Immunohistochemistry standardization of PGF2α-R receptor in the cervix bitch with pyometra

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The prostaglandins action mechanisms are not fully elucidated. It is known that prostaglandins are involved with physiological cervical ripening where cervical fibroblasts are primers of this process by controlling permeability and dilation of small blood vessels. Prostaglandins may also have a direct effect on stimulation of metalloproteinase matrix. This stimulation promotes a vasoactive effect which facilitates cervix leukocyte penetration. Neutrophils are a significant source of collagenase for the final cervical ripening. To better understand the involvement of PGF2α-R in the cervical opening mechanism, pyometra groups of closed and opened cervix were compared by immunohistochemistry. For this it was necessary to standardize this technique for the canine species. Antigen retrieval was in 10mM sodium citrate solution incubated in Pascal pressure cooker (Dako ®). Then, blocks were performed in a solution of 8% H2O2 for 20 minutes, Molico® (3g/100mL) for 60 minutes and protein block for 30 minutes. Primary antibody sc67029 PGF2a-R (Santa Cruz Biotechnology ®) incubation was at 1:200 dilution in a humid chamber for 2 hours at 37°C. The material was then incubated with the secondary antibody Histofine ® for 30 minutes and uncovered with DAB chromogen for 5 minutes. Slides were counterstained with Mayer's hematoxylin for 1 minute. Dehydration in alcohol baths and mounting the slides were performed. The cell cytoplasm stained blue is negative and stained brown are considered positive, thus showing the efficiency of the immunohistochemical technique for evaluation of PGF2a-R receptors in bitches' cervix.

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Effect of prorenin in the epidermal growth factor-like and prostaglandin-endoperoxide synthase 2 mRNA expression induced by LH in bovine granulosa cells

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The renin-angiotensin system is a target for research in physiology of reproduction. In mammals, luteinizing hormone (LH) release increases prorenin synthesis in the blood plasma and follicular fluid (Itskovitz *et al.* 1988, Ann. N. Y. Acad. Sci, 541, 179-89). Our group of research demonstrated that (pro)renin receptor [(P)RR] mRNA is stimulated by LH in granulosa and theca cells of the bovine preovulatory follicle. However, the role of prorenin/(P)RR in the regulation of key genes of the ovulatory process remains unclear. In this regard, in the present study we have addressed the effects of prorenin in the epidermal growth factor-like (AREG and EREG) and prostaglandin-endoperoxide synthase 2 (PTGS2) induced by LH in bovine granulosa cells cultured *in vitro*. Bovine ovaries were collected from adult cows at a local abattoir. Four to five ovaries containing a single large follicle (>10 mm diameter) were selected for each replicate (three replicates). Granulosa cells were obtained by flushing with PBS, pooled and then washed twice by centrifugation. Cell viability was estimated with 0.4% Trypan Blue Stain. Cells were seeded into 96-well tissue culture plates at a density of 5 x 10^4 viable cells per well in 200µl of DMEM-F12 supplemented with penicillin (100IU/ml), streptomycin (100IU/ml), androstenedione (10^{-7}M), FSH (1ng/ml), insulin (10ng/ml) and BSA (0.1%). Cultures were maintained at 39°C in 5% CO_2 for 6h. To evaluate the effect of prorenin in genes regulated by LH, granulosa cells were treated with base medium only (control) and stimulated with LH (alone) plus three different concentrations of prorenin (0.01, 0.1 and 1nM) in the first experiment. The second experiment was performed to investigate the participation of prorenin in the bovine ovulation, pre-incubating bovine granulosa cells with (P)RR inhibitor (10^{-5}M; aliskiren) 1 hour before, adding LH and/or prorenin to the culture medium. In both experiments, we accessed the expression of key genes in ovulation (AREG, EREG, ADAM17 and PTGS2) by RT-PCR comparing to the constitutive gene GAPDH at the end of the culture period (6 hours). The data from different treatments were compared by ANOVA. Data were tested for normal distribution using the Shapiro-Wilk test and normalized when necessary. All analyses were performed using JMP software (SAS Institute Inc., Cary, NC) and $p < 0.05$ was considered statistically significant. In prorenin dose-response experiment, ADAM17 mRNA levels were not different between treatments, as well as supplementation with prorenin (0.01, 0.10 and 1.0nM) did not stimulate EREG mRNA expression (5.82±0.68; 7.27±1.80; 5.75±1.33; respectively), compared to LH treatment (4.73±0.57). In all groups of granulosa cells stimulated with LH, including the prorenin treatment group, EREG mRNA levels were higher than the control group (0.53±0.15). The stimulation provided by LH was sufficient to induce the expression of key ovulation genes in our cell culture system. The AREG mRNA expression was upregulated in the granulosa cells stimulated by LH plus prorenin (5.30±0.38; 13.26±5.10; 7.08±1.95) compared to the control group (0.57±0.40), which was a tendency of increase when compared to LH group (4.98±0.73). The PTGS2 mRNA levels were significantly higher than the control group (1.36±0.87) only in the concentration of 0.10nM of prorenin (6.24±1.80). Therefore, prorenin was used in the second experiment at the concentration of 0.10nM. The supplementation with aliskiren seemed to reduce the AREG (214.19±181.45) and PTGS2 (0.76±0.68) mRNA when the cells are stimulated by LH plus prorenin (547.83±462.97 and 1.30±0.78, respectively). Furthermore, we observed that prorenin did not stimulate EREG mRNA induced by LH. In conclusion, our results suggested that prorenin participates in the cascade of events induced by LH-AREG-PTGS2 system in bovine granulosa cells.

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Hormonal evaluation of progesterone, triiodothyronine, thyroxine in bitches witch eutocia and distocya

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Mechanisms related to the endocrine control during pregnancy and childbirth in bitches is not yet fully elucidated. Studies have already demonstrated the main endocrine changes occurring during pregnancy and parturition bitches. The thyroid gland performs important functions in some stages of life, particularly during pregnancy and childbirth, and these functions are essential differentials in the early stages of gestation. Thyroid hormones (TH) are liberated from thyrotropin-releasing hormone (TRH) originating from the hypothalamus that acts on the anterior pituitary stimulating release stimulating hormone (TSH), which stimulates the release of hormones thyroxine (T4) and triiodothyronine (T3), which act specifically on the cardiovascular physiology in the gestation. Based on this, the present study aimed to demonstrate the hormone findings (progesterone, triiodothyronine and thyroxine) in bitches in eutocia and dystocia evaluated in two moments: M1 (one hour before childbirth or cesarean) and M2 (after childbirth or anesthetic recovery). 28 dogs were used, 22 in dystocia and six in eutocia. Comparing the two groups, progesterone was superior in the dystocia group. Plasma levels of triiodothyronine were higher in M1 in both groups. Thyroxine concentrations were greater in dystocia in M1, and an elevation was observed in eutocias in M2 compared to dystocia. It was concluded that, during childbirth, the elevation of progesterone affect uterine contractions predisposing to dystocia. In the occurrence of dystocia, thyroxine concentrations are high. Just as in finishing the eutocias, in childbirth there is also an elevation of thyroxine concentrations.

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GnRH/GnIH system in zebrafish olfactory epithelium

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GnRH (Gonadotropin-Releasing Hormone) is one of the key hormones that control reproduction in vertebrates. This decapeptide hormone stimulates the release of Fsh (follicle-stimulating hormone) and Lh, (luteinizing hormone) from the pituitary. Based on phylogenetic and molecular analyses, three GnRH variants can be found in many vertebrates: GnRH1, the hypothalamic releasing form (hypophysiotropic preoptic area-GnRH system); GnRH2, the mesencephalic form; and GnRH3, the telencephalic form (TN-GnRH system). In contrast, GnIH (Gonadotropin-inhibitory hormone), a recently discovered hypothalamic neuropeptide, plays an important role inhibiting GnRH. Several studies highlight the importance of the GnRH system to regulate the visual acuity and the sensitivity of the olfactory epithelium. Moreover, it has been demonstrated that the terminal nerve (TN) innervate the retina and the olfactory epithelium, suggesting a direct relation between the olfactory and the visual systems. Previous studies have shown the expression of GnRH system in retina, but their presence in the olfactory system is not characterized yet. Therefore, we investigated the expression of GnRH (*zfgnrh2, zfgnrh3*), GnIH and their receptors (*zfgnrhr1, zfgnrhr2, zfgnrhr3, zfgnrhr4 and gnihr1*) in adult male and female olfactory epithelium. In addition, GnRH system was also characterized in zebrafish retina, olfactory bulb, brain, testis and ovary. Furthermore, RNA extraction, cDNA synthesis and gene expression (RT-PCR) were carried out accordingly and normalized with β-actin. *zfgnrhr1, zfgnrhr2, zfgnrhr3, zfgnrhr4, gnih* and *gnihr1* product were observed in all tissues for male and female. *zfgnhr2* was found only in male and female brain. *zfgnhr3* was expressed in all tissues except male and female retina. Testis *zfgnih* amplified the expected target sequence (133 bp) and also a second faint band (~200bp). In summary, GnRH/GnIH system was characterized for the first time in zebrafish olfactory epithelium; expressing *zfgnrh3* and the receptors *zfgnrhr1,2,3,4*. Interestingly, GnIH and its receptor were also found in zebrafish olfactory epithelium. These results suggest an important modulatory role of GnRH3/GnIH over the sensorial system through the olfactory bulb. Further research will be required to unveil the role of these neuropeptides on the sensorial systems over fish reproduction.

