USA) and 100 U/L of Amphotericin B (Cat# A2942,Sigma-Aldrich Corp., St. Louis, MO, USA), according Dobrinski et al. (1999) and the cells were evaluated using flow cytometry analysis for GFRA1 receptor and Propidium Iodite (PI). The canine SSCs were cryopreserved resorting four different protocols: (1) slow freezing, (2) fast freezing, (3) vitrification and (4) 10% DMSO, all combined with polyethylene glycol (PEG), dimethyl sulfoxide (DMSO), fetal bovine serum (FBS) and sucrose (which according to lee et al. 2016 increased SSC viability after thawed). After two months cryopreserved, the canine SSCs were defrosted and reevaluated by the flow cytometry analysis for the GFRA1 and PI markers. Before cryopreservation, the flow cytometric analysis showed that 86.11% of the germ cells were alive (PI negative) and 12.61% of them were dead (PI positive). After we defrosted them the percentage of germ cells PI negative in mean was of 93.20% and 18.63% for GFRA1 positive. However, analyzing the four experienced protocols, the best results were observed with fast freezing 93.49% PI negative cells and 14.97% GFRA1 positive. Then the cells were placed in culture for another five days and one new analysis were performed, they showed lower viability rates when we cryopreserved the cells only 10% of DMSO (77.02% were PI negative and 10.56% were GFRA1 positive). Overall, our preliminary results showed that the cryopreservation process do not make drastic changes to the cells' viability and the best performance results were obtained, when the SSCs were cryopreserved with fast freezing protocol.

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Arginine effect on seminal production and sperm motility of *Rhamdia quelen* broodstock

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Broodstock nutrition has become prominent in research, presenting positive results at improving reproductive performance of many species. This study aimed to evaluate arginine effect on *Rhamdia quelen* seminal production and sperm motility. For this purpose, 45 male fish were divided among five treatments and four replications. They were kept in cages (40 fish/m^3 density) for six months, fed with diets containing different arginine levels (1.37, 1.67, 1.97, 2.27 and 2.57%). Broodstock underwent hormonal induction (3.0 mg of Carp Pituitary Extract.kg-1), and after 240 Accumulated Thermal Units semen was collected by aid of Falcon centrifuge tubes for volume measurement. Sperm concentration was measured from the semen dilution in buffered saline formaldehyde (1:1000) and counted by Neubauer chamber. Sperm motility test was performed using CASA software (Computer Assisted Sperm Analysis), from free software. Sperm activation was carried out by diluting the semen in water at the ratio of 1: 400 (semen: water). Analyzes were performed by light microscopy (10X) at a rate of 100 *frames*.s^{-1}. Videos were edited and sperm movement parameters were evaluated at the 10th second after sperm activation. The total number of sperm cells was calculated from the ratio between seminal volume and sperm concentration. The total of sperm moving was calculated from the ratio between total sperm cells and motility. Results were submitted to analysis of variance with Statistica 7.0® software, at a significance level of 5%. Fish fed with 2.27% arginine were found to produce more semen and sperm cells – ($p<0.05$) 42,58±12,61 ml.Kg^{-1} relative volume, 30,03±17,1 x10^9 sptz.mL^{-1} concentration – although total production of sperm cells did not differ among treatments. On the other hand, sperm motility was found to be lower in the treatment with 2.27% arginine – ($p<0.05$) 75,22±13,97% – but within the standard of *R. quelen*. Total mobile sperm was 26,35±11,76 x10^{11} for the treatment with 2.27%, superior to the rest ($p<0,05$), showing that even with a lower sperm motility, high sperm concentration offsets motility deficit. Such results indicate that inclusion of 2.27% arginine in *R. quelen* broodstock diet improves reproduction.

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Digestive enzymes of Nile tilapia eggs and larvae: breeders fed with protein levels

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The present study assessed the activity of gastric (pepsin), intestinal (aminopeptidase and alkaline phosphatase) and pancreatic (trypsin, amylase, lipase, protease) enzymes on the embryonic and larval development of Nile tilapia, *Oreochromis niloticus*, obtained from broodstock fed diets with four levels of crude protein. The experiment was carried out at the *Agência Paulista de Tecnologia dos Agronegócios* (APTA) – Research and Development Unit (UPD) in the town of Pirassununga, São Paulo, Brazil (21°55'37,4''S 47°22'10'' O), from January to June 2014, using 144 females and 48 males (3:1) distributed in 16 hapas (12 fish/hapa). Four treatments were used, composed of the following levels of crude protein (CP): 32, 38, 44 and 50 %, with four replications. The eggs were weighed (*mg*), quantified, kept in hatcheries (2.0 L), and separated according to treatment. Twenty-four (24) samples (300.0 mg) per treatment and four (4) samples per stage of embryonic and larval development [S0- cleavage, S1- blastula, S2- gastrula, S3- hatching, S4- 7 days posthatch and S5- 10 days posthatch] were collected, kept in cryogenic tubes and placed in liquid nitrogen (-196.0ºC) until the moment the digestive enzymes were analyzed. There were no differences between the values ($P>0.05$) of pepsin, aminopeptidase, trypsin, and amylase. However, different values were observed ($P<0.05$) for alkaline phosphatase (7 days posthatch), lipase (blastula), and protease (blastula and hatching) with regard to the four treatments. The results showed that diets with levels of crude protein offered to Nile tilapia broodstock influenced the activity of digestive enzymes during embryonic and larval periods, emphasizing that the nutrients ingested by the broodfish were transferred to the progeny. Thus, more studies on diets of broodstock should be conducted in order to provide additional information, not only with regard to levels of protein, but also energy, vitamins and minerals, as well as the interaction between them, and the use of physiological biomarkers for a successful fish farming.

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Effect of dietary supplementation on reproductive parameters and seminal plasma proteins of Morada Nova sheep

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The present study was conducted to evaluate the influence of increasing levels of supplementation with concentrated portions on the semen parameters, scrotal circumference and protein profile of seminal plasma of Morada Nova sheep. Nineteen rams were used, with 7 months of age and initial weight of 16.4 ± 0.25 kg. The trial lasted three months and at the end of the experiment the animals had an average final weight of 29.8 ± 0.53 kg. Supplementation levels with concentrated corresponded to 0.0% (treatment 1 with n=5); 0.6% (treatment 2 with n=4); 1.2% (treatment 3 with n=6) and 1.8% (treatment 4 with n=4) of body weight (BW) of the sheep, based on dry matter, with n being equal to the number of animals per treatment. The highest level of supplementation was associated with 50:50 forage:concentrate. The concentrated feed was formulated based on corn, soybean, urea, limestone and dicalcium phosphate. Irrigated pasture consisted of *Panicum maximum* cv. Aruana with rotational stocking. Semen was collected by electroejaculation and parameters such as sperm motility, progressive motility, vigor, sperm concentration and sperm morphology were analyzed individually for each animal. Semen samples were centrifuged (700 x g, 4°C, 20 min.; 5000 x g, 4°C, 60 min.) for separation of seminal plasma. After the last centrifugation, the supernatant was collected and precipitated with acetone for two hours at -20°C. The precipitated supernatant was centrifuged at 5000 xg at 4 ° C for 40 minutes, being discarded after this. The pellet was dried overnight at 4 °C and then resuspended in 50 uL of sample buffer. After precipitation, samples of seminal plasma were stored at -20 °C. Protein concentration in seminal plasma was determined by the Bradford's method. Samples containing 400 ug protein were mixed with hydration buffer and subjected to isoelectric focusing in 13-cm strips (pH 4-7), followed by SDS-PAGE (12.5%). One two-dimensional gel was done for each animal and was scanned with a ImageScanner II (GE Lifesciences, USA) and analyzed using PDQuest® version 8.0.1 (Bio-Rad Laboratories, USA). Data was analyzed using ANOVA and Tukey test ($p < 0.05$). There was no significant differences between treatments for sperm concentration, motility, progressive motility, morphology and scrotal circumference. Two-dimensional gels of ram seminal plasma had an average of 174 ± 11.62 spots per gel, according to the PDQuest® application. There were no spots with differential expression among the treatment groups. In conclusion, increasing levels of concentrate fed to rams raised in pasture did not influence the expression of testis size, sperm parameters and seminal plasma proteins. Thus, locally-adapted rams, such as the Morava Nova breed, can be maintainded with forage to produce semen of good quality.

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Effect of inclusion of cashew nut meal on semen parameters and protein profile of seminal plasma and sperm cells of crossbred goats

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Goat production has great importance in the Brazil's Northeast. Cashew nut meal (CNM) is a good alternative feeds. The study was conducted to evaluate the effect of inclusion of CNM in the diet on semen parameters and seminal plasma proteins of crossbred goats. Eighteen animals were used with an average age of 18 months, divided into two groups: goats fed diets containing 11% of CNM (CNG); control group fed diets without CNM (CG). Scrotal circumference was measured and semen samples were collected by electroejaculation on experimental days 0, 30, 60 and 90. Sperm concentration (SC), progressive individual motility (PIM) and total motility (TM) were evaluated. To obtain the seminal plasma, the ejaculate was centrifuged twice (700g, 20 min, 4°C; 5000g, 1 h, 4°C). Sperm cells were resuspended, washed in PBS (700g, 20 min, 4°C) and stored at -20°C. To extract protein from sperm cells, the pellet was resuspended in 1.5 mL of PBS with 1% (v / v) of a protease inhibitor cocktail, then was homogenized 20 times using a crusher-type device. It was added 1% (v / v) of Triton X-100 and incubated (2 h at 4ºC) with mild agitation. Afterwards, samples were sonicated in cold water (30 min) and, subsequently, centrifuged (5000g, 1 h, 4°C). The supernatant was retained, proteins were precipitated with cold acetone for 2 hours and centrifuged (5000g, 1 h, 4°C). The pellet was air-dried overnight and resuspended in a sample buffer (7 M urea, 2 M thiourea, 40 mM DTT, 2% free ampholytes, 4% 3-[(3-cholamidopropyl) dimethtylammonio]-1-propanesulfonate). Protein concentration was determined by Bradford's method. Seminal and sperm samples containing 20 µg protein were diluted in sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 20% (v / v) glycerol, 0.2 M DTT , 0.02% bromophenol blue) totaling 20 µl. Then the samples were pooled using three different collections per animal and one of each was separated by 1D SDS-PAGE by placing the samples on polyacrylamide gel (12.5%), with 25 mA/gel and 90 W. Subsequently, gels were stained with a solution of Coomassie brilliant blue R-250. After destained, the gels were scanned and analyzed with Quantity One®, v.4.6.3 (Bio Rad, USA). The experimental design was completely randomized. Data normality was verified by D'Agostino Pearson and Shapiro-Wilk test, accepting quadratic or logarithmic transformations if necessary. T-test was used to evaluate the differences between groups, at the same experimental periods and Tukey's test was employed to verify differences between experimental periods within the same group ($p < 0.05$). Related to scrotal circumference, PIM and TM, there were no statistical difference between periods and treatments. Regarding to scrotal circumference, it was found for CG and CNG, respectively, 25.10 cm and 25.72 cm, for PIM, 3.46 and 3.79, and for TM 73.89% and 78.34%. A statistical difference was found for SC on days 30 ($2.05x10^9$/mL and $1.86x10^9$/mL) and 90 only ($1.80x10^9$/mL and $1.76x10^9$/mL). Concerning the seminal plasma, the gels of CNG showed 63 bands and those from CG, 56 bands. Among the expressed bands, the highest intensities were 15 kDa, 22 kDa and 24 kDa, which are described in the literature as goat seminal plasma (GSP) proteins. Eighteen bands were found only in seminal plasma of CNG. However, gels of sperm cells had 47 bands for CNG against 39 bands for CG, whose the band of 14 kDa presented the highest intensity for both groups. There were 14 bands only in the CNG for sperm cells. Our results demonstrate that there are differences in the protein profile of seminal plasma and sperm cells of goats submitted to the addition of cashew nut meal in the diet.

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Effect of Jabuticaba Peel Extract on Senescence and Obesity of FVB Mice, observed in the Epididymis

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Aging is closely related to problems in the male reproductive system. In another context, obesity is the most frequent chronic medical problem that has been affecting the world population. Thus, senility and obesity, constitute two factors that favor the development of alterations in several parameters in the male reproductive system, ranging from morphological to the endocrine and physiological changes as well as the commitment of sperm functions in the epididymis. These factors favor the considerable increase of male infertility. The Brazilian fruit, jabuticaba, is rich in phenolic compounds with antioxidant, anti-inflammatory and anti-carcinogenic activity, which could counteract such debilities. Considering particularly the morphological damages caused by senescence and adiposity on epididymal parameters, this study evaluated the histological changes in the epididymis caput and cauda region, caused by such factors. Therefore, 70 FVB mice were distributed into seven groups: Young (YG), Senile (SE), Senile+ High fat diet (SHP), Senile+ Jabuticaba peel extract (JPE) (SJ) and the senile+High fat diet+JPE (SHJ). The treated groups were divided in two subgroups, as follows: SJI/SHJI– senile mice that received a dose of JPE/kg/animal and SJII/SHJII – senile mice that received twice the dose given to SJI/SHJI. The treatment lasted 60 days and, after euthanasia, the epididymides were processed for histological analysis. This material was sectioned and stained with the HE technique (hematoxylin-eosin). The stereological and morphometric analyses were performed with IMAGE PRO PLUS 6.0 software and statistical analysis was run with the PRISM STATISTIC 5 software. With caput region stereological evaluation, there was a significant reduction in tubular epithelial volume for SHP group compared to SE. Moreover, such analysis revealed a significant increase in the tubular epithelial volume of SHJI and SHJII compared to the control SHP. The intertubular percentage showed a significant decrease for SE compared to the YG; and a significant increase for SHP in relation to SE. Between the treated groups, this parameter was increased for SJI compared to SE, and it was decreased for SHJII in relation to SHP. The tubular lumen volume was reduced for SE compared to YG, though the same parameter was increased for SHP in relation to SE. Also, in the treatment groups, SJI and SJII, there was a significant increase in the lumen volume when compared to SE. On the other hand, at the epididymis cauda, the epithelium proportion was increased for SE compared to JV as well as for SJI in relation to SE. The intertubular parameter was significant different between the groups. An increased intertubular proportion was observed for SE compared to YG, while for SHP this measure was reduced in relation to SE. Between SHP and SHJI, the intertubular volume was significant reduced to SHJI. Also, the same parameter was reduced for SJI compared to SE and SJII. The lumen proportion was reduced for SE in relation to YG and it was increased in SHP compared to SE. The epididymis caput morphometric analysis revealed significant decrease of the tubular diameter for SE in relation to YG, in addition to an increase of this measure for SHP compared to SE as well as for SJI and SJII compared to SE. At the cauda, this parameter was increased for SJII in relation to SJI, and for SHJII compared to SHP. Therefore, it can be pointed out that aging negatively influences tubular volume, as noted by the volume reduction of luminal components in the epididymis caput and cauda for SE compared with the YG group. On the other hand, JPE administration to senile animals favored the recovery of such parameters as well as the tubular diameter, observed in SJI and SJII. The intimate association between obesity and the development of interstitial edema, and therefore, inflammatory processes, allows us to indicate the fat diet as one of the possible causes for the increase of the intertubular proportion observed in the caput region of SHP animals. Nevertheless, the JPE treatment in senile mice fed with high fat diet (SHJ), promoted an intertubular volume reduction in comparison to the senile group that received only a high fat diet (SHP), throughout the epididymis length. A similar change occurred between the senile group (SE) and their corresponding treated groups (SJI and SJII), at the cauda region. Thus, the JPE seems to act as the reducing agent of intertubular injury, effect attributed to its anti-inflammatory properties. Moreover, the decrease in luminal volume observed for SE compared to YG, both the cauda and caput region, may indicate a tubular atrophy signal and reduction of testicular activity. In consideration of such results, the increase in the luminal parameter, at the caput epididymis of the SJI and SJII groups, points to the effect of the epididymal functional recovery through the action of polyphenols concentrated in the JPE. Therefore, these data corroborate the anti-inflammatory and recuperative activity of phenolic compounds present in the JPE, and highlight the progressively negative effect of senility and obesity on the body. (CEUA/UNICAMP/Protocol#4038-1).

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Effect of short-term nutritional supplementation on oocyte quality in superovulated goats

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Energy-dense diets in small ruminants have significant effects on the reproductive physiology, including the ovarian function and fertility. Thus, the use of nutritional strategies for a short period (*short nutritional flushing*) can be used to stimulate folliculogenesis, through the use of dietary supplements with activity in the ovary, such as glycerin or linseed, increasing the levels of glucose and fatty acids in the plasma and consequently the number of follicles and viable oocytes. Therefore, the aim of this study was to evaluate the effect of *short nutritional flushing* on the viability and oocyte quality in goats superovulated and fed with different nutritional sources. Thirty adult mixed breed goats, with homogenous body condition score (3.0 ± 0.2; Mean ± SD), body mass index (7.2 ± 0.8) and age (28.2 ± 3.4 months), were grouped in three (n = 10) shaded collective boxes and fed with a total mixed ration (TMR) based on chopped elephant grass (*Pennisetum purpureum spp.*) and concentrate (10% Crude Protein and 67% Total Digestible Nutrients). Feed mixtures were provided to satisfy the requirement for breeding of adult non-dairy goats (NRC, 2007), during 15 days, from estrus synchronization to oocyte recovery. Seven days prior to oocyte recovery, the three groups of animals (n=10 each) were fed on a daily basis with: (a) TMR diet (Control Group, CG); (b) 200 mL/goat of crude glycerin (80% glycerin) and water in a 9:1 ratio (Glycerin Group, GG), with each dose of crude glycerin equivalent to 1.03 Mcal of metabolizable energy; and (c) ground flaxseed (*Linum usitatissimum L.*) on a 30% Dry Matter basis (Linseed Group, LG). The TMR feed mixture in the CG and LG groups were prepared in a water solution. Estrus synchronization was initiated by the insertion of an intravaginal progesterone (CIDR®) insert on Day 0 (D0), 12 days prior to slaughter. On Day 6 (D6) the intravaginal insert was removed and 0.075 mg PGF2a (Prolise®) and 150 IU eCG (Folligon®) were given i.m. After 36 h (Day 7), a 0.125-mg GnRH (Gestran®) dose was given i.m. For superovulation, a total of 200 mg pFSH (Folltropin®) was applied i.m. at 12 h intervals (5 applications of 40 mg/mL) from Day 9 (D9) thorugh Day 11 (D11) (Menchaca *et al.*, 2007). Ovaries were collected upon slaughter on Day 12 (D12), 6 h after the last FSH injection, and transported to the laboratory in saline solution at 25°C. Then, cumulus-oocyte complexes (COCs) were recovered by follicular aspiration, classified morphologically according to the characteristics of cumulus investment and cytoplasm as non-viable (degenerate) and viable (grade I, oocytes with multilayered compact cumulus oophorus and an homogeneous cytoplasm; grade II, compacted COCs with at least 3 layers of cells and homogeneous or heterogeneous cytoplasm; grade III, COCs partially denuded and/or with cytoplasm having few pyknotic areas). Data were analyzed by the Chi-square test ($P<0.05$). The LG group had significantly higher rates of oocyte viability (88.9%, 152/171) when compared with the GG (80.1%, 137/171) and GC (78.4%, 149/190) groups. Furthermore, proportion of grade I COCs did not differ between treatment groups (LG: 7.9%, 12/152; GG: 5.1%, 7/137; GC: 10.7%, 16/149). However, the LG group had significantly lower and higher percentages of grade II (44.7; 68/152) and grade III (47.4%; 72/152) COCs, respectively, compared with the CG group (56.4 %; 84/149), grade III (CG: 32.9%; 49/149)], being not different from the GG group for grade II (52.6%; 72/137) or grade III (42.3; 58/137) COCs. The short nutritional flushing using polyunsaturated fatty acids (linseed) appear to stimulate folliculogenesis and oocyte development. Thus, nutritional strategies using this feed stuff may safeguard oocyte viability, increasing reproductive efficiency in superovulated female goats. Further studies are needed to confirm the effects of nutritional additives, which may interfere with the rate of maturation and subsequent embryonic development.

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Electrophoretic characterization of the uterine proteins of goats fed with different levels of energy

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The uterus plays an important roles in the female reproduction, such as hormone production and maintenance of pregnancy, and it is known that these functional attributes are influenced by nutrition. Thus, the aim of this study was to assess the effect of different levels of energy intake on the protein profile of goat uteri. All procedures used in this study were approved by the Ethics Committee in Animal Experimentation of the Ceará State University (no 12066667-7/18). Anglo-Nubian cross-bred (n=24), non-lactating, pluriparous and cycling adult does were selected from a farm herd and grouped according to feed levels (n=6 per group; 4 groups). Such groups were homogeneously distributed based on ($P > 0.05$) live weight, (30.0 ± 2.7 kg), body condition score (2.8 ± 0.2), sternal subcutaneous fat thickness (9.15 ± 1.1 mm) and age (28.9 ± 5.6 months),. Experimental groups received a diet composed of Bermudagrass hay and concentrate (67% TDN and 10% CP), supplied during four weeks to satisfy 1.0 (Group A), 1.3 (Group B), 1.6 (Group C), and 1.9 (Group D) times the nutritional requirement for maintenance of adult non-dairy goats (NRC, 2007). At slaughter, uterine tissue samples were collected, stored in sterile tubes and frozen at -80^{o}C until protein extraction. Tissue fragments were frozen-dried and macerated. Protein extraction was performed in cold PBS containing 0.1% triton X-100. Samples were centrifuged for 30 min at 5000 rpm and the supernatant eliminated. Proteins were then re-suspended in ultrapure water and protein concentration was determined according to the Bradford's method. Uterine proteins (400 µg) were subjected to 2D-SDS PAGE. The samples were analyzed individually for each treatment. All gels were stained with Comassie G250 (BioRad Laboratories, USA) and analyzed by the PDQuest™ 8.0.1 software (BioRad Laboratories, USA). Spots of interest were excised from gels, trypsin-digested and subjected to tandem mass spectrometry. Data were analyzed by ANOVA or the Kruskall-Wallis test. On average, 101 proteins were detected in the gels, regardless the treatment groups. However, the average intensity of the spots were lower for group D (7761817,5 pxs) in comparison with group A (9146241,0 pxs), B (9680350,7 pxs) and C (9869550,7 pxs). Major proteins present were between 12.0 kDa and 128.6 kDa molecular weight. A total of 8 differential spots were identified, between groups, with molecular weights between 14.0 kDa/4.8 PI and 73.6 kDa/6.9 PI. No differential spot was present in a unique group. Therefore, for a better understanding of the results, the next step of this work is the identification of the differential protein spots.

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In vitro development of parthenote embryos derived from goats supplemented with short nutritional flushing

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The use of short period feed supplementation (short nutritional flushing) influence the reproductive performance of domestic ruminants, improving follicular development and oocyte quality by stimulating folliculogenesis. This dynamic supplementation is mediated by metabolites acting in the ovaries, influencing subsequent embryo development and contributing to the increase in reproductive efficiency. Thus, the objective of this study was to investigate the effects of short nutritional flushing in goats on the development of parthenote embryos produced *in vitro*. Thirty adult mixed breed goats, with homogenous body condition score (3.0 ± 0.2; Mean ± SD), body mass index (7.2 ± 0.8) and age (28.2 ± 3.4 months), were grouped in three (n = 10) shaded collective boxes and fed with a total mixed ration (TMR) based on chopped elephant grass (*Pennisetum purpureum* spp.) and concentrate (10% Crude Protein and 67% Total Digestible Nutrients). Feed mixture was furnished to satisfy the requirement of breeding for adult non-dairy goats (NRC, 2007), during 15 days, from estrus synchronization to oocyte recovery. Seven days prior to oocyte recovery, the three groups of animals (n=10 each) were fed on a daily basis was maintained with: (a) TMR diet (Control Group, CG); (b) 200 mL/goat of crude glycerin (80% glycerin) and water in a 9:1 ratio (Glycerin Group, GG), with each dose of crude glycerin equivalent to 1.03 Mcal of metabolizable energy; and (c) ground flaxseed (*Linum usitatissimum L.*) on a 30% Dry Matter basis (Linseed Group, LG). The TMR feed mixture in the CG and LG groups were prepared in a water solution. Estrus synchronization was initiated by the insertion of an intravaginal progesterone (CIDR®) insert on Day 0 (D0), 12 days prior to slaughter. On Day 6 (D6) the intravaginal insert was removed and 0.075 mg PGF2a (Prolise®) and 150 IU eCG (Folligon®) were given i.m. After 36 h (Day 7), a 0.125-mg GnRH (Gestran®) dose was given i.m. For superovulation, a total of 200 mg pFSH (Folltropin®) was applied i.m. at 12 h intervals (5 applications of 40 mg/mL) from Day 9 (D9) thorugh Day 11 (D11) (Menchaca *et al*., 2007). Ovaries were collected upon slaughter on Day 12 (D12), 6 h after the last FSH injection, and transported to the laboratory in saline solution at 25°C. Then, cumulus-oocyte complexes (COCs) were recovered by follicular aspiration, classified morphologically and selected (viable) COCs were placed to *in vitro* maturation (IVM). After 24 h, matured oocytes selected by the extrusion of the first polar body were parthenogenetically activated by exposure to 5 mM ionomycin for 5 min, followed by an incubation in 2 mM 6-DMAP for 4 h. After activation, oocytes were *in vitro*–cultured (IVC) in G-1 medium (Vitrolife®), in an incubator at 39°C and 5% CO_2 for seven days. Cleavage and blastocyst rates were evaluated on Days 2 and 7 of IVC, respectively. Embryos were classified as viable and non-viable, and in compact morula or blastocyst stages, in accordance with embryonic development. Data were analyzed by the Chi-square test ($P<0.05$). The LG treatment had significantly lower maturation (73%; 86/118 *vs.* 84%; 98/116) and cleavage (86%; 74/86 *vs.* 96%; 94/98) rates than the CG, respectively, with results in GG group being similar to the other two groups. Morula (LG:23%, 8/35; GG:34%, 11/32; CG: 23%, 12/52) and blastocyst rates (LG: 77%, 27/35; GG:66%, 21/32; CG:77%, 40/52) were similar between groups, with a total mean embryo yield efficiency of 44% (119/269). We conclude that the short nutritional flushing protocols used in this study were not able to improve embryo production efficiency in goats. However, embryo production rates were excellent for the goat species, irrespective of the diet.