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Expression of regulating factors of apoptosis, leptin and leptin receptor in ovine cumulus-oocyte complex

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Factors that regulate apoptosis can determine oocyte viability; however, few studies on the gene expression and action of these factors in antral follicle cumulus-oocyte complex (COC) development or their relationship with leptin (LEP) and its receptor (LEPR) in sheep have been published. The aim of this study was to evaluate the mRNA expression of *LEP*, *LEPRb* and apoptosis-regulating factors (*Bcl-2*, *Bax*, *p53* and Caspase-3) genes in oocytes (OOs) and cumulus cells (CCs) from antral follicles of different sizes. Twenty pools each of OOs (20 unities) and CCs (from 20 COCs) that were harvested from > 3 - mm and≤ 3 - mm follicles were evaluated. Gene expression was determined after mRNA extraction and further cDNA amplification using real-time PCR. The mRNA expression of factors related to apoptosis (*Bcl2*, Caspase-3 and *p53*) was detected in OOs and CCs obtained from small and large follicles. The mRNA expression of *Bax* was only observed in OOs, regardless of the follicle size. The mRNAs of *LEP* and *LEPRb* were not expressed in OOs. *LEP* mRNA was only expressed in CCs from small follicles, and *LEPRb* mRNA was expressed in CCs from follicles of both sizes but tended to be higher in follicles > 3 mm ($P = 0.10$). *Bcl2* mRNA expression was higher in OOs from small follicles than large follicles and higher compared with CCs from smaller follicles ($P < 0.0001$). The *Bcl2* mRNA expression did not differ between CCs obtained from large and small follicles ($P > 0.05$). The *Bax* and *p53* mRNAs showed higher expression in OOs from larger follicles than smaller follicles ($P < 0.01$). Although *p53* mRNA expression was detected in CCs, no difference was found between the two follicle sizes. Caspase-3 mRNA showed higher expression ($P < 0.0001$) in OOs from larger follicles than smaller follicles; however, there was no difference between CCs obtained from large and small follicles ($P > 0.05$). There was a positive correlation between *p53* and *Bax* mRNA expression in oocytes from small and large follicles ($r = 0.66$, $P = 0.02$). Additionally, a positive correlation between *Bax* in OOs and *LEPRb* in CCs was observed ($r = 0.64$, $P = 0.03$). In conclusion, this study showed that apoptosis-regulating genes are present in the OOs and CCs of antral follicles and that apoptosis-promoting genes (*Bax* and Caspase-3) show higher expression in large follicles, suggesting that COCs harvested from large follicles are affected more by the cell death process. *LEP* and *LEPRb* mRNA are expressed only in CCs, which indicates that *LEP* has a role in follicular growth regulation and oocyte maturation in sheep; however, further studies are necessary to determine the pathways associated with these mechanisms. The study evaluated the transcript expression (or the mRNA) of the genes instead of the molecule expression (LEP, LEPR or caspase expression). A better description should be used throughout the abstract.

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Analysis of cell-secreted vesicles present in bovine ovarian follicles during early follicular development

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Intercellular communication is crucial to allow ovarian follicles to grow and ovulate. Lately, cell-secreted vesicles called microvesicles (MVs) and exosomes (EXOs) were suggested as mediators of cell communication, and are present in different body fluids. MVs (100-1000 nm) and EXOs (30-150 nm) are secreted by cells using different cellular pathways and contain similar bioactive material such as mRNAs, miRNAs, and proteins. Recently, these vesicles have been identified in ovarian follicular fluid of mares, cows and women. The presence of these vesicles in ovarian follicular fluid presents an opportunity for the discovery of novel diagnostic markers, as well as therapeutic tools to improve fertility. Cows are good models to study follicular cell communication due to the easy access to samples and well-established protocols for artificial reproductive techniques. In order to study and characterize cell-secreted vesicles in bovine follicular fluid, we isolated MVs and EXOs from immature bovine follicles and determined the purity of the preparations based on morphology and RNA/protein contents. Ovarian follicles (5 follicle per ovary) ranging from 3-6 mm were dissected and follicular fluid was recovered without significant cell damage. Follicular fluid samples were processed individually, or in pools of 5 and 10 follicles. To isolate MVs and EXOs we started with differential centrifugation (300 x g for 10 min, 2000 x g for 10 min) followed by 16,000 x g during 30 min (first protocol). The second protocol used the supernatant from the previous protocol, filtered through a 0.2 μm filter and centrifuged two times at 100,000 x g. Following vesicle isolation, samples were processed for transmitted electron microscopy and RNA/protein isolation. As expected, a larger number of follicles used to isolate vesicles resulted in higher yields of RNA and protein, although a minimum of 5 follicles in a pool resulted in sufficient material for RNA and protein analysis. Transmission electron microscopy (TEM) on vesicle sample preparations using the first protocol demonstrated the presence of MVs (100-1000 nm) but also EXOs and mitochondria. The second protocol resulted in precipitation of a homogeneous preparation containing vesicles resembling EXOs (30-150 nm). The RNA profile was different between MVs and EXOs. MVs presented enrichment of RNA molecules smaller than 25 nucleotides, while the EXOs contained a larger variety of RNA sizes suggesting the presence of small RNAs, miRNAs, and tRNAs. Coomassie stained SDS/PAGE gels revealed similar molecular weight proteins between MVs and EXOs. In conclusion, we used two protocols to isolate and characterize vesicles in bovine ovarian follicular fluid. TEM demonstrated the presence of two distinct preparations of cell-secreted vesicles namely MVs and EXOs. Using the first protocol resulted in isolation of MVs and EXOs, as well as mitochondria. The presence of mitochondria probably resulted from cell lysis during follicular development or follicle content collection. This last possibility is considered unlikely due to the healthy appearance of the selected follicles and the collection methodology, which only ruptures the wall of the follicle to aspirate follicular fluid. The second preparation yielded a homogeneous EXO sample without the presence of MVs or mitochondria. These different isolation techniques for cell-secreted vesicles from ovarian follicular fluid will benefit current ongoing and future experiments to understand the biology and function of MVs and EXOs in ovarian folliculogenesis.

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Induction and synchronization of estrus in pluriparous goats utilizing hCG

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Goats are an important domestic species for tropical regions because of their significant supply of quality products. The most widespread hormonal method to synchronize estrus in goats is the use of intravaginal progesterone followed by the administration of exogenous gonadotropins. The biological activity of human chorionic gonadotropin (hCG) is primarily similar to LH, with effects similar to those caused by FSH. Its administration at the early luteal phase also leads to ovulation of the dominant follicle of the first wave and the formation of a functional accessory *c*orpus luteum (CL). This study aimed to evaluate the early signs of estrus of pluriparous Saanen and Alpine Brown goats, through the induction and synchronization of estrus with the use of protocols that employ intravaginal progesterone-based intravaginal device (MPA) associated with the application of human chorionic gonadotropin (hCG). The experiment was conducted in the Sector of Goat Husbandry of the Department of Animal Science, in the Universidade Federal de Viçosa, located in Viçosa, Zona da Mata of Minas Gerais. Two hundred and nineteen multiparous lactating goats were used, out of which 143 were Parda Alpina and 76, Saanen. The induction was performed according to the following protocol: D0 (day 0), placement of the Medroxyprogesterone Acetate-based intravaginal device (MAP); on D5, application of 75 mg of synthetic prostaglandin (D-cloprostenol®). On D6, the intravaginal device was removed and on D7, 24 hours after device removal, animals received the application of 250 IU of hCG. The observations of estrus were performed using ruffians, in the morning (06:00 to 07:00 a.m.) and in the afternoon (05:00 to 06:00 p.m.). They started 12 hours after the removal of the intravaginal devices for a period of three days, the appearance of estrus responses were analyzed every 12 hours and expressed as percentages. Mating was performed by artificial insemination with fresh diluted semen 12 hours after estrus detection. The majority of animals showed estrous behavior between 48 and 60 hours after removal of the device. Since hCG physiological activity is similar to that of the luteinizing hormone (LH), the follicles must be at an advanced stage of growth, when there will be receptors specific for LH, which trigger the final growth and maturation of the follicle and consequently, ovulation. Therefore, this property may explain the late response observed this experiment compared to experiments by other authors using other gonadotropins in goats, because the response to this treatment depends on the animal stage of follicular growth.

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A specific adenil cyclase inhibitor (DDA) and a cyclic AMP-dependent protein kinase inhibitor (H-89) block the action of equine growth hormone (eGH) on *in vitro* maturation of equine oocytes

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Immature equine oocytes are capable of completing meiosis *in vitro*, but subsequent *in vitro* fertilization (IVF) and embryonic development of those oocytes are questionable. The effect of growth hormone (GH) on equine oocytes has demonstrated that equine oocytes resumed meiosis in the presence of equine GH (eGH) *in vitro*. Recently, we reported the presence of eGH-R in equine ovarian follicular structures, such as cumulus cells and oocyte, which may mediate a positive effect when eGH is used in culture during equine oocyte maturation *in vitro*. Furthermore, investigations are needed to acquire a better understanding of the developmental competence of equine oocytes when eGH are added during *in vitro* maturation (IVM) to improve the efficiency and the use of assisted reproductive technologies (ART) in the horse. The aims of this study were to determine whether stimulatory function of equine growth hormone (eGH) on equine oocyte maturation *in vitro* is mediated via cyclic adenosine-monophosphate (cAMP) and if the addition of eGH *in vitro* influences oocyte nuclear maturation and if this effect is removed when GH-inhibitors are added to culture. Cumulus oocyte complexes (COCs) were recovered from follicles <25mm in diameter and randomly allocated as follows: a) control (no additives) and b) 400 ng/ml of eGH. A specific inhibitor against cyclic AMP-dependent protein kinase (H-89; 10^{-9}, 10^{-11}, and 10^{-15} M) and a specific adenylate cyclase inhibitor (DDA; 10^{-8}, 10^{-10}, and 10^{-14} M) were used to observe whether they could block the eGH effect. After 30 h of *in vitro* maturation at 38.5°C in air with 5% CO_2, oocytes were stained with 10 µg/ml of Hoechst to evaluate nuclear status. More (p <0.05) mature oocytes were detected when COCs were incubated with eGH (29 of 84; 34.5%) then the control group (18 of 82; 21.9%). The H-89 inhibitor used at 10^{-9} M (4 of 29; 13.8%) decreased (p <0.05) the number of oocytes reaching nuclear maturation when compared to eGH (11 of 29; 38%). The DDA inhibitor at 10^{-8} M (2 of 27; 7.4%) also reduced (p <0.05) the number of oocytes reaching maturity when compared with the eGH group (9 of 30; 30%). Results from this study demonstrate that a significant number of equine oocytes achieved nuclear maturation status under the presence of eGH into the IVM system at 30 h of culture. We conclude that the addition of either DDA or H-89 in culture was accompanied by inhibition of eGH-induced oocyte nuclear maturation. These findings provide strong evidence that the signaling transduction pathway in equine oocyte was inactivated possibly by the inhibition of the activation of protein kinases or by blocking the cAMP production. These results provide a physiological and biological mechanism of the maturation and development of horse oocytes when *in vitro*.

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Survival of cattle preantral ovarian follicles after culture *in vitro* with medium supplemented with recombinant Bovine Somatotropin (rBST)

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The development of the Brazilian cattle industry has led to an increase in productive and reproductive efficiency of species of economic interest. Therefore, is necessary to develop and application of biotechnologies for increasing the reproductive potential of animals of high value livestock. In this context, the biotech of isolation and culture of preantral follicles from bovine ovaries (PFBO), which has as main objective the recovery of preantral follicles in the physiological *in vivo* process, would undergo atresia. Different hormones and growth factors have been used in these follicles *in vitro*, in order to define an appropriate system that enables the viability and growth of the same. So, the aim of this study was to evaluate the viability of bovine preantral follicles after *in vitro* culture for seven days with different concentrations of recombinant bovine somatotropin (rBST). Ovaries (n = 12) were obtained from bovine slaughtered in a slaughterhouse and transported to the laboratory in a water-bath at 4 ° C, up to one hour after collection. In laboratory, the parenchymal region of the ovary was fragmented and one fragment of each ovary was immediately fixed for classical histology (T1 - uncultivated control) and the others cultured in α-MEM (T2 - cultivated control) and α-MEM plus 10 (T3), 100 (T4) and 1000ng/ml rBST (T5). The cultures were grown for seven days, with the medium completely replaced every two days. After one and seven days, ovarian fragments were fixed and stained for subsequent histological analysis. After one day of culture, the percentage of normal follicles in non-cultured fragments (92.22%) was higher ($P < 0.05$) than that observed in cultured fragments (71.66; 81.66; 74.44 and 79.44% for treatments 2, 3, 4 and 5, respectively). Likewise, after seven days of culture, the percentage of normal follicles in non-cultured fragments was higher ($P < 0.05$) when compared to cultured fragments (72.78; 78.34; 83.88 and 76.66 for treatments 2, 3, 4 and 5, respectively). These results demonstrate that *in vitro* culture compromises the number of normal follicles. However, despite this reduction in survival compared to non-cultured control group, rates observed for normal follicles are still considered high. It was found also that although the rate of follicular survival was reduced in cultured follicles compared to control this rate was maintained when the follicles were evaluated after seven days of culture ($P < 0.05$). This demonstrates that the culture time does not affect the viability of these follicles. The results showed that the addition of rBST in the culture media of *in vitro* bovine preantral follicles has no beneficial effects on follicular viability.

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Gamma receptor peroxisome proliferator-activated (PPARγ) mRNA expression in follicular deviation in cattle

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Gamma receptor peroxisome proliferator-activated (PPARγ) is associated with different functions in the body, such as fat cell differentiation, inflammation, cell growth and steroidogenesis. It is known that this family of receptors is expressed in reproductive tissues from different species. In the mouse, the expression of this receptor increased according to follicular growth and decreased after the LH surge. Conversely, in sheep, PPARγ expression is primarily restricted to granulosa cells of developing follicles, and its expression is higher in small follicles. PPARs have been shown to participate in the regulation of apoptosis and cell cycle control and affect the synthesis and metabolism of estradiol. Therefore, the decline of PPARγ during follicular growth facilitates the progression of the cell cycle and contributes to the dominant follicle selection process. Nevertheless, these results suggest that PPARγ may have an important role during follicular deviation, since the dominant follicle has higher concentrations of estradiol compared to the subordinate follicle. The aim of this study was to investigate the expression profile of PPARγ in dominant and subordinate follicles during the period of follicular deviation. Cows had the estrous cycle synchronized and were ovariectomized on days 2, 3 or 4 relative to the follicular wave (4 cows per day). Therefore, with this experimental design, samples were collected before follicular deviation (day 2) and when the first and second largest follicles had a slight (day 3) or marked difference (day 4). Follicular fluid and granulosa cells from these two largest follicles were recovered to determine estradiol concentration and gene expression respectively. Real-time polymerase chain reaction (qRT-PCR) was conducted in a CFX 384^{TM} Real Time System, Bio-Rad. Samples were run in duplicate and expression values were reported relative to Histone H2A as the reference gene. The differences in continuous data were assessed by two-way ANOVA including the effects of follicle type (dominant or subordinate), day of follicular wave and their interaction. The mRNA abundance levels of CYP19 in granulosa cells increased in the dominant follicles and decreased in the subordinate follicles during development. These results confirm that the ovaries obtained at days 2, 3, and 4 of the first follicular wave were before, during, and after follicular deviation, respectively. There was no follicle vs. day interaction or follicle effect on PPARγ mRNA expression. However, on day 4 when estradiol levels are higher compared to day 2, PPARγ mRNA expression reduced 45% and 46% respectively on dominant and subordinate follicles ($p<0.001$). This data is consistent with studies demonstrating the involvement of this receptor in the synthesis and metabolism of estradiol. In conclusion, the results showed that this receptor may be involved in the selection of the dominant follicle in cattle and deserves to be explored.

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NPPC mRNA expression is upregulated by LH through EGF-R during ovulation in cattle

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Recently the Natriuretic Peptides (NPs) have been described in the local regulation of reproductive functions in polyovular mammals, beyond its systemic activity. In rodents, NPC precursor (NPPC) mRNA expression has been downregulated by hCG in granulosa cells. Furthermore, NPPC mRNA expression is downregulated by amphiregulin in granulosa cells *in vitro*. In monovular species the role of NPs during ovulation is not fully understood. The aim of this study was to evaluate the pattern of NPs precursors (NPPs) and receptors (NPRs) mRNA expression in granulosa cells after GnRH-induced ovulation *in vivo* in cattle and their interaction with the EGF system. Cyclic beef cows were synchronized using a progesterone-based protocol. After intravaginal device removal (day 9), ovaries were examined by transrectal ultrasonography and the cows that had GnRH-responsive preovulatory follicles (≥ 12 mm) were challenged with 100 μg of gonadorelin acetate IM 12 h after removal of intravaginal device. Treated cows were then ovariectomized 0, 3, 6, 12 and 24 h post-GnRH by colpotomy (n=5 to 6 animals in each time-point). Immediately after ovariectomy, follicular fluid was recovered and each cell type (granulosa and theca) was isolated. The effect of NPs on EGF-like factors (epiregulin and amphiregulin) mRNA expression and the effect of EGF-R (EGF-receptor) signaling blockade on LH modulation of NPPC mRNA expression was evaluated using a model of intrafollicular injection and granulosa cell culture. Data were tested for normal distribution using Shapiro-Wilk test and analyzed by ANOVA. NP precursor type-A (NPPA) mRNA expression was not regulated after GnRH treatment *in vivo* but its receptor (NPR1) expression increased ($P<0.05$) at 24 h compared to 0 h (time of GnRH treatment). The mRNA coding for NP precursor type-B (NPPB) was not detected in bovine granulosa cells. Interestingly, NPPC was increased at 3 and 6 h after GnRH treatment ($P<0.05$), returning to levels similar to hour 0 at 12 and 24 h whereas its receptor (NPR2) was not regulated. *In vitro*, granulosa cell treatment with NPPA and NPPC alone or combined with LH did not modulate amphiregulin and epiregulin expression. The addition of LH to granulosa cell culture induced NPPC mRNA expression ($P<0.05$), as observed *in vivo* after GnRH treatment, being the LH effect completely abolished after addition of EGFR blocker (AG1478) both in the intrafollicular environment and also in the granulosa cell culture. In summary, NPPC mRNA is upregulated by LH *in vivo* and *in vitro* and the LH-effect on NPPC expression is mediated by activation of EGF-R. Our results suggest that NPs are involved in the ovulatory process in bovine, as well as the regulation and function of NPs during ovulation may differ between monovular and polyovular species.