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Sperm parameters and seminal plasma proteome of rams undergoing intermittent testicular heat stress

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The present study evaluated the effects of testis heat stress on rams by means of intermittent scrotal insulation (SI). Six adult and reproductively sound Morada Nova rams had their testis insulated during four consecutive nights (6:00 p.m. to 6:00 a.m.). Semen was obtained by electroejaculation 14 days before and weekly for 120 days after the last night of SI. Scrotal circumference (SC), sperm motility and morphology were evaluated, as well as sperm DNA integrity, using the sperm chromatin dispersion test. Day 0 was the first day of insulation. Then, seminal plasma was obtained on days -14, 0, 7, 18, 28, 48, 77 and 120 and analyzed by 2D-SDS-PAGE. Gels were stained with Coomassie G-250 and evaluated using PDQuest software (Bio-Rad, USA). Proteins were identified by tandem mass spectrometry (ESI-Q-ToF). Data obtained over time in relationship to SI was evaluated by LSM ($P \leq 0.05$) using SAS software (v. 9.0; 2002) procedures. *In silico* analysis of protein-protein networks were evaluated by STRING v. 10.0. SC averaged 30.5 cm at pre-insulation, increased to 31.8 cm at the end of insulation (day 4), reached 27.9 cm on day 28 and returned to normal values on day 57 (30.6 cm). Sperm motility decreased from 75% to 33% between days 0 and 4, respectively. Motile sperm were undetectable in the ejaculates on days 23 and 28. However, sperm motility was 10% on day 35 and increased to 52% on day 57, returning to normal (67%) on day 77. The percentage of normal sperm decreased from 96% on day 0 to 77% and 6% on days 7 and 35, respectively, returning to normal on day 91 (87%). Sperm DNA integrity decreased from 86.5% (day 0) to 52.3% on day 7, reached the lowest scores between days 11 and 63 (10.7%) and returned to normal on day 120 (80.4%). Considering that spermatogenesis and the sperm transit into the epididymis takes close to 60 days, it is necessary two sperm cycles to for the reestablishment of the normal sperm DNA integrity. After SI (day 7), the amount of seminal plasma proteins identified as carboxypeptidase Q precursor (CPQ) and superoxide dismutase [Cu-Zn] isoform X1 (SOD) decreased while albumin precursor (ALB) increased. Protein CPQ had the lowest expression from day 7 to 48, returning to normal on day 77, coinciding with the return of normal sperm motility. Protein CPQ interacts with natural resistance-associated macrophage protein 1, which controls resistance to pathogens through mechanisms that involves sequestration of Fe^{2+} and Mn^{2+}, cofactors of catalases and superoxide dismutases. Protein CPQ may act to protect macrophages against their own generation of reactive oxygen species (ROS). The decrease of CPQ could explain the reduction of SOD expression; in addition to the reduction in sperm motility after SI. SOD had the lowest expression on day 18. This protein destroys ROS produced by cells, which is toxic to biological systems. Additionally, SOD interacts with peroxiredoxin-6 and thioredoxin. Peroxiredoxin-6 is an antioxidant with a protective effect against oxidative stress on DNA and cells. Studies shows thioredoxin regulates caspase-3 activity, thus reduced levels this protein is associated with high CASP3 activity which in turn is related to higher sperm DNA fragmentation. In our investigation, high DNA fragmentation was observed when there was lower CPQ and SOD expression in the seminal plasma of rams (from days 18 to 48). Albumin is characterized as a non-enzymatic antioxidant and it cloud bind to ROS and protect sperm from lipid peroxidation. Seminal plasma ALB had higher expression after SI (day 7), showing higher values on day 28 (no sperm motility) and returning to normal when seminal parameters were reestablished. Thus, intermittent testicular heat stress caused significant changes in sperm motility, DNA integrity and seminal plasma proteome of rams. Proteins with changed expressions during the study were likely involved in protective processes against oxidative stress on sperm cells.

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Characterization of Sertoli cell proliferation in iNOS$^{-/-}$ mice during the postnatal development

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Sertoli cell (SC) proliferates until two to three weeks after birth in mice, and it is known that the inducible nitric oxide synthase (iNOS) and/or the nitric oxide acts indirectly as a mediator of its proliferation during postnatal life. It's been shown that iNOS deficiency in mice results in an increase in testicular weight and gonadosomatic index, due to an increase in the SC proliferation index up to 10 postnatal days (Pnd) which generates higher SC number in adulthood, but, in our knowledge, there are no studies concerning how this proliferation behaves after Pnd10. Therefore, the intention of this study was to characterize with more detail the period of Sertoli cells proliferation in iNOS deficient mice, to see if, besides the higher proliferation index, these cells also have a longer proliferation period, leading to the much higher number of SC observed in the adults. Wild-type and iNOS$^{-/-}$ mice with 1, 5, 10, 15, 20 and 25 postnatal days were evaluated through biometric and immunohistochemistry analyzes, with incorporation of the cell division marker BrdU and calculation of the proliferation index in SC. As expected, iNOS$^{-/-}$ mice showed a significant increase in testicular weight in Pnd1, Pnd15, Pnd20 and Pnd25, and an increase in the gonadosomatic index in Pnd10, Pnd20 and Pnd25. Regarding the proliferation index, we observed a reduction in the Sertoli cell proliferation in the iNOS$^{-/-}$ group from 10 postnatal days, but in both groups it is still possible to find proliferating cells stained for BrDU until Pnd25, although with a very reduced percentage. These results demonstrate that the Sertoli cell proliferation period in iNOS$^{-/-}$ mice is not extend, being similar to what was already described in literature for wild-type mice.

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Climatic factors and their correlation with the superficial digital thermal imaging of the scrotum and seminal quality in Nelore bulls (*Bos taurus indicus*)

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Bulls should produce a large number of morphologically normal sperm in order to optimize reproductive function for natural breeding or artificial insemination. However, brazilian herd consists predominantly of zebu cattle, which their fertility is challenged by climatic factors in the extensive management conditions. Bull testis temperature must remain between 4 and 5ºC below rectal temperature to maintain efficient semen production, and the scrotal surface temperature is linked to testicular temperature, providing detailed information about bull's ability to maintain testicular temperature. The infrared digital thermal imaging is a noninvasive imaging test with high accuracy that can detect scrotal surface temperature and, therefore, testicular temperature. The use of infrared digital thermography in Brazil's zebu cattle has been little studied and reported in scientific articles. Thus the aim of this summary was to study the relation between climatic factors, scrotum surface temperature and seminal quality through infrared digital thermography in Nellore bulls, raised extensively. Scrotal thermography by Flir E40® camera, blood samples for serum testosterone dosage and semen collection by eletroejacultion was performed in six bulls every 10 days with six replications. Climatic factors as temperature, relative humidity, dry globe temperature and temperature of the wet globe were recorded by a globe thermometer (InstruTemp®,ITWTG-2000). Scrotum thermograms were analyzed with Flir Tools® software for surface, letf and right sides, and scrotal neck temperatures; middle, dorsal and ventral testicles regions; and epididymis tails. Semen and thermograms data were analyzed by ANOVA and Tukey's test at 5%. Pearson correlation was applied to scrotum surface temperature, rectal temperature, semen quantitative and qualitative characteristics and climatic factors. Positive correlation ($P<0.05$) was observed for sperm motility *vs* scrotum temperatures, sperm concentration *vs* scrotum temperatures and climatic factors *vs* rectal temperature. The increase in scrotal temperature influenced sperm motility improvement. A study made with *Bos taurus* bulls reported that moderate increase in testicular temperature can cause marked reduction in sperm production with concentration decrease. Extrinsic factors can act on rectal temperature variations as the time of day, food and water intake, nutritional status, temperature, density, shading, wind speed, season, exercise and sunlight. Negative correlation ($P<0.05$) was observed for ambiente temperature *vs* sperm concentration, showing that elevated rectal temperature results in decrease of semen quality, probably due to heat stress with cortisol increase. It was concluded that scrotum surface temperatures and climatic factors, temperature and air humidity influence semen quality. Thermography is recommended as complementary exam for bulls reproductive evaluation. (CEUA/ UNOESTE/ Protocol 1700).

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Effects of birth weight on sperm quality in postpubertal boars

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In recent years, breeding has focused on increasing litter size. However, selection for larger litter size favored the occurrence of lighter piglets at birth. These animals have impaired muscle development, higher mortality rates and lower carcass quality. However, reports of birth weight effects on the reproductive system is scarce, specially in boars. Since low birth weight piglets are a reality in pig farms and given the importance of breeding males for reproductive efficiency, the objective of this study was to investigate the effects of birth weight on semen quality in young males. Forty four littermate boars were selected according to their birth weights and allocated to two experimental groups: high (HW, n = 22): 1.8-2.1 kg; and low (LW, n = 22): 0.8-1.1 kg. They were reared in pens separately by birth weight group until 180 days old. At this time, a subset of eight animals from each experimental group was randomly chosen, and eight ejaculates per male were collected within a two-week interval for further semen quality assessment. Boars were castrated at 10 months old for evaluation of testicular biometrical and histological data. Blood samples were also collected for testosterone analysis. Data were analyzed as a randomized complete block design, where litter of origin was blocked, and treatment effects on the parameters evaluated were analyzed using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). Least square means were compared using the Student T test with $P< 0.05$ being considered significant. LW males showed lower body and testicular weights compared to their HW littermates ($P<0.05$). Seminiferous tubules diameter and epithelium height were similar between both treatment groups. Regarding semen quality, all parameters investigated (motility, volume, concentration, DNA and acrosome integrity and sperm morphology) were similar between experimental groups. Testosterone levels were not affected by birth weight either. These findings suggest that even though testes size is compromised in LW boars, birth weight does not affect semen quality in post pubertal boars. (CETEA/UFMG/ Protocol #65/2011).

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Effects of the saturated lipids intake from weaning on the metabolic and sperm parameters of gerbils at adulthood

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Both clinical and experimental data had shown that obesity at adulthood alters the circulating steroids hormones and affects the reproductive capacity, reducing the semen quality and increasing the infertility taxes; however, experimental studies are concentrated in conventional rodent models, as rats and mice. Thus, the evaluation of the effects of excessive lipids intake in other animal models is important, particularly during sexual maturation phase, a critical period and still understudied. Therefore, this study investigated the consequences of a high saturated-fat diet intake during sexual maturation on the metabolic and sperm parameters of Mongolian gerbils (*Meriones unguiculatus*) at adulthood. Twenty male gerbils (4 weeks old) were fed for 12 weeks with balanced diet (Control group – C) or high-fat diet containing 31.2% saturated lipids derived from lard (Diet group - D). The main endpoints evaluated were adiposity index, blood lipid and hormonal profiles, testes histology, expression of peroxisome proliferator-activated receptor gamma (PPARγ), and sperm parameters. High saturated-fat diet intake did not alter neither the body weight (C: 71.7±5.6; D: 73.3±6.6g) nor the adiposity index (C: 3±0.7; D: 3.4±0.9%) of the animals. Nevertheless, there were a tendency to enhance the body weight gain (C: 43.4±2.3; D: 45.4±4.7g; p=0.08) and an increase in the retroperitoneal (C: 794±300; D: 1015±351mg) and visceral (C: 140±67; D: 213±122mg) fats deposits. D animals also had alterations in their lipid profile; total cholesterol and non-HDL cholesterol levels were higher (C: 60.8±11.5; D: 96.2±12.4mg/dL and C: 24.4±5.2; D: 50.5±11.4mg/dL, respectively), and there was a tendency to raise the triglycerides level (C: 101.6±37.5; D: 135.2±72.1mg/dL; p=0.07). Excessive saturated lipids intake did not change the serum testosterone level (C: 1.5±0.5; D: 1.3±0.3ng/mL), but enhanced the serum 17β-Estradiol level (C: 30.1±6; D: 36.4±5.1pg/mL). Histopathological analysis of the testes did not reveal marked alterations in the seminiferous tubules structure in the animals from D group except that 4% of them showed premature detachment of germ cells. Expression of testicular PPARγ did not differ between the groups (C: 1.8±1.6; D: 1.6±0.8% from β-actin), as indicated in the Western blotting analysis. No changes were detected in daily sperm production (C: 13.3±2.5; D: 13.5±2.3 $x10^6$), sperm reserve (C: 360±57.1; D: 364±50.1 $x10^6$) and sperm transit time through the epididymis (C: 13.7±1.7; D: 13.4±1.3 days), but there was impairment of the sperm motility. The number of spermatozoa with progressive movement was reduced (C: 62.2±6.3; D: 48.9±5.8) while the number of spermatozoa with non-progressive movement (C: 17.9±4.4; D: 25.7±5.8) and spermatozoa immotile (C: 19.9±5.4; D: 25.4±4.4) increased in the D group. Taken together, our data show that the saturated lipids intake causes dyslipidemia and damages the sperm motility, even without induce obesity. Seen the importance of this parameter for sperm quality and fertility evaluation, it is possible to suggest that the high saturated fat intake be able to impair the reproductive capacity of the animals. (CEUA Protocol #093/2014).

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Electrophoretic profile of the testicular fluid from ruminants

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The proteins in testicular fluid are basically of Sertoli cell origin and these cells secrete both testis-specific and serum proteins into the luminal compartment of the testis. The developing germ cell are embedded by the fluid and proteins secreted by Sertoli cells. That fluid also leads the sperm through the tubules towards the rete testis. The objective of the study was to evaluate the electrophoretic profile of the proteins from testicular fluid of rams and bulls. Testicles were collected from six adult animals (three rams and three bulls) in a local, commercial abattoir and rapidly transported on ice to the laboratory.Testicles were dissected and fluid was obtained. Fluid from testicles of each species were pooled and centrifuged at 1500 x *g* for 20 minutes at 4°C to remove sperm cells. Thereafter, proteins were precipitated with 3 volumes of acetone. Aliquots of testicular fluid were diluted 30 times in distilled water for quantitation of protein by Bradford assay (Bradford, 1976), using bovine serum albumin as standard (Sigma-Aldrich, St. Louis, MO, USA).20 µg of proteins from testicular fluid were separated by 1-D gel electrophoresis. Then, gel was stained using Coomassie R-250 and scanned and analyzed using Quantity-One® software (BioRad, USA).In the gel of testicular fluid from rams were detected 23 bandsthat ranged between 15.26 and 210.93 kDa with an average 67.74 kDa. 65.2% (n= 15) of the bands were found above 30 kDa, corresponding to 38.5% of the intensity of all bands detected. 30.4% (n= 7) of the bands were under 30 kDa which represented 10.2% of the intensity. Three more intense bands (80.45, 55.92 and 49.28 kDa) showed 18.71% of total proteins bands detected in the gel.The gel of the testicular fluid from bulls alsoobtained a total of 23 bands, which ranged from 17.6 to 206.5 kDa with an average of 70.5 kDa. 73.9% (n= 17) of the bands had molecular weight above 30 kDa corresponding to 41.1% of the intensity and 26% (n= 6) were under 30 kDa representing 7.2% of the intensity. Three more intense bands (115.17, 79.8 and 57.86 kDa) presented 22.83% of the intensity of all bands detected. The most intense bands in bulls and rams testicular fluid showed molecular mass between 79 and 80 kDa corresponded to 8% of total proteins bands detected in both fluids.Based on approximated molecular weights, those proteins could correspond to isoforms of albumin (67 kDa), which was also identified by Melsert et al. (1988) in the rat testicular fluid. According to those authors,albumin is the main biologically active molecule in rat testicular fluid.This protein has also been identified in rete testis fluid from rams. Albumin acts as a carrier protein for steroids, fatty acids and hormones and it is associated to sperm protection through the absorption of lipid peroxides, thus reducing the damage caused by these compounds in the sperm membrane.The presence of albumin in the testis may be related to its ability to transport androgens. Another possible protein present in that band can be testibumin which was found in high concentration in testis from rats. Based on structural and immunological analysis, studies showed that testibumin may be the albumin homologue in the reproductive tract. In addition, the specific function of the testibuminhas not been elucidated, but this protein appears to increase in response to testosterone and FSH which shows a possible role in testis. Testicular fluid from rams and bulls presents a diversity of proteins and these molecules may play important roles in the process of spermatogenesis, protection of gametes and early maturation.