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AMH and AMHR2 profile in granulosa cells during follicle deviation and in FSH-induced codominant follicles

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Anti mullerian hormone (AMH) is known to be highly expressed in granulosa cells of small antral follicles. However, the expression pattern of AMH and its receptor (AMHR2) during follicular deviation and their regulation by FSH have not been established. In *Experiment 1*, cyclic beef cows were synchronized and ovariectomized on days 2 (before), 3 (at the expected moment) or 4 (after deviation) of the follicular wave to recover the two largest follicles. Follicles obtained before deviation (day 2) were classified as largest (F1) or second largest follicle (F2) and were 7.4 ± 0.16 mm and 6.6 ± 0.44 mm diameter, respectively (P > 0.05). Follicles obtained at the expected time (day 3) or after deviation (day 4) were classified as dominant (DF) or subordinate follicle (SF). Diameters of DF and SF were significantly different (P < 0.05) on day 3 (7.9 ± 0.44 and 6.9 ± 0.16 mm) and day 4 (9.55 ± 0.32 and 6.75 ± 0.21 mm). In *Experiment 2*, animals were treated (i.m.) with FSH twice daily for 2 days in decreasing doses of 100 mg (30, 30, 20 and 20 mg) or saline starting on day 2 of the first follicular wave of the cycle. The two largest follicles were collected 12h after the last dose of FSH or saline (day 4). The follicles from the control group were classified as largest (DF) and subordinate follicle (SF) and follicles collected from the FSH group were classified as largest codominant follicle (co-DF1) and second largest codominant follicle (co-DF2). As expected, the diameters of DF and SF (control group) differed (9.5±1.3 and 6.1±0.6 mm diameter, respectively; P<0.05). However, diameters of co-DF1 and co-DF2 were similar in the FSH group (8.6±0.9 and 7.5±0.7 mm). Follicular status (healthy *vs*. atretic) was confirmed by *CYP19A1, LHCGR, CCND2 and CASP3* mRNA expression and cleaved CASP3 protein abundance in granulosa cells. In *Experiment 1*, *AMH* mRNA expression was similar in F1 and F2 before deviation (Day 2). On the other hand, the *AMH* mRNA levels were higher in DF than SF at the expected time (Day 3) and after (Day 4) deviation (P<0.05). There was no statistical difference of *AMHR2* mRNA expression during the deviation process (P>0.05). However, after deviation (day 4) AMHR2 mRNA tended to be more expressed in DF than SF. Surprisingly, the AMH protein concentration in SF was higher than in DF (2348.67±255.2 and 659.1±261.6 ng/ml, respectively, P<0.001). In *Experiment 2*, *AMH* mRNA expression in granulosa cells were similar among the follicles within groups. However, FSH supplemented follicles had greater *AMH* abundance than control follicles. These data were complemented by AMH protein, which was higher in FSH supplemented follicles (co-DFs) and DF than SF (P<0.05). On the other hand, the *AMHR2* mRNA expression was higher in DF than in SF (P<0.05) and similar between co-DFs (P>0.05). AMH concentration at the follicular fluid was more abundant in SF follicle than in DF but was similar in co-DFs. Our results suggest that AMH expression is regulated during follicular deviation and FSH can induce AMH at granulosa level *in vivo*.

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Effect of camel seminal plasma and mechanical stimulus on ovulating response of rabbit does

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Induced ovulation is regulated by complex neuroendocrine factors. In this regard, two main mechanisms are generally recognized: animals where ovulation is mainly induced by neuroendocrine stimulus (rabbit, cat) and species where the seminal plasma is responsible for triggering the GnRH release (llama, alpaca, camel). The presence of an ovulation-inducing factor (namely β-NGF) in the seminal plasma of several species has been widely documented. Intramuscular (i.m.) injection of rabbit seminal plasma in female llamas induces ovulation. At the same time, the occurrence of occasional ovulation (about 10%) has been found in artificially-inseminated rabbit does without GnRH administration indicating that the ovulatory responses could be affected by mechanical stimulation and by seminal plasma components. Thus, the present study was conducted to evaluate the effect of the camel seminal plasma to induce ovulation and changes in LH and progesterone concentration in rabbits. The β-NGF concentration of the camel SP was 127 pg/mL (ELISA method). Four multiparous New Zealand does (body weight = 3.6 kg) were synchronized and submitted to intramuscular injection of 1 mL of camel seminal plasma and vaginal introduction of an empty catheter. For LH determination (EIA method), blood samples were collected every 30 minutes until 3 hours after treatment. Progesterone was determined (RIA method) 30 minutes before treatment and every 4 days until day 12. All does were receptive to artificial insemination (turgid and reddish vulvar lips) and peripheral concentrations of both LH and progesterone indicated that none of the does ovulated. This fact does not confirm the hypothesis of a synergic effect between seminal plasma and vaginal stimulation in inducing sporadic rabbit ovulation. However, the low number of does treated and the possible low content of β-NGF could be responsible for the lack of effect.

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Effect of hCG administration on accessory corpus luteum formation and area in estrous induced nulliparous Santa Inês ewes

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The effects of hCG on accessory corpus luteum (CL) formation have been reported in cattle (Fonseca et al., 2001, Arq Bras Med Vet Zoo, 53:451-458) and goat (Fonseca et al., 2006, Anim Reprod, 3:410-414). In these studies, the main aim was to evaluate the effect of greater plasma progesterone concentrations (P4) on pregnancy rate. The luteotropic effect of hCG administration on cell populations in the ovine corpus luteum was also reported (Farin et al., 1988, Biol Reprod, 38:413-421). Nowadays, with the use of real time ultrasonography, it is possible to monitor CL development and the associated physiologic events like luteogenesis (increasing P4 production) and luteolisys (decreasing P4 production). The aim of this study was to evaluate the effect of hCG administration seven days after breeding on accessory CL formation and CL area in Santa Inês sheep. Estrus was synchronized in nulliparous ewes (n=14) using an intravaginal sponge with 60 mg of medroxyprogesterone acetate (Progespon®, Syntex S.A., Indústria Bioquímica e Farmacêutica, Buenos Aires, Argentina) for six days. One day before sponge withdrawal, all ewes received 300 IU eCG i.m. (Novormon® 5.000, Syntex S.A., Indústria Bioquímica e Farmacêutica, Buenos Aires, Argentina) and intra-vulvo-submucosal injection of 22.5 µg d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina). After removal, the females were monitored twice daily for detecting the onset of estrus and mated with fertile males. Seven days after breeding, the ewes were assigned into two groups according to body condition score (BCS; scale: 0 to 5) and treatment: hCG group received 250 IU of hCG (n=7; BCS of 3.14±0.20; Vetecor®, Hertape-Calier do Brasil Ltda, São Paulo, Brazil) and the control group received same volume of saline solution (n=7; BCS of 3.25±0.35). CL area was measured by transrectal ultrasonography exams (M5 Vet® equipped with a 6.5 MHz transducer, Mindray, São Paulo – SP, Brazil) performed once a day on days 7, 10, 13, 16, 19 and 22 after breeding. CL area was considered the sum of the area of all CL present in each animal. When present, luteal cavity areas were subtracted. Data were evaluated by one way analysis of variance with Tukey test and 5% minimum significance. The number of CL on day 7 was similar between hCG treated and control groups (1.28±0.46 and 1.28±0.48, respectively). The number of CL was greater ($P < 0.05$) in the hCG group than in the control group on days 13 and 16 (2.28 ± 0.48 vs. 1.28 ± 0.48, respectively). When pregnant ewes from the hCG group (n= 4) were compared with the control group (n= 7), a greater ($P < 0.05$) CL area (cm2) was detected in hCG-treated ewes on days 16 (1.99 ± 0.17 vs. 1.16 ± 0.18), 19 (1.65 ± 0.25 vs. 1.14 ± 0.18), and 22 (1.82 ± 0.19 vs. 1.26 ± 0.39) in hCG and control ewes. The treatment with hCG 7 days after natural breeding was efficient to induce the CL accessory formation, increasing the total luteal area in ovaries of pregnant ewes. The study of the associated repercussion of this phenomenon on P4 and pregnancy rate is encouraged in Santa Inês ewes.

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Morphology and cytoplasmic granules of the corpus luteum in pregnant and non-pregnant Nelore cows

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There are histological differences between cyclic and gestational corpus luteum (CL) in cows. However, these differences are not completely known in Nelore cows. The purpose of this study was to evaluate the volumetric proportion of the CL, the mean nuclear diameter of lutein cells and the distribution of lutein cells with cytoplasmic granules in the CL throughout pregnancy, and in cyclic Nelore cows. Twenty four ovaries with CL were collected in Santa Vitória slaughterhouse (Contagem, MG). The ovaries were equally split into four groups: animals until 90 days of pregnancy (1st trimester), animals with gestation between 91 and 180 days (2nd trimester), animals with gestation between 181 and 270 days (3rd trimester) and cyclic animals. The ovaries of non-pregnant animals were selected based on the presence of an active CL, with intense peripheral vascularization and dark red coloration, and the phase of diestrus was not determined. The period of gestation was determined based on the measurement of the foetus length. The CL were fixed in 10% neutral buffered formalin and processed for inclusion in paraplast. The tissues were cut in 3μm sections and stained in Hematoxilin-eosin, Gomori's Trichrome, Toluidine blue, Bromophenol blue and Xylidine Ponceau for analysis in light microscope. The CL volumetric proportion was evaluated by the esthereometric method and the constituents analyzed were: lutein cell cytoplasm and nucleus, connective tissue and fibroblasts, endothelial cells and pericyte, and blood vessels. The frequency of CL in the right ovary was 79.16% (19/24). The volumetric proportion of lutein cell cytoplasm decreased ($P<0.05$) from 46.13±2.84% to 37.88±3.50% and the volumetric proportion of the nucleus decreased ($P<0.05$) from 9.16±1.03% to 6.21±1.02% between the first and the second third of pregnancy. The amount of connective tissue and fibroblasts significantly increased ($P<0.05$) throughout gestation, with 20.93±5.39% on the first third and 34.04±4.17% on the second. This observation is compatible with the natural process of luteal regression during gestation. An increase ($P<0.05$) in the nuclear diameter of large lutein cells was observed between the first (23.66±1.73%) and the last third (26.27±1.33%) of pregnancy. Small lutein cells were not different among the groups. Cytoplasmic granules were found only in the large luteal cells. The amount of lutein cells with granules increased ($P<0.05$) throughout gestation, but significant difference was observed only between the first (2.33±1.94%) and the second third (25.50±20.34%). In the group of non pregnant animals just 2 lutein cells with granules were observed. Considering the distribution of granules in the CL throughout pregnancy found in this experiment, it is possible to suggest that these granules have importance in the maintenance of pregnancy, luteolysis or parturition (CEUA/UFMG/Protocol#247/2013).

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Intrauterine development of mice exposed to fluoxetine during pregnancy

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Depression is a psychiatric disorder that results from the deficiency of neurotransmitters in the central nervous system, characterized by mood swings and loss of enjoyment of general activities. It mainly affects women and can appear during pregnancy. Within the therapeutic options for the treatment of this disorder are the selective serotonin reuptake inhibitors, which has fluoxetine as its most famous representative. The present study evaluated maternal toxicity and intrauterine development of mice exposed to fluoxetine during pregnancy. For that purpose, Swiss mice were mated and, after confirmation of pregnancy, females were divided into 3 groups: a control group (G0) that received saline (used for dilution of the drug), and the others, that received different doses of fluoxetine (Daforin®) – 10 mg/kg (G1) and 20 mg/kg (G2). Drug administration was performed by gavage from the 8th to 17th day of gestation, a period that comprises organogenesis. The animals were weighed throughout pregnancy to monitor weight gain and on the 18th day of pregnancy the females were euthanized. They were first anesthetized with ethyl ether and then killed by cervical dislocation. Then the laparotomy and hysterectomy procedures were performed for analysis of embryo development. Data were analyzed by Student's t-test, ANOVA followed by Tukey and Fisher's exact test. Statistical analysis showed that there was a statistically significant reduction in maternal weight gain (G0 - 3.505±1.117; G1 - 2.597±0.8704 and G2 - 1.821±0.8445). There was a statistically significant difference in rates of post-implantation between control and the two treated groups (G0 - 8.882±1.615; G1 - 17.13±5.747 and G2 - 31.63±6.670), rates of reabsorption (G0 - 7.764±1.807; G1 -13.14±4.795 and G2 - 31.21 ± 6.458) and rates of fetal viability (G0 - 91.12±1.615; G1 - 82.87 ± 5.747 and G2 - 68.37±6.670). The weight of the placenta, fetal length and placental index showed no significant statistical difference between the groups. However, when the group G2 fetuses were related to the age of pregnancy, it was found that 29.3% of these fetuses had birth weights below the ideal. It is inferred that fluoxetine 20 mg/kg decreases maternal weight gain and can increase the rate of abortions during pregnancy. Thus, fluoxetine should be avoided during pregnancy, unless the benefits outweigh the risks, as in the cases of depression during pregnancy in which pharmacotherapy cannot be interrupted.

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Evaluation of the teratogenic effects of exposure to captopirl in offspring of Swiss mice

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Captopril is a drug which pertains to the class of Angiotensin Converting Enzymes (ACE), highly used as an anti-hypertensive, to treat cardiac insufficiency, post-infarction and diabetic nephropathy therapy, due to its low cost and mainly because of its satisfactory action as an anti-hypertensive. The use of captopril during gestation potentially impacts the embryo/fetal development due its capacity of permeating the placenta. The present study aimed to evaluate the teratogenic effects on mice offspring exposed to captopril during gestation. Swiss mice were mated and pregnant females were separated into four experimental groups: control group, which received distilled water, and three other groups receiving 12.5mg/kg (G1), 25mg/kg (G2) and 50mg/kg (G3) of captopril (Londrifórmula). The drug was administered daily, by gavage, three hours after fasting to ensure maximum absorption. Treatment began post-implantation, on the fifth day of gestation, and ended on the seventeenth day. The females were euthanized on the eighteenth day followed by laparotomy and hysterectomy to facilitate removal of fetuses and congenital malformation analysis. Data were analyzed by Fisher's exact test with $p<0.05$ significance. Regarding external malformations, cleft palate was observed but was not greater (G1 0%, G2 1.66% and G3 0%) than the control group (0%). Results of skeletal analysis showed that the palate, axial skeleton and superior members of treated groups was not greater than the control group. However, cranial malformations were greater (G1 p=0.3370, G2 p=0.0222 and G3 p=0.0071) in Captopril treated groups (G1 61.3%, G2 62.5% and G3 66.7%) than the control group (8.5%), being that the most frequent malformation was incomplete ossification of the occipital bone. The clavicle alterations were greater (G1 p=0.0018, G2 p=0.0095 and G3 p=0.0320) in treated groups (G1 12.0%, G2 7.5% and G3 12.0%) than in the control group (0%). Analysis of inferior members showed incomplete ossification or absent metatarsal and metacarpal phalanges which was greater (G1 p=0.0002, G2 p=0.0009 and G3 p=0.0001) in groups exposed to captopril (G1 16.3%, G2 13.8% and G3 24.0%) than the control group (0%). Regarding the visceral analysis, the following parameters were analyzed: cerebral hemisphere, ventricles, palate, nasal cavities, respiratory organs, digestive and urogenital systems, but none of the structures presented alterations greater than the control group. Thus, captopril promotes congenital malformations mainly in the skeletal system of the Swiss mice offspring.

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Paregoric Elixir exposure during intrauterine development of mice

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Narcotics in general when used during pregnancy can cause miscarriage, encourage premature birth and cause congenital malformations. The Paregoric Elixir (Catarinense ®) is a drug in the class of narcotics and used as antidiarrheal and analgesic. It consists of *Papaver somniferum* L., equivalent to 0.05% of morphine, the main active ingredient of opium, which causes adverse effects such as sedation, muscle stiffness, euphoria, vasodilation and in extreme cases generates overdose. The phytotherapeutic medicine has been used as a narcotic by addicts, mainly by young people at reproductive age. Although this drug should be sold with a prescription, some pharmacies do not require a prescription. Considering the harmful effects of this drug, this study aimed to investigate the possible maternal toxicity, as well the possible changes in the intrauterine development of mice subjected to Paregoric Elixir during pregnancy. For this purpose Swiss mice were mated and pregnant females were divided into four groups: G0 (control) which received saline and G1, G2 and G3 groups that received, respectively; 5, 20 and 35 mg / kg of the drug. The intraperitoneal administration was held from the 5th to the 17th day of gestation, corresponding to the period comprising post-implantation and late gestation. Females were weighed during pregnancy to monitor weight gain and on the 18th day they were euthanized, the organs (heart, lung, kidney, liver and gravid uterus) were removed and weighed and the uterine contents examined. Data were subjected to ANOVA followed by Tukey and Bonferroni. Regarding maternal toxicity, statistical analysis showed no significant changes for maternal weight gain in the groups G0 (4.71 ± 0.763), G1 (4.94 ± 1.223), G2 (4.93 ± 0.783), G3 (3.79 ± 0.701); as well as the other parameters: body weight and clinical signs of toxicity. As intrauterine development showed a significant reduction in the rate of fetal weight in G3 (0.99 ± 0.081) compared to groups: G0 (1.33 ± 0.042), G1 (1.29 ± 0.032) and G2 (1.27 ± 0.045), the parameter rates of post-implantation loss, resorption rates and fetal viability were not statistically significant. Just as the weight of the placentas, fetal length and placental index showed no significant statistical difference between the groups. Based on these results, it is inferred that the Paregoric Elixir at a dose of 35 mg / kg caused growth retardation in fetuses. Due to morphine, which is a highly lipophilic low molecular weight, it passes the placental barrier and the immature blood brain barrier of fetus causing respiratory depression, making fetal development difficult.