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Evaluation of *Sry* gene silencing in adult testis

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Sry gene is located on the Y sex chromosome and its expression is crucial for male sex determination during the embryonic stage of mammals. Besides encoding the major testis-determining factor in embryos, Sry transcripts are found in different tissues of adult mice, including testis. Although several hypotheses have been raised regarding the function of *Sry* gene in testicular physiology of adult animals, few scientific evidences are conclusive in this subject. In this study, we assessed the morphofunctional aspects of adult mice testis treated with siRNA targeted against *Sry* gene expression. Carbon nanotubes conjugated with siRNA were injected in the tail vein of six balb-c adult mice. Control group (n=6) was treated with only carbon nanotubes. Because the half-life of siRNA ranges between 48 to 72 hours, the testis, epididymis and seminal vesicle were collected three days after treatment. To clarify the specific role of the *Sry* in spermatogenesis, the samples were analyzed through morphometry, immunohistochemistry and TUNEL. Body and testicular biometric parameters did not vary between the groups. Regarding testis morphometry, we observed a smaller tubular and luminal diameter, an enlargement of the tunica propria, higher frequency of germ cell apoptosis and increased Leydig cell nuclear diameter in the treated group when compared to the control group. In addition, in situ staining of DNA fragmentation (detected by TUNEL assay) showed a high density of apoptotic elongated spermatids in the treated group. Remarkably, two animals presented a very low testis size and showed severe alterations in testicular morphology three days after *Sry* silencing. Their seminiferous tubules were devoid of germ cells and showed a very small tubular diameter. Evaluating their epididymis, a high number of non-apoptotic germ cells was observed in the lumen of the epididymis cauda, suggesting that these cells had been released from the seminiferous epithelium (Sertoli cells) since the beginning of treatment. In summary, this study shows that, in addition to its crucial role in testis development, Sry also controls adult testis maintenance and the spermatogenic process. Further genetic (RT-PCR/qPCR) and physiologic (Laser Doppler) studies are being developed in order to better elucidate the specific role of this gene in the adult testis.

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Evaluation of the role of CCR2 expression in the transition region between the seminiferous tubules and the *rete testis*

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The transition region (TR) is the area where the seminiferous tubules (ST) connects to the *rete testis*. This region and its surrounding area in the testis parenchyma is considered to exhibit distinct immuno-morphological properties and previous data from our laboratory showed that Sertoli cells (SCs) located in the TR express CCR2 in Wistar rats. In this context, it is known that its ligand (CCL2; monocyte chemoattractant protein 1) can recruit lymphocytes and macrophages to the testis interstitium/intertubular compartment leading to a naturally higher numbers of these immune cells in the TR. In order to better understand the role of CCR2 in the TR, we have evaluated transgenic mice (C57BL/6) for this receptor, and simulated orchitis via lipopolysaccharide (LPS) treatment (injection of a single dose of 0.5mg/kg body weight LPS (Sigma-Aldrich) through the ocular vein for 6h) in the wild type mice (C57BL/6). Testes from the wild type ($CCR2^{+/+}$) and transgenic ($CCR2^{+/-}$/$CCR2^{-/-}$) mice were routinely processed for histological analyses, which were performed using the Image J software. Initially, we observed a reduced number of SCs in the TR in transgenic mice when compared to the wild type group ($P < 0.05$). Furthermore, we also observed that degeneration/alteration of the seminiferous epithelium begins from this specific area, and were associated with high numbers of immune cells (macrophages, lymphocytes) after LPS stimulation. It therefore appears that the TR is an immunologically vulnerable region of the testis. We are currently evaluating the expression of CCR2 in the TR in the LPS-treated group aiming to elucidate if this chemokine receptor has a modulatory role under pro-inflammatory stimuli. We expect to demonstrate if CCR2 has a dual role in the TR - related to cell proliferation/down-modulation of inflammatory response - as has been shown in other organs.

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Evolutionary diversification and interaction of proteins from the rete testis fluid of rams

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Rete testis fluid provides the biochemical environment in which the sperm are kept before entering the epididymis. The rete testis has a close connection with the development of male gametes and, as a result, it contains proteins that potentially contribute to spermatogenesis. The present study was conducted to determine evolutionary diversification and interaction of rete test fluid proteins that contribute to ram sperm development using bioinformatics approaches. Six Morada Nova rams with 1.2 years of age were slaughtered according to instructions of the Technical Regulation for Industrial and Sanitary Inspection of Animal Products (RIISPOA, 2008). Testicles were removed, dissected and the fluid from rete testis was obtained. The fluid was centrifuged and supernatant was collected and precipitated with acetone. Then, the pellet was resuspended in sample buffer. Protein concentration was determined by the Bradford assay and then, proteins were separated by 2-D SDS-PAGE and identified by tandem mass spectrometry. Multiple sequence alignments and percent identity scores for four proteins was performed using all sequences from ovine, bovine, human, mouse and rat. Phylogenetic trees were produced using Molecular Evolutionary Genetics Analysis (MEGA, version 6.0). Percentual of identity among species was perfomed by Culstal Omega, version 1.2.2. and the conserved domain database (CDD) were determined by NCBI/BLAST (Basic Local Aligmenment Search Tool). Protein-protein interaction network was analyzed using the STRING, version 9.0. Seventy-six spots were identified by mass spectrometry, which represented forty-eight different proteins and four of them, including retinal dehydrogenase, translationally-controlled tumor protein, prostaglandin-H2 D-isomerase and vitamin D-binding protein are the major ones that potentially contribute to spermatogenesis. The phylogenetic trees showed more similarities of amino acid sequences of the retinal dehydrogenase and translationally-controlled tumor protein among *Ovis aries, Bos taurus* and *Homo sapiens* than that among *Ovis aries* and bovine, human, mouse and rat. Prostaglandin-H2 D-isomerase and vitamin D-binding protein phylogenetic trees demonstrated similar sequence of amino acids among *Rattus norvegicus, Mus musculus and Homo sapiens*. Results from Clustal Omega showed that retinal dehydrogenase has 99.80% identity score for *Ovis aries vs. Bos taurus*, 91.62% for *Ovis aries vs. Homo sapiens*, 86.23% for *Ovis aries vs. Rattus norvegicus* and 86.63% for *Ovis aries vs. Mus musculus*. Translationally-controlled tumor protein exhibited 100% identity score for *Ovis aries vs. Bos taurus* and *Homo sapiens*, and 95.54% for *Ovis aries vs. Rattus norvegicus* and *Mus musculus*. Prostaglandin-H2 D-isomerase has 90.58% of identity scores for *Ovis aries vs. Bos taurus*, 68.42% for *Ovis aries vs. Homo sapiens*, 58.73% for *Ovis aries vs. Rattus norvegicus* and 61.38% for *Ovis aries vs. Mus musculus*. Vitamin D-binding protein has 80.34, 73 and 71.73% identity scores for *Bos taurus vs. Homo sapiens, Bos taurus vs. Mus musculus* and *Bos taurus vs. Rattus norvegicus*, respectively. Retinal dehydrogenase, translationally-controlled tumor protein, prostaglandin-H2 D-isomerase and vitamin D-binding protein contain conserved domains in all species and they belong to the following superfamilies, respectively: aldehyde dehydrogenase superfamily (ALDH-SF), translationally controlled tumour protein superfamily (TCTP), lipocalin and albumin, respectively. The interaction networks of retinal dehydrogenase, translationally-controlled tumor protein, prostaglandin-H2 D-isomerase and vitamin D-binding protein showed interactions with molecules involved in structural support, antioxidant and catalytic activity, transport, metabolism, adhesion, proliferation and signalization cell. Vitamin A plays an important role in spermatogenesis and it is converted to retinoic acid in Sertoli and germ cells through retinal dehydrogenase activity. Translationally-controlled tumor protein is related to spermatogenesis, since it had high level of expression in spermatogonia and peritubular cells of rats, according to the literature. Prostaglandin-H2 D-isomerase acts in intercellular transport of retinoids and lipophilic substances for developing germ cells in the seminiferous tubules. Vitamin D-binding protein acts in the transport of vitamin D and its metabolites which may be involved in spermatogenesis. In conclusion, proteins of the ram rete testis such as retinal dehydrogenase, translationally-controlled tumor protein, prostaglandin-H2 D-isomerase and vitamin D-binding protein are very similar among the species studied and they have conserved domains during evolution.

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Immunolocalization of AMH and cell quantification in seminiferous tubules of buffalo fetuses

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The testicular development of buffalo fetuses has been studied by several authors (Abdel-Raouf et al, 1974; Kaur et al, 2011) and anti-Muleriano hormone (AMH), also known as Mullerian Inhibiting Substance (MIS) expressed by Sertoli cells plays an important role in sexual differentiation by inducing regression of the Mullerian ducts in the male fetus (Jost, 1953). AMH was identified in Sertoli cells of equine fetuses between 5 and 11 months of gestational age (Ball et al, 2008), however there are no studies in the literature with immunolocalization of AMH in buffalo fetuses. This work aims to make the morphometry of the seminiferous tubules (sexcords), to quantify the support cells (Pre-Sertoli) and gonocytes and make the immunolocalization of AMH in buffalo fetal testis of different ages. Testis of buffalo fetuses (n = 12) aged between 3 and 8 months (15-98cm CRL) (Abdel-Raoulf et al, 1974) were collected in slaughterhouse processed for conventional histology and stained with H.E for morphometry. The imunolocalization of the AMH was performed on histological sections deparaffinized using anti-AMH antibody 1:50 (SC 28912) according to manufacturer instructions. For morfometry and quantification were analyzed 100 tubules in each age. The average of tubular diameter was 38.06 (± 5.4), 46.27 (± 8:47), 44.11 (± 6.83), 32.56 (± 5.65) and 35.5 (± 4.26) µm; the average of supporting cell (pre-Sertoli) was 7.72 (± 1:53) 8.13 (± 1.68) 9.24 (± 3.10) 05.08 (± 1:56) and 8.86 (± 1.73) and the average of gonocytes was 0.82 (± 0.77) 0.94 (± 0.78); 1:24 (± 0.95), 0.70 (± 0.64) and 1.13 (± 0.86) to 3-4, 5, 6, 7 and 8 months, respectively, showing a a statistically significant difference between different age groups ($p \leq 0.05$). Immunohistochemistry for AMH in testicular parenchyma sections showed intense labeling in the cytoplasm of pre-Sertoli cells within the sexual cords (seminiferous tubules in development) in all ages analyzed and immunostaining was not observed in gonocytes and interstitial tissue. Herein, the results showed that AMH is present in testis of buffalo fetuses between 3 and 8 months, however further studies are needed to clarify its role in testicular development.

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Intratesticular injection of silver nanoparticles as a potential neutering agent for male Wistar rats

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Advances in nanomaterials technology has enabled a wide range of studies. Silver nanoparticles (AgNP) are being widely used, having the ability to cross barriers such as hematotesticular, and a documented toxicity on male germ cells. Knowing the problem of the increasing number of stray animals, which are potential disease transmitters, this study aimed to produce a neutering effect on male animals through an intratesticular injection of AgNP. A solution of AgNP functionalized with PVP with mean diameter of 17.5±0.3 nm and concentration of 0.46mg Ag/mL was used in this work. Thirteen Wistar rats (six to nine weeks of age) were used. Nine animals received intratesticular injection of 220µL AgNP solution into each testis, and were euthanized 7 (N=3), 14 (N=3) and 28 days (N=3) after the injections, while the control group (N=4) received the same volume of saline (0.9%), and were euthanized 7 days after the injections. The animals were observed and weighed daily until the day of euthanasia. After the euthanasia blood, testes and epididymis were collected. The left epididymis was used for collecting sperm for motility and pathologies analysis. The testes and right epididymis were fixed in Bouin for 24 hours, dehydrated in ethanol, clarified in xylene and embedded in Paraplast®. Five µm-thick sections were obtained fixed onto slides and stained with hematoxylin-eosin, and taken to evaluation in a light microscope. All animals gained weigh after the injections, and no significant differences were found between treated and control animals. Hematological parameters remained stable, except for the numbers of white blood cells, percentage of lymphocytes and number platelets, all dropping 7 days after the injections and returning to normal afterwards. Concerning semen analysis, treated animals showed a significantly ($P<0.05$) smaller percentage of sperm cells with normal morphology (40±6 after 7 days, 36±5 after 14 days, and 49±6 after 28 days of the injections) than control animals (73±3). The most common sperm pathologies observed were curved tail and bent tail. Mean sperm motility was 90.0±7.1 on control animals, and dropped to 46.7±40.4 and 46.7±41.6 on days 7 and 14 after the injections, returning to 88.3±7.6 on day 28 after the injections. Despite these numerical differences, a great individual variation was present and no significant difference was observed. Histological analysis showed no significant changes in the morphology of seminiferous tubules, with the presence of all sperm cell lineage and spermatozoa within the lumen of the tubules in all analyzed animals. In conclusion, some negative effects on sperm motility and morphology were found on animals that received AgNP intratesticular injections, but those effects were mild and reversible. Therefore, although the neutering effect could not be achieved, AgNP showed potential as a neutering agent. Further studies are necessary to test different formulations of AgNP. (CEUA/UnB/Protocol#102854/2015).