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Physiological characterization of the postpartum period, estral cycle and early pregnancy based on progesterone levels and sexual behavior in four different ovine breeds under high altitude conditions in Colombia

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The postpartum period, estral cycle and early pregnancy in ewes involve endocrine changes mainly evidenced on luteal function and progesterone secretion. The aim of this study was to characterize physiological phenomenons and determine the effect by type of lambing based on the evaluation of progesterone profiles and sexual behavior during ovaric reactivation postpartum, estral cycle and the beginning of pregnancy in four different types of breeds under high altitude conditions in a tropical country. 32 adult ewes of four breeds: Colombian Creole, Romney Marsh, Hampshire y Corriedale, were followed up since 5-7 days after lambing and until confirming pregnancy by ultrasound. During this time, blood collection was performed three times a week in order to evaluate progesterone levels; also a teaser ram was used to detect heats. A breeding period was scheduled for 34 days since the estral regularity was achieved by most of the ewes of the study. Data of body weight and body condition were collected once a month during the entire experiment. A commercially available ELISA kit immunoassay (EIA) DSI® RH-351 was selected to assess plasma progesterone levels in duplicate samples; a linearized calibration curve was performed in order to adapt the test providing a range containing from 0.148 to 9.5 nmol/l of progesterone with 95% certainty and inter-assay and intra-assay coefficients of variation of 6.2 and 3.5 respectively. This experiment utilized an analysis of variance with a factorial structure using the statistical program SAS (PROC GLM). There were significant differences for the time of ovarian reactivation with the type of lambing effect (P= 0.032); Comparison of means for breeds showed a wide difference between Creole and Corriedale (P= 0.0252) and other genotypes. The first ovulation postpartum appeared around 28 days in Creole ewes, 43.4 in Romney Marsh, 54.9 in Hampshire and 56.1 in Corriedale. The percentage of heat detection had a higher value in the Hampshire 83.49% and Creole 74.07% and a lower value for Romney 55.12% and Corriedale 59.31%. The Creole showed less time for ovaric recuperation, larger numbers of normal estral cycles (P= 0.0082) and better regularity into cyclicity. The time for reactivation could be related with reproductive performance, because conception rate was higher for Creole (100%), meanwhile the Corriedale group showed the lowest (87%). In conclusion, progesterone profiles can be a useful tool to evaluate luteal activity, especially to assess the duration of acyciclicity before ovarian reactivation. This way was possible to identify physiological patterns based on progesterone profiles and sexual behavior (teaser ram) in the genotypes involved in the experiment. This is the most complete study of ovarian reactivation in ewes under high altitude conditions in Colombia.

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Lipid profile of follicular fluid and follicular cells by mass spectrometry (MALDI) associated to oocyte competence

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The follicular microenvironment comprises the follicular fluid, oocyte, and its surrounding cells - these cells are organized as a functional unit essential to follicular growth and production of oocytes with development potential. Lipids are important components in the follicular environment, and can influence embryo viability, cryopreservation, implantation and act as signaling molecules. In the present study the correlation between development potential of oocytes and the lipid profile of follicular fluid (FF) and follicular cells (FC) was investigated. Lipid profiles were acquired by mass spectrometry (matrix-assisted laser desorption/ionization - MALDI-MS) with 2.5-dihydroxybenzoic acid matrix (DHB) as the organic matrix. Retrospective analysis of oocyte development was performed by monitoring individual embryo development after parthenogenetic activation. We performed MALDI-MS analysis in 30 FF samples and 21 FC samples originated from routines with a blastocyst/cleaved rate of at least 35%. Experimental groups were determined according to the stage of embryo development in non-cleaved (NCLIV), cleaved (CLIV) and blastocysts (BLAST) and data analyses were performed by multivariate statistics, specifically Principal component analysis (PCA) and analysis of variance (ANOVA), considering the *m/z* values of lipids previously identified using the same methodology in biological samples, mainly oocytes and embryos. Two lipids that were relevant for the experimental groups' separation were the potassium adduct of the triacylglycerol (TAG) containing 54 carbon and 4 unsaturation in the fatty acyl residues [TAG (54:4) + K], which was previously identified in embryos by Ferreira et al. (2010) and by Hayasaka et al. (2009). The second relevant and previously identified lipid was the TAG (54:3) of *m/z* 923.99, reported by Ferreira et al. (2010). These TAG have stearic, oleic and linoleic acid fatty acyl residues and both were more abundant in the non-blastocyst group (NCLIV+CLIV) compared to the BLAST group. Comparative analyzes considering only CLIV and NCLIV groups indicated protonated phosphatidylcholine containing 40 carbon and 7 unsaturation [PC(40:7)] of *m/z* 836.20 as a relevant lipid for group differentiation. This lipid has been reported by Fuchs et al. (2008). Regarding the lipid profile of FC, the TAG of *m/z* 909.5542, 925.5828 and 923.6035 were more abundant in CLIV and BLAST groups. These lipids were identified according to Saraiva et al. (2009), Schiller et al. (2003) and Ferreira et al. (2010). There were other relevant lipids for group differentiation which could not been identified yet. The data suggest higher abundance of TAG mainly containing fatty acids such as oleic, linoleic and stearic in FF and FC to be present in non-blastocyst groups. These differences in the lipid profiles obtained from single oocytes and embryos by MALDI-MS may be related to oocyte developmental capacity. This approach can lead to the identification of lipids to be used as non-invasive biomarkers of oocyte quality or development capacity if embryo biopsy is used as well as to monitor the effect of culture systems and their possible effect on gamete and embryo cyropreservation.

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Bovine embryos produced *in vitro* with sexed sperm by flow cytometry: evaluation of embryonic development and expression of gene candidates for pregnancy recognition

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Doses of semen from 4 bulls, enriched with X-bearing chromosome spermatozoa are used in artificial insemination (AI) and *in vitro* production embryos (PIVE). However, field studies using this type of semen demonstrated a reduction in pregnancy rates, which can be justified by injuries during sperm sexing and alterations in DNA, interfering with the expression of paternal genes affecting embryonic development and resulting in pregnancy loss after 90 days. The aims of this study were to compare the cleavage and blastocyst rates produced *in vitro* using conventional semen and sexed semen by flow cytometry, and check differences in the expression of genes related to pregnancy recognition (*AKR1B1*, *PTGS1*, *PTGS2*, *mPGES-1*, *mPGES-2*, *TNF-α*). A total of in 120 embryos were produced *in vitro* using the two types of semen. Bovine embryos were produced by *in vitro* fertilization, 48h after fertilization the cleavage rate was evaluated, and on the seventh day after fertilization the blastocyst rate was measured. Subsequently, a pool of 30 blastocysts per bull was subjected to RNA extraction for evaluation of gene expression by qPCR. It was possible to extract an average of 38.75 ng/μL. No significant differences (P>0.05) for cleavage (P=0.4778) and blastocyst (P=0.7011) rates between the experimental groups were observed. Regarding the expression pattern, flow cytometry did not alter the transcript levels of AKR1B1 (P=0.1124), PTGS1 (P=0.8892), PTGS2 (P=9484), mPGES-2 (P=0.7978) and TNF-α (P=0.709) genes, suggesting that embryos produced with sperm sexed by flow cytometry do not cause harmful changes in genes involved in recognition of pregnancy. However, the mPGES-1 (P=0.0248) gene was 4.54 times less expressed in embryos produced with sexed sperm by flow cytometry, suggesting the possibility of pregnancy loss, since studies indicate that high levels of mPGES expression are required for maintenance and pregnancy recognition. There are no reports in literature assessing the expression of mPGES-1 and mPGES-2 genes in bovine embryos produced *in vitro* with sexed sperm by flow cytometry. However, SAINT-DIZIER et al. (2011) showed that grade 1 embryos produced *in vitro* with conventional semen have higher levels of PTGS2 and m-PGEs, suggesting that these genes may be candidate genes to indicate the quality of embryos produced *in vitro*.

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A new model for evaluation of oxidative stress in *Bos indicus in vitro* produced embryo

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Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) and antioxidants. It is one of the factors associated to the low blastocyst rate of *in vitro* produced embryos when compared to the *in vivo* counterparts (1). As the in vitro system moderately induces oxidative stress itself, it is necessary to develop strategies in order to fully induce and evaluate this type of stress. In this study, we propose a new semi-quantitative method for analysis of ROS in in vitro bovine embryo models submitted to an induced oxidative stress by menadione. Oocytes were designated to *in vitro* maturation (IVM), under mineral oil, incubated at 38,5°, 5% CO_2 in air, for 22-24 hours. Following IVM, oocytes were transferred to *in vitro* fertilization (FIV) droplets and inseminated with $1x10^6$ spermatozoa/ml, after Percoll gradient selection. After 18h of IVF, presumptive zygotes were transferred to groups of 15 embryos/droplet of KSOM-aa, under mineral oil. Embryos were cultured *in vitro* (IVC) in a low oxygen tension atmosphere (low O2 ATM; 38.5°C, 5% O_2, 5%CO_2, 90%N_2) to minimize ROS formation due to high oxygen tensions (2). On day 3, embryos were supplemented with 5% of FCS. Forty-eight hours later, only embryos showing more than 16 cells were treated with different doses of the oxidative stress inductor menadione (0, 1, 2.5 and 5µM), during 24h in low O_2 ATM, without mineral oil (3). After incubation, embryos were returned to IVC droplets under mineral oil until Day 8 of culture, when blastocyst rates and embryo grades were assessed. In order to measure levels of ROS, we used semi-quantitative analysis of expanded blastocysts stained with CellROX® Green (CRG) fluorescence probe (Life Technologies) and imaged at an epifluorescence microscope. Embryos were incubated with 5µM of CRG and Hoechst 33342, for 30 minutes at 38.5°C, 5% CO_2. After incubation, embryos were washed in PBS with 1g/L PVP, fixed with formaldehyde 3.5%, washed again in PBS/PVP, placed on a slide and evaluated. Analyses were performed with Image J. The number of nuclei was recorded and the measurement of pixels/blastomere was defined by the equation: (total pixel per area of nuclei/background)/number of blastomere. Ninety four embryos were analyzed from 5 replicates. Results were analyzed with SAS 9.3 by polinomial regression, post-hoc Tukey test and Spearman test ($p<0.05$). Significant effect of menadione dose was observed for blastocyst rates ($R^2=0.55$; $p=0.0039$; $y= 1801.14 – 167.93x$), and for the number of blastomere per embryo ($R^2= 0.31$; $p < 0.0001$; $y=127.23 + 17.98x + 2.13x^2$). A dose dependent effect of menadione dose was also observed for pixel/blastomere ratio ($p<0.001$; $R^2=0.81$; $y= 1.24+0.13x$). There was a strong inverse correlation between pixel/blastomere and blastocyst rate ($p=0.014$; Roc = -0.54), and also between pixel/blastomere and number of blastomeres ($p=0.008$; Roc = -0.57). Furthermore, a positive correlation was found between blastocyst rate and number of blastomeres ($p=0.02$; Roc = 0.53). Results showed that menadione possibly disrupted the oxidative homeostasis as it decreased blastocyst rates and the number of cells per embryo, thus it may be an efficient inductor of oxidative stress. CRG analysis was shown efficient to evaluate ROS levels of *in vitro* produced bovine embryos. The proposed technique was also able to correlate ROS levels with embryo development. . Results found in the present study reinforce the deleterious influence of high levels of ROS on blastocyst rates and embryo development ([1]Takahashi M. Oxidative Stress and Redox Regulation on In Vitro Development of. J Reprod Dev 2012;58:1–9.;[2]Arias ME et al. Evaluation of different culture systems with low oxygen tension on the development, quality and oxidative stress-related genes of bovine embryos produced in vitro. Zygote 2011:1–9.[3]Moss JI et al. Insulin-like growth factor-1 protects preimplantation embryos from anti-developmental actions of menadione. Arch Toxicol 2009;83:1001–7).

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Pregnancy in Hystricomorpha: preliminary study of doppler mesearusement during the last third of gestation in agouti (Dasyprocta prymnolopha, Wagler 1831)

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Research that addresses more precisely the gestational development in wild species are essential for the understanding of their reproductive physiology. Raising animals in captivity in an attempt to preserve the species becomes a challenge when there is the need to maintain the natural characteristics of these animals in the wild. These factors often hinder reproduction in captivity, due to problems of stress management, which can lead these animals to abortion. Signaling these factors can aid in prior clinical management, which can mean the difference in the development of a successful pregnancy. The agouti (Dasyprocta prymnolopha, Wagler, 1831) is an animal that covers a wide area of the country, from Amazonia to the semiarid, and currently is considered among animals that are listed as endangered. This study aimed to evaluate the placenta and fetal vessels (aortic and portal vein) of 8 pregnant agouti in the last third of gestation, from the Center for Research and Preservation of Wild Animals of the Federal University of Piauí-UFPI. The animals were subjected to physical restraint in dorsal recumbency. The abdominal scanning was performed in sagittal and transverse planes, in order to evaluate the fetus and the placenta as well as the vascular flow. The animals were calm during the procedure. 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 average diameter of placentas studied was 26.3±1.35mm. Heart rate showed a mean of 222±8.2. The flow rate and the average resistivity index in the umbilical artery were respectively 21.11±3.97cm/s and 0.86±0.2. In the fetus, the mean flow velocity in the aorta was 29.24±3.25cm/s. Portal vein flow velocity and the resistivity index were respectively 9.65±1.8 cm/s and 0.9±0.32. To our knowledge this is the first study about the uteroplacental blood flow in Hystricomorpha rodents, as the agouti. Similar to that observed in rabbits there is a low resistance flow, providing a large increase in placental perfusion. This observation can also be seen in bitches, mares and even in humans. The blood flow has shown to be discontinuous and with monophasic waveforms. The venous flow, however, showed well defined systolic and diastolic peaks, with typical deceleration time in all animals studied, similar to that observed in rabbits, cats, bitches, and despite the differences in placentation, in women. The aortic and portal vein flow of the fetus showed high systolic peak with an apparent increase in the resistivity index for gestational age, since this index was found to be higher than that observed in the descriptions made in rabbits for the same period and closer to what is observed in humans. The inherent increase in intraplacentary flow mechanisms showed no positive correlation to the diameter of the placenta growing in our preliminary results (r = 0.3). These data are not yet fully defined in the literature, especially by placental and subplacenta component in this species. This study allowed us to collect preliminary values of placental and fetal vascular flow in agoutis. We believe that these initial findings can help to gestational management of these animals as a way to provide the appropriate gestational development for the species.

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Dynamic reprograming of 5-methylcytosine during canine germ cell development

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Epigenetic reprogramming in mammalian germ cells, zygote and early embryos plays a crucial role regulating expression patterns during critical stages of development. Genome-wide demethylation in primordial germ cells (PGCs) is a unique reprogramming event essential for erasing epigenetic memory and preventing the transmission of epimutations to the next generation. In addition, PGCs undergo a wave of global methylation at the 5 carbon of cytosine of the CpG dinucleotide (5mC) reprogramming, one of the important mechanisms to regulate gene expression and genomic imprinting. However, there is little information about canine germ cell development, especially in regions of the coelomic epithelium and cells derived from subjacent mesenchyme, which are the main regions that find primordial germ cells during their migration to the formation of gonadal cords. Thus, in the present study, we investigated the dynamics of 5mC, during development of canine primordial germ cells. Six canine embryonic cells were collected between 24 and 30 days post-coitum (dpc) and later we performed immunohistochemistry for the 5mC marker. For the expression analysis, five random fields of each sample were photo documented. The scores were done using the ImageJ software. The difference between the positive marker cell averages, in the different embryo ages, were evaluated with students t-test with 1% of significance level. The 5mC was present in the nuclei of cells that compound embryonic urogenital system, such as in the hindgut epithelium, mesonephric glomeruli and tubules. The embryo with 24 dpc had significantly more cells stained than other ages. The ages 25 and 26 had the lowest number of stained cells and did not differ between them. Nonetheless, 27 dpc demonstrate more stained cells than 28, which in turn had more than 30 ($p<0,01$).These data reported reduction in global levels of DNA methylation (5mC) in migrating PGCs from 25 and 26 dpc. Thus, the study demonstrates the first steps the epigenomic reorganization. These events help understand the process of sequential reprogramming of PGC in the canine and can facilitate the derivation of embryonic germ cell in this specie.

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Uterine vascularization and fetal position along the uterine horn in gilts and its relation to fetal weight

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Important changes are observed in the vascular architecture of the uterus of pregnant sows compared to non-pregnant females. Birth weight is highly dependent on the amount of nutrients supplied through the placenta, and this nutritional supply is affected by the area of contact between the placenta and the endometrium and by the blood flow. Thus, the aim of this study was to evaluate the uterine vascularization and fetal position along the uterine horn and its relation with fetal weight, in different gestational stages of gilts. For evaluation of the uterine vascularization and fetal position fifteen gestating gilts were slaughtered at different gestational stages (50, 80 and 106 days), totaling five sows per group. One hundred seventy-five fetuses from these fifteen gestating gilts were evaluated according to the gestational age. The gilts were housed in individual gestation crates in the Swine Experimental Center of Federal University of Lavras, and were artificially inseminated at its fourth estrus at 150 days. These gilts were kept in that location until the day of slaughter. After the slaughter, the entire reproductive tract was removed and collected. Subsequently in the uterine opening, the fetuses were identified according to the uterine position: apex (region closest to the ovary), base (region closest to the uterine body) and the medium part (situated between these two). After identification, fetuses were weighed. For quantification of the number and diameter of the vessels in different regions of the uterine horns (apex, medium part and base) a latex solution (Neoprene®) was injected into the uterine arterial system. The data was statistically analyzed. According to the findings, there was a larger number of vessels ($P<0.05$) in the medium part of the uterine horns, regardless of the gestational age or uterus side. There were no differences between the base and the apex of the uterus in relation to vascular density. Differences between the pregnancy ages were not observed for the number of uterine arteries. Regarding the diameter, arteries with larger caliber were most abundant ($P<0.05$) in the apex of the uterine horns and arteries with smaller caliber in the base. However, the fetal weight was not influenced ($P>0.05$) by the position that the fetus was in the uterus. It was concluded that there are differences in the uterine vascularization between the different uterine segments, but this fact is not able to influence fetal weights. The medium part of the uterine horns is the segment which is destined with the highest number of vessels, regardless of gestational age, and the apex region has a greater tendency to take vessels of larger caliber.