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Isolation and in vitro culture of spermatogonial stem cells of *Canis lupus familiaris* testis

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Spermatogenesis is the process that occurs throughout the life of the individual, by which sperm are produced due to the presence of a population of spermatogonial stem cells (SSCs) in the seminiferous tubules, comprising 0.03% of the germ cell population in the testis of adult individuals. In this study was to isolate and maintain *in vitro* culture SSCs of dog testis for prolonged periods, using two methods of cell isolation, one enzyme (type IV collagenase, hyaluronidase, trypsin) and other nonenzymatic (mechanical disruption syringe) a yield of 93 % was obtained for both methods, and no significant differences between isolation methods ($P < 0.05$) were detected. It was observed that those cells isolated by nonenzymatic method are generating on average 4,4 colonies, but not resist more than 3 sub cultures whereas cells isolated by the enzymatic method resistant to 7 sub cultures but form on average 2,24 colonies. These results indicate that it is possible to isolate and maintain dog spermatogonial stem cell, however is need prolong the sustainable viability *in vitro* culture.

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Laterality of spermatogenesis in pampas deer testicles (*Ozotoceros bezoarticus*)

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There are previous reports of gonadal asymmetry in various mammal species. In pampas deer, the right testicle is significantly heavier and has greater volume than the left testicle. The aim of the study was to determine if there is laterality in the spermatogenic processes between both pampas deer testicles. The study was performed with 7 pampas deer males allocated at the Estación de Cría de Fauna Autóctona (ECFA, Maldonado, Uruguay). They were anesthetised with a combination of ketamine, xylazine and atropine in darts flowed from a blowpipe during the breeding season (February), and fine needle aspiration (FNA) was performed in each testicle. The scrotal skin was rubbed with an antiseptic, and a sterile needle (23 G x $1^{1/4}$", SAKIRA S.A.) connected to a syringe (5 mL) was inserted into the centre of the testicle. Half length of the needle was introduced into the parenchyma and the testicular content was aspirated through the syringe. Smears were prepared with the content collected from each testicle (approximately 20 µL) were dyed with McGrunwald-Giemsa and examined under 100 x 1.250 magnification in the optic microscope. A minimum of 200 (range: 200 to 265) spermatogenic cells as well as the total number of Sertoli cells present in the same images were counted, and the germ cells were classified as spermatogonia, spermatocyte, early spermatid, late spermatid and spermatozoas. The percentage of each stage of the germ line, the number of Sertoli cells, the relationship spermatozoa/spermatogonia and of spermatozoa/Sertoli cells in each testicle were compared with a paired t test. The right testicle tended to have more spermatogonias (3.4 ± 0.8 vs. 1.9 ± 0.4, right and left testicles respectively, P= 0.06) and had a greater percentage of early spermatids (25.6 ± 4.5 vs. 14.8 ± 5.2, right and left testicles respectively, P= 0.004) than the left testicle. On the other hand, the left testicle had a greater percentage of spermatozoas (5.3 ± 2.1 vs. 12.3 ± 4.8, right and left testicles respectively, P= 0.05), a greater spermatozoid/spermatogonia ratio (1.5 ± 0.5 and 8.2 ± 2.6, right and left testicles respectively, P= 0.02), and tended to have more spermatozoas/Sertoli cells (0.5 ± 0.2 and 1.0 ± 0.3, right and left testicles respectively, P= 0.07). While the right testicle had a greater incidence of cells from the earlier stages of spermatogenesis, the left one had the inverse relationship, with greater efficiency in spermatozoa production. Therefore, we concluded that although the right testicle is bigger and heavier, the spermatogenesis processes are more efficient in the left one.

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Morphometry testicular sheep supplemented with detoxified castor bean cake (*Ricinus communis L*) replacement in the soybean meal

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The breeding of ovine in the Northeast is inefficient to fill the meat demand of the consumer market in the region due to low productivity and low use of the herd created in extensive system. In this context, an alternative to reduce the costs of feeding is the use of leavings generated in the production of biodegradable fuel, the castor bean cake, a bioproduct of castor oil, is one of them. However, the presence of anti-nutritional factors in the endosperm of castor seeds becomes an obstacle in the reuse of these in animal feeding, but you can make it happen through their detoxification. Thus, the aim of this study was to evaluate the effect of using detoxified castor bean cake in replacement of soybean meal in sheep's supplement through the testicular morphometry. Were used 24 uncastrated, Santa Inês and Dorper's crossbreed sheep, with an average weight of 29 ± 0.8kg, distributed in three groups, that receiving, during 90 days, isonitrogen diets formulated according to the National Research Council, containing 0; 50 and 100% of detoxified castor bean cake replacing soybean meal supplement. Animals stayed in Aruana grass pasture (*Panicum maximum cv Aruana*) and received supplement provided for the group in the amount of 1.2% of body weight once a day (07:00h) and water *ad libitum*. After the supplementation period, the animals were weighed and slaughtered in specialized slaughterhouse and their testicles were collected and the weight was measured. Then, fragments of 5mm wide by 10mm length of the testicles of each animal were obtained from the midportion for testicular histological processing. The parameters evaluated were: body weight (kg), testicular average weight (kg), gonadosomal index (%), diameter of the seminiferous tubules (µm) and height of the germinal epithelium (µm), volumetric proportion of the components of testicular parenchyma (%); total length of seminiferous tubules (m), length of seminiferous tubule per testicle gram (m/g), leydigosomatic index (%) and tubulossomatic (%). The Data were evaluated by analysis of variance at 5% of probability. There was no difference (P>0.05) for body weight at slaughter (37.74±0.27Kg); testicular average weight (0.12±0.09Kg); gonadosomal index (0.0017%); diameter of the seminiferous tubules (271.10±28.13µm); height of the germinal epithelium (66.01±10.30µm); volume ratio of the components of the testicular parenchyma (91.57% of tubular components being composed of 3.98% of own tunic, 54.59% of the seminiferous epithelium and 33% lumen; and 8.43% of intertubulares components being composed of 0.33% Leydig cells, 0.24% and 7.65% of vessels tissue); total length of seminiferous tubules (79.05±8.20m); seminiferous tubule length per gram of testis (0.66±0.17m/g), leydigosomatic index (0.0010%) and tubulossomatic (0.2494%). Thus, the substitution of soybean meal for detoxified castor bean cake can be made in feed formulation for sheep supplementation up to 100% without testicular morphometric changes, being important the seminal evaluation in future studies.

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Relation of sperm quality, scrotal temperature and testicular ultrasonography features in Nelore bulls

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The breeding soundness exam can be complemented by testicular ultrasonography and scrotal thermography exams. B-mode ultrasound can supply images of the structure and echogenicity of the testicular parenchyma, while the color Doppler mode is an effective method to evaluate the vascular changes in testes and pampiniform plexus. Thermography exam assess the temperature of scrotal superficies and turn possible to indicate a possible failure on testicular thermoregulation. The present study aimed to investigate if the sperm quality in bulls has been related to alterations of testicular ultrasonography and scrotal temperature. For this, the study was conducted in October 2015, in tropical region with an average daily temperature of 25.9±1.15°C and average relative humidity of 52.95±3.74%. Four ejaculated from nine Nelore bulls (n=36) aged between 22 and 25 months were utilized. Semen was collected by electroejaculation (Autojac V2 Neovet®) with intervals of 1 to 4 days. Before the semen collection, scrotal superficies (SSMT) and eye area mean temperature (EAMT) were measured by thermography exam using the camera T620 (FLIR Systems, USA). Thermography was performed respecting 0.9m of distance of the scrotum and camera. Images were analyzed in FLIR Quick Report® software. Rectal temperature (RT) was also measured at this time using a digital thermometer. Immediately after thermography exam, testicular ultrasound was performed using B-mode and color Doppler (M5vet, Mindray®). The testicular parenchyma was evaluated for the presence of hyperechoic points (0 to 3 score, being 0: no hyperechoic points and 3: presence of diffused over five points hyperechoic), homogeneity (0 to 2 score, being 0: without presence of anechoic points and 2: with the presence of many anechoic points) and vascularity (0 to 4 score, being 0: absence of visual vascularization and 4: more than 2 points of vascularization with larger caliber that appear in at least 2/3 of the video). The pampiniform plexus was assessed by vascularization score (1 to 5, being 1: 0 to 20% of pampiniform plexus filled by vascularization and 5: 81 to 100% of pampiniform plexus filled), and Resistance Index (RI, 0-1) At the end of ultrasound exam, semen was collected and evaluated on total motility (TM), major (MA), minor (MI) and total sperm defects (TO), plasma membrane integrity (PMI), acrossomal membrane integrity (AMI) and high mitochondrial membrane potential (HMMP), and sperm showing simultaneous integrity of plasma and acrossomal membranes and mitochondrial potential (PIAIH). Ejaculates were separated in two groups according to sperm quality: good quality (GOOD; n=21) (<20% of major defects, <30% of total defects and/or >60% of total motility); and regular quality (REG; n=15) (≥20% of major defects, ≥30% of total defects and/or ≤60% of total motility). The groups were compared by analysis of variance by the MIXED procedure of SAS software (SAS Institute, 2004). Difference was considered significant when P≤0 .05. In concern to TM, the GOOD group (91.07±0.82%) presented higher (P=0.01) than REG group (84.81±2.68%). REG group had a higher (P<0.0001) percentage of major (29.83±3.14%) and total defects (36.03±3.15%) than GOOD group (MA= 7.23±0.78%; TO= 13.47±1.28%), but there was no difference (P=0.59) in minor defects (GOOD=7.00±0.90%; REG=6.20±1.23%). Besides, GOOD group showed more quantity of sperm cells with PMI (P=0.02; 79.87±2.01%), AMI (P=0.03; 80.22±1.98%), HMMP (P=0.0031; 83.95±2.03%) and PIAIH (P=0.01; 77.78±2.02%) than REG (PMI=71.00±3.37%; AMI=71.63±3.57%; HMMP=68.56±4.88% and PIAIH=65.73±4.69%). In relation to ultrasound exam, REG (0.20±0.09) showed higher (P=0.04) quantity of hyperechoic points than GOOD (0.02±0.02). Besides, REG (0.06±0.04) tended (P=0.08) to be minus homogenous parenchyma than GOOD (0±0). No other ultrasonography feature presented difference. As for RT, SSMT and EAMT, the results showed no difference (P>0.05). Thus, it is possible to conclude that the quality of semen is not influenced by variation in scrotal temperature at the time of harvest. Furthermore, the study shows that bulls with the greatest presence of hyperechoic points in testicular parenchyma, suggestive of calcification points, have reduced semen quality.

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Scrotal surface and eye area temperature are effective in identifying testicular thermoregulation and physiological changes in Nellore bulls (*Bos indicus*)

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Bos indicus cattle is a native animal from South and Southeast Asia, regions with tropical climates. However, high environmental temperatures can compromise body and testicular thermoregulation, changing the physiological parameters, such as decreasing sperm quality. The temperature of the scrotal surface and the eye area measured by infrared thermography, has become a useful technique in the evaluation of thermoregulatory capacity and diagnosis of both testicular and physiological disorders caused by increased temperature. It is important to add new tools to evaluate the impact of environmental factors such as heat stress and its impact in reproduction. Accordingly, this study aimed to evaluate the scrotal temperature changes and the eye area temperature of Nellore bulls (Bos indicus) and its relation to physiological parameters and environmental temperature. For such, were used 12 Nellore bulls with mean of 34 ± 1.16 months of age. Evaluations were made for three non consecutive days .The scrotal surface mean temperature (SSMT, ºC) and eye area mean temperature (EAMT; ºC) were measured by infrared thermographic camera, T620 model (FLIR Systems, USA). The images were evaluated using the FLIR Quick Report® software (FLIR Systems, USA), and were captured at a distance of 0.9 m between the camera and the scrotum. The environmental temperature (ET, ºC) was verified by a data logger device (OPUS 20 THI - 8120.00, Lufft, Germany). Were also assessed rectal temperature (RT, °C) using a digital thermometer, heart rate (HR, bpm) and respiratory rate (RR; breaths/min) using a stethoscope. The data were submitted to Pearson correlation analysis using the Statistical Analysis System (SAS, 2004). The significance level was 5%. Physiological values of RT, RR and HR changed according to environmental temperature variations. The results showed a positive correlation ($p<0.0001$) between the ET and RT ($r = 0.73$), also ($p = 0.0001$) to ET and RR ($r = 0.40$), however the correlation between ET and HR showed no statistical difference ($p = 0.2617$). It was showed positive correlation ($p<.0001$) between RT and EAMT ($r = 0.63$). Regarding SSMT, were observed that there was positive correlation for all parameters, SSMT x ET ($r = 0.87$, $p <0.0001$); SSMT x RT ($r =0.63$, $p <0.0001$); SSMT x RR ($r = 0.29$, $p = 0.0072$); and SSMT x EAMT ($r = 0.83$, $p = 0.0001$); differently to SSMT x HR, that was found negative correlation ($r = -0.23$, $p = 0.03$). Thus it can be consider that SSMT is affected directly by ET, RT, RR, as well as EAMT values, and it can be allow identifying the heat stress. Thus, were conclude that it is possible to evaluate the physiological changes of thermoregulation through the variation of temperature of bovine skin, in this case the mean surface of the scrotum and the eye.

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Scrotal surface mean temperature differ throughout the day in Nellore bulls (*Bos indicus*)

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The Nellore cattle are characterized, in general, like animals more adapted to tropical environments. Meanwhile, environmental high temperatures may cause elevation of testicular temperature harming sperm quality. Scrotal superficies mean temperature (SSMT) measured by infrared thermography camera has been became a useful technology in assessment of thermoregulatory capacity and the diagnosis of testicular disorders caused by increased temperature. However, few studies demonstrated variation of SSMT throughout the day. Thus, the aim of this study was to evaluate scrotal temperature changes of Nellore bulls (*Bos indicus*) during different periods of day (morning and afternoon) and, their relationship with their physiological parameters and environmental temperature. For this, twelve Nellore bulls were used with 34±1.16 months of age. The assessments were made during three nonconsecutive days evaluating on the morning (7 a.m.) and afternoon (12 p.m.). The SSMT (ºC) was measured using an infrared thermography camera, T620 model (FLIR Systems, USA). The images were evaluated using the FLIR Quick Report® software (FLIR Systems, USA) and captured from a distance of 0.9 m between the camera and the scrotum. The environmental temperature (ET) was measured by Data Logger (OPUS 20 THI - 8120.00, Lufft, Germane). It was also evaluated the rectal temperature (RT, °C) by a digital thermometer; heart rate (HR; bpm); and respiratory rate (RR; breaths/min) using a stethoscope. Data were submitted to analysis of variance using the MIXED procedure of Statical Analysis System (SAS, 2004). Significance difference was considered when $P \leq 0.05$. SSMT measured on the morning (28.76±0.19ºC) presented lower (P<0.0001) than SSMT measured on the afternoon (32.70±0.12ºC). In the same way, on the morning ET (13.91±0.29ºC), RT (37.02±0.12ºC) and RR (24.02±1.02 breaths/min) were lesser (P<0.0001) than on the afternoon (ET = 25.58±0.3ºC; RT = 38.46±0.07ºC; RR = 30.09±1.20 breaths/min). However, there was no difference in heart rate (P=0.19) measured on the morning (79.95±2.47 bpm) and on the afternoon (75.58±2.62 bpm). Considering the previous results during the studied periods, morning and afternoon, respectively, of RR (24.02±1.02; 30.09±1.20 breaths/min) and HR (79.95±2.47; 75.58±2.62 bpm) were observed the influence of environmental temperature on RR and HR values obtained during both periods according to normal parameters suggested to Nellore bulls during winter (33.6±0.67 breaths/min and 68.9±0.80 bpm). Knowing that, for spermatogenesis normally occurs, the testicles temperature must be between 2 and 6°C lower than body temperature and the environmental temperature should be between 27 and 32ºC, were noted that, there is a difference of more than 6°C between SSMT (28.76±0.19ºC) and body temperature (37.02±0.12ºC), and a noticeable decrease in environmental temperature during the morning (13.91±0.29ºC). Subsequently, during the afternoon, the SSMT values, RT and ET were significantly different, and, still were kept within normal parameters. About the differences in the values of SSMT during the two periods of the day, in the morning (28.76±0.19ºC) and afternoon (32.70±0.12ºC), we can conclude that the SSMT varies according the temperature of the day, indicating that it is precise to standardize the period in which the assessment is made and thus minimize variations caused by environment temperature on the day. In this manner, these results can contribute attempts to establish selection criteria of the reproductive capacity of animals.

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Sertoli cell and spermatogenic efficiencies in the "common vampire bat", *Desmodus rotundus* (Geoffroy, 1810) (Chiroptera: Phyllostomidae: Desmodontinae)

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The *Desmodontinae* subfamily comprises of three species of haematophagous bats, monotypic and sympatric, in which *Desmodus rotundus* is the most prevalent. Because it feeds on livestock and is a potential carrier of rabies, this common vampire bat is considered as a pest. Despite of the distinct reproductive strategies of bats, little emphasis has been given to the study of testis function in these animals. Therefore, in the present study our main objectives were to evaluate the morphology, kinetics and efficiency of spermatogenesis in the common vampire bat. Sertoli cell and spermatogenic efficiency particularly important parameters. Therefore, testes from six adult males were routinely processed for histological, immunohistochemical and stereological analyses. In order to determine the duration of spermatogenesis, intraperitoneal injections of bromodeoxyuridine (BrdU) were performed at 1 hour and 14 days before the sacrifice. All data are presented as the mean ± SEM. The mean body and testis weights of *D. rotundus* were, respectively, 31.7 ± 0.04g and 0.81 ± 0.1g, providing a gonadosomatic index of 0.51 ± 0.07%. In general, the values observed for several important parameters that positively correlate with high sperm production were found for this bat species, such as: short length of spermatogenic process (29.3 ± 0.7 days), high percentage of seminiferous tubules (95±0.2%) in the testis parenchyma, high Sertoli cell efficiency (number of spermatids per Sertoli cell; 16 ± 1:1) and low frequency of germ cell loss (15%) during the two meiotic divisions. As a summation of all these data, the daily sperm production per testis gram (spermatogenic efficiency; daily sperm production (DSP)/g/testis) was 176 ± 4 million, by far the highest values found for the mammalian species investigated up to date. In order to better understand this strikingly high spermatogenic efficiency in this species, we are currently evaluating other important parameters related to testis physiology such as for instance the number of spermatogonial generations.

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Testicular needle aspiration: is it a safe method for breeding soundness evaluation of bulls?

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Testicular needle aspiration (TNA) represents a complementary method of testicular analysis, providing objective information on spermatogenesis. Although TNA is considered a low-cost and rapid execution technique, there is no consensus regarding the optimal diameter of the needle or the possibility of damage to the testicles after multiple punctures. The aim of the study was to evaluate the possible side effects of successive testicular punctures performed with hypodermic needles of different gauges. For this, 15 bulls were simultaneously submitted to a monthly TNA using a 18-gauges (40 × 12mm) or 22-gauges (25 × 7mm) needle, respectively in left and right testicles. The animals were randomly divided into 3 groups and castrated two days after the first punctures (G1, n = 5); after third punctures (G2, n = 5) and after sixth punctures (G3, n = 5). Fragments from the central portion of the testicular parenchyma (coincident with the path of the needles) were excised, fixed in Bouin's fluid and embedded in paraffin for histological processing. The sections were cut, stained with haematoxylin and eosin and evaluated under a light microscopy at ×100 magnification. Data (%) were analyzed by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS Institute™. No difference was observed in the percentage of degenerated seminiferous tubules between G1 (19.33^{a}), G2 (21.00^{a}) and G3 (23.89^{a}) (P = 0.7366). However, a higher amount of intra-tubular erythrocyte (3.67^{a}, 2.60^{ab}, 0.55^{b}; P = 0.0020), inter-tubular erythrocyte (22.33^{a}, 21.20^{a}, 3.33^{b}; P = 0.0368) and a higher tubular dilation (13.00^{a}, 10.00^{a}, 3.33^{b}; P = 0.0312) was observed in G3 compared to G2 and G1, respectively. There was no interaction between the needle gauge and the occurrence of testicular damage in animals submitted to one (G1) or three (G2) punctures. However, a higher percentage of tubular degeneration (41.87) was associated to 18 gauge needles in G3 in relation to fine needles (22-gauge) used in the same group or in relation to the other groups. In conclusion, successive punctures can compromise the testicular arquitecture and functionality of cattle and the use of thick gauge needles should be recommended only for one TNA, since multiple punctures can result in a higher percentage of tubular degeneration.

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The effect of Brazilian propolis and ametryn on Leydig cells and testosterone production of Wistar rats

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Brazilian green propolis is an important chemical defense used by bees against pathogenic microorganisms. In popular medicine propolis have been used as a remedy in wound treatment, stomach ulcers, mouth rinse and in the preparation of food and beverages. The indiscriminate use of propolis can cause damages in male gonads. Preview studies showed that propolis can increase sperm production. Ametryn is a triazine herbicide that inhibits photosynthesis in plants and it is used in many countries as a selective herbicide to control broadleaf and invasive grasses in corn and sugarcane crops. Wistar rats treated with ametryn showed decrease in Leydig cell proportion such as and sperm decrease. Studies show the effect of these two compounds (ametryn and propolis) applied separately in Wistar rat testis. The aim of the present work was investigate the effect of concomitant application of these two compounds on Leydig cells morphology and quantify PSA and testosterone hormones in blood. Leydig cells are located in the interstitial region of the testis and are responsible for testosterone production. The treatment was performed for 90 days old-male Wistar rats (*Rattus norvegicus*), administered by gavage. The groups were divided into four groups (n = 6) C: control group (5mg of water/day), P: propolis group (6mg/kg/day of propolis extract), A: ametryn group (15mg/kg/day os ametryn) and P+A: propolis and ametryn group (association of propolis and ametryn doses). They were anaesthetized, euthanized and perfused after 56 days, before perfusion, blood was collected by cardiac puncture. Testis were fixed with Karnovsky and routinely processed for paraffin inclusion. The blood analysis were performed by São Lucas Laboratory, in Rio Claro, SP. Leydig cells stereology showed significant differences comparing P+A group and the other groups. The proportion of cell nucleus increased and cytoplasm proportion decreased. In the blood analysis, testosterone hormone production decreased significantly in the P+A group compared to the other groups ($P < 0.0001$) and increased in the ametryn group compared to the propolis group ($P = 0,0395$). In previous investigations of our research group with different propolis doses, all exposed groups showed a decrease in nuclear proportion and increased Leydig cell cytoplasm proportion. According to previous studies did not observe any change in the relative proportions of nucleus and cytoplasm with Ametryn treatment. Therefore, our results show that Propolis along with Ametryn interfered in the composition nucleus/cytoplasm of Leydig cells, consequently in testosterone production, which may negatively affect spermatogenesis. Further analysis such as spertmatides count in testis are necessary to confirm these results.

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The marmoset as an experimental model for comparative study on human spermatogenesis

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The black tufted-ear marmoset, *Callithrix penicillata*, is a small primate which belongs to the Callithricidae family, and has been widely used in biomedical researches due to its phylogenetic proximity to humans. This fact makes it an interesting experimental model. In the reproductive field, their testes present seminiferous epithelium organized in multi-stage tubules as in human, despite having different number of stages of the seminiferous epithelium cycle - SEC (marmoset IX and human VI). The present study aimed to investigate, in a comparative way, if the IX stages of the marmoset SEC could be divided into VI stages, like human, and therefore use this species as an animal model for further studies related to human spermatogenesis. Testis of five adult male marmosets were processed by a high resolution light microscopic (HRLM) method being fixed by heart perfusion with glutaraldehyde, embedded in Araldite epoxi resin, sectioned at 1µm thickness and stained with toluidine blue borate, as previously standardized by our group. The IX stages of marmoset SEC were redefined into VI stages with the following correspondence (human-marmoset): I-I; II-II/III; III-IV; IV-V/VI; V-VII/VIII and VI-IX. To verify if testicular parameter could be comparable between them, morphological and morphometrical analyses were performed as follows: (1) spermatogonial subtypes characterized by their nuclear morphology; (2) spermatogonial numbers; (3) mitotic and apoptotic spermatogonial numbers and (4) efficiency of spermatogenesis (mitotic, meiotic, Sertoli cell workload and efficiency of spermatogenesis indexes). The indexes were determined by germ cell count at the same position in the SEC, once stage IV of marmosets corresponds exclusively to stage III in human. Spermatogonial number of both SECs showed similar germ cells distribution, when the proposed correspondence was applied. A_{dark} spermatogonia maintained their population constant along all stages of the SEC. On the other hand, A_{pale} spermatogonia numbers varied, decreasing their number when dividing to type B_1 spermatogonia. Subsequent germ cell divisions were B_1 to B_2 spermatogonia and B_2 to preleptotene primary spermatocyte, when the number of each one was almost the double of the predecessor cell, coinciding with mitotic peaks at specific stages. Regarding spermatogenesis efficiency, as the stage III (human) and IV (marmoset) occupy the same position in the SEC, the results were similar. The (1) mitotic index was ~6, when it was expected to be 8; (2) meiotic index obtained was ~3, when it was expected to be 4; (3) Sertoli cell workload was ~6 and (4) spermatogenic efficiency was ~45, which is considered a high index. In conclusion, we observed that IX marmoset stages of the SEC can be fully adapted to VI man stages due to similar spermatogonial kinetics (number, mitosis and apoptosis) and spermatogenesis efficiency. Thus, this finding suggests that marmosets can be used for further experimental studies related to testicular analysis, and is an appropriate experimental model for comparative study on human spermatogenesis.

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Edition of TFAM gene by CRISPR Cas9 technology

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The mitochondrial transcription factor A (TFAM) is a member of HMGB subfamily, that bind to promoters of mtDNA. It is a very important gene that maintains mtDNA, regulates the number of copies and is essential for the initiation of transcription mtDNA (CHOI et al., 2011). Recently, gene editing tools such as nucleases have been used as gRNA- known as CRISPR / Cas9 (Regulary interspaced clustered short palindromic repeats). CRISPR / Cas9 technology uses a short gRNA that is a junction sequence of tracrRNA with crRNA, containing 20 nucleotides complementary to the DNA sequence as well as a RNA nuclease guided Cas 9. When gRNA binds to the target site, the Cas9 protein is recruited to bind in the chosen location and induce double strands breaks in DNA (JINEK et al., 2012). In this context, this study proposed to edit the TFAM gene aiming to generate ROS zero cell (cells without to reactive oxygen species) through the knock-out in bovine fibroblasts. Bovine fibroblasts used in this study were derived from a skin biopsy collected from an adult. The sequence obtained from the database GenBank (www.ncbi.nlm.nih.gov) was inserted in the CRISPR direct site (crispr.dbcls.jp) and in the rgenome site (rgenome.net) to design the RNA guide. The gRNA was designed in the CRISPR direct site (crispr.dbcls.jp) for the Exon 1 of the gene TFAM bovine and after was performed the CRISPR cloning. Fibroblasts cells were electro-transfected with Cas9 (Addgene 48668) and control plasmids using the Nucleofector ^{TM} Kit for Primary Mammalian Fibroblasts (VPI-1002) and electro-transfected with Amaxa Nucleofector 2B equipament using the Program U-012. Post transfected cells with more than 70% of confluence were further split into 1 cell/well (96- well plates for cell cloning). The genomic DNA was extracted from cells using the ENZA Tissue DNA Kit- D3396-01, according to the manufacturer's protocol. To assess the mutation frequency, T7 endonuclease assay were performed. We observed that the cells were efficiently transfected since they have a rate of 90% transfection. The T7 test of the fibroblast transfected cells showed 40% of mutation rate. In conclusion, 1 clone of the cells had the deletion mutation in the CRISPR design site and other clones have been analyzed.

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Lentivirus-mediated generation of mosaic transgenic fish

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Fish transgenesis is performed since the beginning of the 80's by using the standard technique: microinjection. Commonly, a mosaic animal is first generated and then full transgenic organisms (fishes). However, microinjection is a technique that possesses low efficiency rate and requires highly trained people to inject correctly using the glass needle and the micromanipulator. So, in the present study we genetically modified Nile tilapias using lentiviral particles, in order to obtain a protocol to generate the mosaic transgenic animal in a faster and easier way. The lentivirus were generated in 293FT cells by using a plasmid DNA developed from pLenti6.3/V5™-TOPO® to contain the DsRed2 fluorescent protein codifying sequence. Nile tilapia newly fertilized eggs were exposed to different concentrations of lentivirus particles (from $6.0x10^5$ TU/mL to $6.0x10^4$ TU/mL) for 24 hours (under agitation at 28ºC) in two different systems, using Petri dishes or Falcon tubes. After that, the eggs were kept under agitation in tank water until the hatching. Larvae were analyzed under fluorescence microscope during its development and the presence of DsRed2 fluorescent protein could be noticed. The death rate was reduced when the Petri dish system was used. The protein presence was also accessed through immunohistochemistry using the antibodies Living Colors Anti-RCFP Polyclonal Pan Antibody (primary 1:1000) and Goat anti-Rabbit IgG HRP conjugate (secondary 1:500). The desired codifying sequence presence in the mosaic fishes was confirmed by analyses of mRNA through RT-PCR. So, lentivirus particles are important tools for the development of new biotechnologies in aquaculture as they can be used to quickly generate mosaic transgenic tilapias: which can be later outcrossed to produce the full transgenic fish, or which can provide gametes containing the transgene to obtain the full transgenic animal through *in vitro* fertilization.

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Targeted mutagenesis using easy-to-use CRISPR/Cas system in rainbow trout, *Oncorhynchus mykiss*

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A highly efficient technology for inducing mutations, CRISPR/Cas9 system, has been widely used in many species. This technique consists of using a synthesized short guide (sg) RNA by in vitro transcription, which encodes both CRISPR targeting (cr) RNA and trans-activating crRNA (tracrRNA) as a single chimeric transcript, injected together with Cas9 protein mRNA. Here, instead of a sgRNA, a combination of synthetic crRNA, tracrRNA and Cas9 protein were injected. This new approach simplifies the workflow by eliminating the time-consuming construction of sgRNA expression vectors. Therefore, this study aimed to induce targeted mutagenesis in Salmonidae fish using the CRISPR/Cas9 system through synthetic crRNA, tracrRNA and Cas9 protein. Appropriate crRNA oligonucleotides were designed to target rainbow trout *dead end (dnd)* gene, essential for proper primordial germ cells (PGC) migration towards the gonadal anlagen and PGC maintenance and survival during embryogenesis, and *slc45a2* gene, essential for melanin synthesis. For the *dnd* gene, Cas9 protein, tracrRNA and crRNA were mixed at concentrations of 1.0, 0.2, and 0.2 ng/nL, respectively for High (H) group and 2.0, 0.4, and 0.4 ng/nL concentrations, respectively for Extra High (EH) groups, being injected 4 nL solution per embryo. For *slc45a2* gene, Cas9 protein, tracrRNA and crRNA were mixed at concentrations of 1.0, 0.2, and 0.2 ng/nL, respectively, and were 2 (Medium group, M) and 4 (High group, H) nL solution were injected. For both genes, solutions were microinjected into one-cell stage rainbow trout embryos. At 35 days post fertilization (dpf) stage, embryos were collected to evaluate the efficiency of *dnd* knockout. At 47 dpf stage, pigmentation phenotypes of larvae were observed. After extraction of genomic DNA, *dnd* and *slc45a2* fragments were amplified by PCR. CRISPR/Cas9-induced mutations were then detected by T7 endonuclease I (T7EI) digestion and genotyping analyses (for *slc45a2*). For *dnd* gene, the two groups (H and EH) of microinjected rainbow trout embryos showed survival of ≥42.5%. After cleavage with T7EI, we found some mismatches, indicating that *dnd* gene KO worked in H and EH groups in some embryos. The T7EI assay is easy and fast and does not need any specialized equipment. For *slc45a2*, the two groups (M and H) showed survival of ≥74%. At hatching stage, 84% of the M group larvae showed partial loss of pigmentation. In the H group, 68% and 4% of larvae showed a complete and partial loss of pigmentation, respectively. T7EI digestion suggests a high rate of mutation for *slc45a2*. Various types of mutations and no wild-type DNA were detected around the target site by direct sequencing of DNA from the H group. Thus, we conclude that the CRISPR/Cas9 system using synthetic crRNA, tracrRNA, and Cas9 protein can successfully introduce mutations into the *slc45a2* and *dnd* gene in Salmonidae fish. Particularly, we could effectively introduce bi-allelic and non-mosaic mutations into the *slc45a2* gene.

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Biology of reproduction of crab eating fox (*Cerdocyon thous*) and cryopreservation of genetic material for enrichment of animal germplasm bank

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The sprawl of urbanization and road network process without building ecological corridors contributes to the high mortality rates observed today, and a threat to the population decline of wild species such as the crab eating fox (*Cerdocyon thous*). A strategy for the ex situ conservation is the study of the reproductive biology of the species and cryopreservation of their genetic heritage through the formation of an Animal Germplasm Bank. Obtaining biological material of this research met in accordance with the principles adopted by COBEA (Brazilian College of Animal Experimentation), under the approval of the Research Ethics Committee (CEP - FZEA) No. 14.1.1465.74.6/SISBIO No. 47183-1. Reproductive systems of *Cerdocyon thous* females (n = 2) were examined macroscopically, and microscopically by histological techniques (hematoxylin-eosin, Masson's Tricrhome, Schiff Reactive or "PAS" and Alcian Blue) and scanning electron microscopy. The macroscopic study of the female reproductive system showed that the format of the ovaries were similar to a bean and the oviducts lengths were between 5 and 8 cm in the specimens analyzed, the presence of bihorn uterus (3 cm) and they were long and narrow (9-11 cm). As observed in other species (wild and domestic), the cervix was as a single annular conformation carrying out communication between the uterus and the vagina. The vagina has lengthened and circular muscle and the vulva with dense anatomical conformation with a quite pronounced clitoris. In addition, with regard to the establishment of a cell line (fibroblasts) to the genebank enrichment, the results showed that cells *Cerdocyon thous* showed a low clonogenic capacity, especially when compared to the domestic dog, which can be explained by the "in vitro" environment action, and also wild specimens are unknown history (age, diet) where such factors could lead to the size of the telomeres of cells (fibroblasts) and thus be a hindrance to a satisfactory cell proliferation. Still, it was possible to create a bank of limited cell number, but feasible (to the 3rd pass). The present study had preservationist character, and aimed to help at long-term in the conservation of genetic resources of the species belonging to the Brazilian biomes, as the starting point for the implementation and continuity of new researches correlated with the specie in question.

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High-performance liquid chromatography as a novel tool for assessing ovarian function in jaguars (*Panthera onca*): development and validation of the method and quantification of ovarian steroids

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Of the four species of big cats, jaguars (*Panthera onca*) are the least studied in terms of reproductive physiology; this, along with the fact that their conservation status is of concern, can justify the search for novel methods that could facilitate the gathering of reproductive physiology data in the species. High-performance liquid chromatography (HPLC) is a method of considerable sensitivity and high precision that can be used to quantify compounds such as steroids. Other advantages of the method include the fact that the use of an automatic chromatographer can be much less labor-intensive than other techniques, such as enzyme immunoassays, and unlike the latter, HPLC can analyze multiple compounds at once. Therefore, the objectives of this study were to develop and validate an HPLC method for quantification of fecal steroids in jaguars. Two adult female jaguars were used in this study. Food dye was added to the diet of one female two to three times a week for identification of fecal samples, which were frozen before lyophilization. Hormone extraction was performed by adding 10 mL of a methanol/water solution (80/20) to 2.0 g of pulverized feces and homogenizing the mixture overnight. After centrifugation, the supernatant (fecal extract) was transferred to vials for partition with hexane, which was performed twice. The separated hexane phase was evaporated and the extract mass was re-suspended in 400 μL of methanol/water (80/20), from which 20 μL were injected into an automatic chromatographer. Parameters analyzed during the validation of the method were as follows: precision, accuracy, selectivity, limits of detection and quantification, linearity, and robustness. Concentrations of hormone metabolites (estradiol, estrone, progesterone) were considered to be elevated if they exceeded the sum of the mean plus two times the standard deviation of the mean. This formula was used repeatedly in an iterative process to exclude all values that were considered elevated until only baseline values remained for calculation of the mean baseline concentration. Any elevated concentrations of estrogen (estradiol or estrone) metabolites detected at the start of a series of elevated values marked the beginning of estrus. The method validation confirmed its reliability for all parameters analyzed. The method was able to quantify a compound in fecal samples that was likely an estradiol metabolite, based on its retention time and UV spectrum. Although some chromatograms revealed the presence of peaks with retention times comparable to those of estrone and progesterone standards, they were not able to be quantified due to concentrations being below the limit of quantification. Four estrous cycles were monitored (1 from Female #1 and 3 from Female #2). Mean interval between two estruses was 40.2 (± 0.2 SD) days. Mean baseline concentrations (± SD) and range of peak values of estradiol metabolite in Females #1 and #2, respectively, were as follows: 9,610 (± 7,001) ng/mL (peak range, 25,010 to 57,911 ng/mL); and 3,737 (± 1,161) ng/mL (peak range, 6,424 to 239,574 ng/mL). The method developed and validated herein demonstrates that HPLC is sufficiently sensitive to detect variations in estradiol metabolite concentrations in jaguar fecal samples. Partition with hexane was essential to remove the food dye from the fecal extract, and this step was necessary because of the interference of the dye in the chromatograms. Based on the mean interval observed between estruses, the length of the estrous cycle observed in this study was in agreement with what has been reported in previous studies in the species employing other methodologies of hormone analysis; thus, HPLC can be a tool for monitoring estrous cyclicity in jaguars noninvasively.

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Physiological and biochemical responses to repeated anaesthesia and electroejaculation in adult and yearling pampas deer (*Ozotoceros bezoarticus*) males

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As pampas deer (*Ozotoceros bezoarticus*) is an endangered species, gamete preservation is a useful tool for preservation and maintenance of genetic diversity. However, semen should be collected by electroejaculation (EE) under general anaesthesia, for which the animals should be captured with darts. We previously reported that EE induces pain and stress in this species, even under general anaesthesia. The objective of this study was to determine if the repetition of captures, anesthesia and EE affects heart rate (HR), pulse rate (PR), respiratory rate (RR), rectal temperature (RT), oximetry (SpO_2), cortisol, alkaline phosphatase (AP), and creatinekinase (CK) responses in adult and yearlings pampas deer (*Ozotoceros bezoarticus*) males. The study was conducted from June to March of the following year at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar, Maldonado, Uruguay with 11males. From these, 6 were adults [4-7 years old; 29.6 ± 2.7 kg (mean ± SEM)] and 5 yearlings (1.5-2 years old; 25.0 ± 2.2 kg), each group allocated in a 0.5 ha enclosure. The animals were captured with darts flowed from a blowpipe containing 2mg/kg of xylazine, 1.6 mg/kg of ketamine, and 0.013 mg/kg of atropine every ~3 months (June-July, September-October, January, late March). Once asleep, EE was performed applying series of 1v lasting 4-5 s each, with rest intervals of 2-3 s, increasing 1v every 10 pulses, until achieving ejaculation or a maximum of 6 v. After the process ended, yohimbine hydrochloride (0.26 mg/kg) was administered iv to reverse the anesthesia. Heart rate (HR), PR, RR and SpO_2 were measured before EE (BEE), in each voltage serie, and after EE (AEE), and cortisol, AP, CK, and RT were measured BEE and AEE. All animals survived at least for 6 months after the last manipulation. Adult and yearling animals had similar induction times (11.4 ± 6.8 min vs. 11.3 ± 8.4 min, ns) and remained anesthetized 80.0 ± 5.0 min and 75.8 ± 3.2 min (ns). The erection occurred at 4.2 ± 0.9 v and 4.5 ± 1.0 v in adults and yearlings (ns). Semen was collected from all animals in all the procedures, beginning when 3.0 ± 1.5 v and 3.8 ± 1.8 v were applied in adult and yearling males, respectively (P = 0.06). Heart rate, PR, RR, CK, and cortisol concentration increased AEE. Heart rate increased from 52 ± 8 to 151 ± 2 bpm (P <0.0001); PR from 64 ± 5 to 109 ± 4 bpm (P <0.0001); RR from 24 ± 4 to 31 ± 3 bpm (P <0.001); CK concentration from 303 ± 25 to 788 ± 112 UI/L (P <0.001) and cortisol concentration from 0.21 ± 0.01 to 0.99 ± 0.45 µg/dl (P = 0.003). On the other hand, RT decreased AEE from 37.5 ± 0.2 to 36.7 ± 0.2 ºC (P = 0.004). Heart rate was maximum in the first 118 ± 5 bpm and third collections 122 ± 6 bpm (P < 0.01), RR in the fourth 44 ± 3 bpm (P < 0.001), and RT in the third 38.1 ± 0.1 ºC and fourth 38.3 ± 0.2 ºC (P < 0.0001) procedures. Creatinekinase was maximum in the first 405.1 ± 48.1 UI/l (P = 0.02), and AP in the second 741.1 ± 193.7 UI/l (P < 0.0001) procedures. We concluded that EE under general anesthesia in pampas deer males is stressful, although the procedures can be repeated with the intervals used in this study without accumulated negative effects.

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Seasonal variation of electroejaculatory response and semen parameters of collared peccaries (*Pecari tajacu*) bred under semiarid conditions

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It is known that the reproductive activity of various mammals can be affected by several factors such as the climate and its changes, which are decisive for the semen quality in several species. In semiarid regions, animals suffer the effect of high temperatures, especially during dry season. This research was conducted in order to obtain information regarding a possible influence of seasonality on semen collection efficiency and sperm kinetic parameters in collared peccaries (*Pecari tajacu*) bred under semiarid conditions. These are preliminary data obtained from September 2015 to June 2016. The experiment was conducted in the Centre for Multiplication of Wild Animals/UFERSA, located in Mossoró, RN, in the Northeast Brazil, under the following geographical coordinates 5º10'S, 37º10'W. Eleven sexually matured male were monthly monitored. The animals were isolated from females 3 months before the commencement of the study and kept under a 12 h natural photoperiod. They were maintained outdoors in groups of five and six animals in paddocks (20m×30m) with a covered area of (3m×3m). The animals were fed with sow food and seasonal fruits. Water was freely available. For occasion o semen collection, they were anesthetized with propofol (5 mg/kg; Propovan®, Cristália, Fortaleza, Brazil) and the semen was collected with the aid of an electroejaculator (Autojac®, Neovet, Campinas, SP, Brasil) connected to a source of 12 V using a rectal probe. Semen was collected in graduated plastic tubes and immediately evaluated for the kinetic parameters of sperm motility by a computer assisted semen analysis (CASA IVOS 12.0, Hamilton-Thorne, Beverly, USA). Environmental parameters such as ambient temperature (°C), relative humidity (%) and rainfall were obtained by an automated meteorological station from the National Weather Institute – INMET. The efficiency of semen collection per month was expressed as percentage and analyzed by the Fisher's exact test. Effect of monthly seasonal variation on sperm parameters was checked by two way-ANOVA for repeated followed by the Tukey test ($P < 0.05$). The efficiency of semen collection per month was 81.8% in September, 63.6% in October, 72.3% in November, 63.4 %, in December, 36.4%, in January, 90.9% in February, 72,3% in March, 54.5% in April, 72.3 %, in May and 54.5 %, in June. In February we verified an increase on such efficiency, when compared to January ($P<0.05$), highlighting that it is positively influenced by beginning of the rainy season. Moreover, January presented the most unsatisfactory values for efficiency of semen collection, probably because of the previous long dry season. In fact, from September to November 2015, no rainfall was detected in the region. In December, rainfall values of 13.2 mm were determined, reaching 204.6 mm in January, and 21.8 mm in February. Moreover, significant variations were detected concerning temperature and relative humidity from December (28.2 °C, 65.9 %) to January (27.3 °C, 79.1%) and February (27.9°C, 74.9 %). We believe that the positive effect of rainfall was only verified on the semen collection in February due to the spermatic cycle that lasts 55.1 ± 0.7 days in peccaries. In addition, few variations were verified regarding sperm kinetic parameters, and only a difference on values for velocity straight line (VSL) was found between February (32.8 ± 2.6 µm/s) and June (32.8 ± 3.0 µm/s) when compared to November (19.7 ± 2.6 µm/s). In conclusion, these preliminary results suggest that seasonality can influence the efficiency for semen collection, but not the quality of sperm kinetic parameters in collared peccaries raised under semiarid conditions. These are valuable information to be used for the management of peccaries destined to reproduction, which are not indicated to be used for reproductive assisted techniques during and immediately after the dry season peak.

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Sperm motility of Brazilian-tapir (*Tapirus terrestris)*pre and post-thawing

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The Braziliantapir (*Tapirus terrestris)* is listed as endangered in the Red List of IUCN, therefore, it is important to have reproductive management with the aim of maintaining the maximum genetic diversity. The present study had the goal to describe the andrological parameters in the species, as well as collaborating with the development of assisted reproduction techniques, which could contribute to its conservation. Nine adult males from ItaipuBinational were utilized, four of which kept captive at the Biological Refuge Bela Vista in Foz do Iguaçu – PR, Brazil, and five at the Regional Zoo, in Hernandarias, Paraguay. The animals were submitted to chemical restraint through the association of ketamine hydrochloride, detomidine hydrochloride, butorphanol tartrate, and atropine hydrochloride, all of which administered via IM. Semen collection was performed by means of an electroejaculation machine (PT Electronics™, Boring, OR, USA), with a specific probe for tapirs (5.2 cm diameter), following the protocol described by Pukazhenthi*et al* (2011). Samples were obtained from four of the nine males. The semen collected was divided into two identical samples, and a subsequent dilution in a 1:1 proportion using the following diluents: Botusemen™ and INRA96™, followed by their centrifugation and discarding the supernatant. The precipitate were resuspended by using the freezing diluents Botu-crio™ and INRA96™ + egg yolk, followed by bottling in 0.5 straws. Four protocols were used for the semen cryopreservation, denominated according to the diluent utilized, followed by the cryoprotectant, making the following protocols: BB (Botu-semen + Botu-cryo), BI (Botu-semen + INRA 96 with egg yolk), II (INRA96 + INRA96 + egg yolk) and IB (INRA 96 + Botu-cryo).Each one of the samples had its motility (%) evaluated right after the semen collection. The cryopreservation of the samples was performed through programmed curve for equine semen on proper machine (TK-3000, TK, Uberaba – MG), and stored in liquid nitrogen for further analysis. The thawing and analysis of the samples was realized at the Laboratory of Semen Biotechnology and Andrology, Department of Animal Reproduction, at FMVZ – USP, São Paulo, being evaluated about their motility (%) by means of computerized system of sperm analyses (CASA – Computer Assisted Sperm Analyses; Hamilton Thorne) 10 minutes after thawing. Results are presented as means ± standard error of the mean. The following total motility pre-thawing results were obtained: BB- 23,75±7,46; BI- 47,5±11,27;IB-53,75±11,27; II- 62,5±11,08; on the other hand, post-thawing results were: BB-12,5±4,27; BI- 9,75±4,0; IB-11,25±5,46; II-12,5±4,19. As observed, the protocols showed similar results considering sperm motility after the cryopreservation, making them feasible for tapir semen cryopreservation.

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They are not vampires, but may reproduce during all year: testes and sperm cells of *Artibeus lituratus*, *Platyrrhinus lineatus* and *Sturnira lilium* (Chiroptera: Phyllostomidae) in a Neotropical savanna

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Environmental conditions (temperature, rainfall, relative air humidity and photoperiod) strongly affect the reproduction of bats. Although the effects of environmental factors on the reproduction of different species of bats have been described, there are no studies regarding the seasonal influence on the reproduction of bats in the Cerrado biome. The Cerrado is the most diverse tropical savanna in the world, and presents a seasonal climate with well-defined rainy and dry seasons. The aim of this study was to analyze the reproductive aspects of male bats of three locally common species of the Phyllostomidae family: *Artibeus lituratus*, *Platyrrhinus lineatus* and *Sturnira lilium*, during the dry and rainy seasons. Five males of each species were collected during the dry season. During the rainy season five males of *A. lituratus* and *S. lilium*, and four males of *P. lineatus* were collected. The testicles were fixed in Bouin's solution for five hours. The samples were dehydrated in ethanol, clarified in xylene and embedded in Paraplast Plus®. Three µm-thick sections were obtained at intervals of 45 µm, fixed onto slides and stained with hematoxylin-eosin, and taken to evaluation in a light microscope. Tubular area, lumen area, tubular diameter, and lumen diameter and epithelium height were measured on testicular cross sections using the computer software Image Pro-Plus®. Body weight was significantly higher during the dry season for the three species. Gonadosomatic index (GSI) was significantly different between seasons for all three species, being higher during the rainy season for *A. lituratus* and *P. lineatus*, and during the dry season for *S. lilium*. Testicular weight and length were higher during the rainy season for *A. lituratus* and *P. lineatus*, and during the dry season for *S. lilium*. The tubular parameters were significantly bigger in *A. lituratus* than in the other two species during both seasons. No difference on the tubular/interstitial ratio was observed in any of the studied species during both seasons. In both rainy and dry seasons all sperm cells and germ-cell lineages were visible on seminiferous tubules whereas sperm cells were observed on epididymides of all sampled animals. The percentage of morphologically normal sperm was low (between 35-60%), with no differences between seasons. Spermatozoa from *A. lituratus* presented an arrow-shaped head, while the head was round-shaped in the other two species. In conclusion, despite the fact that some significant differences between seasons could be observed, our data suggest that males from the three studied species may be able to reproduce along the year and do not present reproductive seasonality in the Cerrado biome. (CEUA/UnB/Protocol#116319/2011).

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Ultrasonographic study of ovary in agoutis (*Dasyprocta prymnolopha*) bred in captivity, during the estrous cycle

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The use of native wild animals has been an alternative activity for producer income and subsistence in regions that presents many difficulties to have traditional livestock. The agouti is a rodent that has shown efficiency on captivity production, so deeper technics and studies about its reproductive physiology are tools to amplify and optimize its productive scale. The objective of this paper was characterizing ovarian morphological changes in agouti (*Dasyprocta primnolopha*) species bred in captivity on Patos city, Paraíba, Brazil, by Ultrasonograpy study. The experiment has done in Morphologic Sciences Lab of Veterinary Medicine Academic Unit (UAMV), Federal University of Campina Grande (UFCG). The animals were obtained in partnership with NEPAS (Wild Animals Center of Studies and Preservation), in Federal University of Piauí, Teresina-PI. The methodologic protocols were approved by Environment Ministry, SISBIO Nº 47944-1, and by the UFCG's Ethic Committee on Animals Use, CEP Nº 237 - 2014. Ultrasonography was realized during every experiment phase when ovarian cycle change was detected through vaginal cytology aiming evaluate ovaries morphological changes. The animals were caught using a dip nets, they were mechanically held with leather gloves help and sent to Veterinary Anatomy Lab of Rural Health and Technology Center in Campus – Patos – PB. With the animals mechanically held and previously trichotomized on abdomen's side region (right and left flanks) until sublumbar muscles high. We used 5 females of *Dasyprocta prymnolopha* species and a vasectomised male for stimulate females' cycle. It was followed all phases of females' estrous cycle through vaginal colpocytology, as reference for ultrasonography evaluations realized by a portable Ultrasound equipment, with coler doppler of MINDRAY® brand, linked to a multifrequency linear transducer to identify morphologic changes of both ovaries to each phase of estrous cycle. We observed that the length of estrous cycle was 28.2±12.6 days, with 3.5±2.4 days for proestrus, 1.75±0.5 days for estrus, 5±0.8 days for metestrus and 18±9.4 days for diestrus. The left ovary is dorsally located, next to upper abdominal cavity, caudally to left kidney, attached to up side by the suspensory ligament of the ovary; the right ovary was more caudoventral to right kidney, suspended in the cavity by the suspensory ligament of the ovary. From ultrasound exam was possible stablish access through right and left flank, generating a constant window delimited for the kidneys right and left as well, cranially from dosrsally suspensory ligament of the ovary and from ovary's arteries and veins ventrally, forming a triangle of caudal vertex with the ovary located on this vertex. Measurements were made getting the ovaries length and width, as length average of 0.78 ± 0.09cm and as width average of 0.32±0.05cm to left ovaries and a length average of 0.76±0.09cm and a width average of 0.31±0.05cm to right ovaries. No differences were observed in the ovary size at different estrous cycle phase ($P<0,05$). The follicular phase was well characterized by the appearance of one or two follicles well seen on sonography exam. Neither the corpus luteum nor atreitic follicles was possible to be observed by ultrasound exam. Despite the ultrasonography has shown its efficiency on follicular phase identification, other complementary exams must be realized to other phases distinction.

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Histological comparison of the effect of fragment size in vitrification of cat ovarian tissue: preliminary results

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Cryopreservation of ovarian tissue is an important tool to preserve fertility, once the thawed/warmed tissue may be transplanted or harvested in order to obtain oocytes for IVM-IVF. Vitrification is characterized by a rapid cooling rate and an absence of ice crystal formation, and is considered a technique easy to perform. Vitrification of ovarian tissue has been reported in several species, including the domestic cat and, in this specie, attempts have been made to improve the quality of the warmed tissue. To author's knowledge, up to date there is no reports investigating the effect of fragment size in vitrification of cat ovarian tissue. Hence, the aim of this study was to evaluate whether the size of the ovarian fragment interfere with the permeability of cryoprotectants in a domestic cat model. Ovaries were obtained from four cats submitted to routine ovariectomy. Ovarian cortical tissues were isolated in HTF (Human Tubal Fluid) medium and sectioned into 2 mm and 4 mm cubes. Eighteen fragments were vitrified and 18 were used as control. Vitrification was performed as previously reported (Luvoni et al., 2012). Briefly, fragments were immersed in PB1 with 1 M DMSO (Sigma Chemical Co., USA), at room temperature for 60 sec, placed for 5 min into 1 ml cryotube with 5 µl of PB1-DMSO on ice, added with 95 µl of DAP123 solution (1 M Acetamide, 2 M DMSO, 3 M Propylene Glycol; Sigma) for additional 5 min and plunged directly into liquid nitrogen for storage. At thawing, cryotubes were held for 60 sec at room temperature and added with 900 µl of 0.25 M sucrose (Sigma) prewarmed to 37°C. The thawed fragments were washed in PB1 medium, transferred to HTF medium and then fixed in formol. Histomorphological evaluation was performed. Vitrified/warmed fragments were embedded in paraffin blocks and 4µm thickness serial sections were obtained and stained with hematoxylin and eosin to assess histomorphological features of primordial, primary, secondary and antral follicles. Data were analyzed by chi-square test and differences were considered significant at $p<0.05$. There was no significant difference in the percentage of follicles presenting normal morphology between the 2 mm (58.3%) and 4 mm (64.2%) cubes and also compared to the fresh ones (77.2%), irrespective of the type of follicle. Although the results indicated that both fragment sizes are suitable for maintenance of follicular morphology under the conditions employed in this study, further investigation using a higher number of ovarian fragments and evaluating follicle viability using different tests might be necessary.

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