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Use of a regulator of the PI3K/Akt pathway in the production of bovine embryos with different mediums

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The PI3K/Akt pathway is involved in protein synthesis, cell proliferation, cell differentiation, microtubule dynamics by phosphorylation of initiation factors, components of the cell cycle, transcription factors and proteins involved in microtubule formation and cell adhesion. Recently it has been reported that this pathway can regulate the meiosis of oocytes in metaphase transition, especially I / II, being part of the cascade MAPK3 / 1 and MAPK14 in oocytes and cumulus cells in cattle. The oocyte maturation is crucial for being the first step by which the oocyte passes to become able to be fertilized. In this step numerous nuclear and cytoplasmic events occur. Thus, the aim of this study was to evaluate the influence of the PI3K/AKT pathway in *in vitro* fertilization of bovine oocytes and early embryo development when a chemical regulator of this pathway is added to different maturation mediums. The oocytes were obtained from slaughterhouse ovaries and classified as grade I and II. After selection they were placed for maturation and kept in drops of 100 µL (99.5 µl of IVM medium + 0.5 µl of the pathway regulator -wortmannin) of the modified maturation medium (20-25 oocytes per drop) or 100 µL of control maturation medium under mineral oil for 22 hours at 38.5 ° C in 5% CO2. After IVM, they were subjected to *in vitro* fertilization. The matured COCs were washed in fertilization medium and transferred to drops of fertilization of 100 µL with diluted semen with the concentration adjusted to obtain a fertile dose of 2 x 10^6 spermatozoa / ml. IVF was then held for 18 hours in an incubator under the same maturation conditions. After incubation with spermatozoa, presumptive zygotes were washed in *in vitro* culture medium (IVC) and then cultured for 8 days in drops of 200 µL of this medium, in the same condition of IVM and IVF. After 72 hours, the cleavage rate was evaluated and 50% of the culture medium in each drop was renewed. Two other assessments, in the seventh and eighth days of cultivation, were made to determine the total number of blastocysts. An experimental design with six replications and six treatments was performed. Treatment 1 (LC1): Maturation medium 199-A without the inhibitor and without insulin-transferrin-selenium (ITS), fertilization medium Talp-Fec and culture medium 199; Treatment 2 (LW1): Maturation medium 199-A with inhibitor and without ITS, fertilization medium Talp-fec and culture medium 199; Treatment 3 (CC1): Maturation medium 199-B without inhibitor and with ITS, fertilization medium Fert-Talp and SOF culture medium; Treatment 4 (CW1): Maturation medium 199-B with inhibitor and with ITS, fertilization medium Fert-Talp and SOF culture medium; Treatment 5 (LC2): Maturation medium 199-A without inhibitor and without ITS, fertilization medium Fert-Talp and SOF culture medium; Treatment 6 (LW2): Maturation medium 199-A without inhibitor and without ITS, fertilization medium Fert-Talp and SOF culture medium. First we assessed whether the addition of 20 nM wortmannin on maturation medium interfered with embryo production. The LW2 group had a higher percentage of blastocysts (57.7%) on the seventh day compared to (19.7%) the control-LC1 ($P \leq 0.05$). In assessing the eighth day, the LC2 and LW2 groups showed higher blastocyst rates (56.5 and 63.3%, respectively) compared to (27.7%) the control-LC1 ($P \leq 0.05$). These results show that the combination of medium 199 with wortmannin and without ITS in IVM, Fert-Talp in IVF and IVC in SOF is effective in *in vitro* embryo development, improving the blastocyst rates.

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Re-expansion and hatching rates after vitrification of bovine *in vitro* produced embryos and cultured in medium containing conjugated linoleic acid

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It is considered that the existing inconsistencies in cryopreservation of bovine *in vitro* produced embryos (IVP) are related to the high lipid content in the cytoplasm of blastomeres, originated mainly by the presence of fetal calf serum (FCS) in the *in vitro* culture medium (1). The conjugated linoleic acid (CLA) is a fatty acid that has the property of decreasing lipogenesis in embryo cells, improving the quality of the embryo produced and possibly decreasing their sensitivity to cryopreservation (2). The aim of this study was to evaluate the effect of adding CLA in culture media containing or not the FCS on the viability of IVP embryos after vitrification. 218 complexes *cumulus oophorus* (COCs) were matured, fertilized *in vitro*, and randomly cultured *in vitro* according to three experimental groups: control (FCS without CLA), FCS + CLA and CLA (CLA without FCS). After seven days of culture, the embryos were cryopreserved with the vitrification method, according to the experimental group. To assess the viability of the produced embryo, 74 vitrified embryos were heated and then placed in culture in individual drops for 72 hours. The re-expansion and hatching rates were evaluated at 24, 48 and 72 hours after the outset of cultivation. Re-expansion and hatching rates after heating of bovine *in vitro* produced embryos, according to the experimental group were, respectively, 70.4% and 42.1% for control treatment, 43.3% and 23.1% for FCS + CLA treatment and 47.1% and 25.0% for CLA treatment. Regarding the rate of re-expansion, the CLA treatment was lower than the control group and higher than the FCS + CLA ($P < 0.05$). Regarding the hatching rate the three treatments had similar performance ($P > 0.05$). Unlike what was found in this study, it was expected that embryos cultured in medium containing CLA, in the presence or absence of FCS, presented higher re-expansion and hatching rates, because they have better embryonic cell quality (2.3). The FCS in the *in vitro* culture media provides growth hormones, nutrients and antioxidant component factors, increasing the production of embryos and accelerating embryonic development (4). After 24 hours of culture, the re-expansion and hatching rates, according to experimental groups were, respectively, 73.7% and 37.5% for control treatment, 100.0% and 100.0% for FCS + CLA treatment and 100.0% and 100.0% for CLA treatment. After 48 hours of culture, the remainder of the treatment control embryos re-expanded and hatched. As shown in the results, different than expected, the embryos in the control group progressed more slowly than the embryos from other treatments.

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A fertile female twin to a male calf - case report

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The term freemartin is utilized to designate a cow or sterile heifer, but the term also applies to a female conceived in multiple heterosexual births. As a consequence of the pregnancy, in more than 90% of cases, the pregnancy establishes a vascular anastomosis between the chorionic placentas of twin fetuses around 30 to 40 days of gestation, occurring before the sexual dimorphism. This originates a fetal circulation common in cells and plasmatic substances similar to hormones, leading to a female intersex condition. This case cannot be prevented. However, it can be diagnosed by various methods, ranging from simple examinations of the placental membranes to karyotype reviews. In some cases, the male twin may have been aborted at an early stage of pregnancy (before the 40th day of pregnancy) and in this case, there is no evidence for freemartinism. Many ranchers typically create female-born gestation of twins of different sexes, precisely because they know that most females born in these cases are sterile. Here we relate the case of the Gyr cows, approximately seven years of age and born from a twin pregnancy with a male, product of artificial insemination, in a property located in Coroaci, MG, Brazil. According to the owner, the female that was believed to be sterile was kept in the squad because it has good genetics and morphological characteristics that fit the breed's standard. This female did not present history of any behavioral and phenotypic abnormalities, which are common, and after performing visual examination and ultrasonography, normality can be observed throughout the reproductive tract. At 54 months after the animal has natural heat, the same was inseminated and diagnosed pregnant after thirty days. The male born with that animal also showed no abnormality. The twin pregnancies have a lower frequency than the Zebu race (B. *taurus indicus*) when compared to animals of European origin (*B. taurus taurus*), not corroborating the described case where the animal is a Zebu. It is cited in literature that it is expected that only 10% of the female bovine born from a twin pregnancy with a male are normal. This mature female, presenting characteristics of a normal female gave birth at approximately 63 months old and this suggests that vascular anastomosis did not occur in this animal. The male born with the female in the case in question also did not present any abnormalities, being used as the breeder. In intersex cases, the external genitals are feminine in appearance, and the degree of impairment of the internal genitals are varied, a hypertrophied clitoris may be observed, the presence of long hair on the vulva, a shorter vagina, an absence of the cervix, and this was not found in the described animal, noting that the internal and external genitalia are normal.

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Placental efficiency in Piau breed and Commercial Line pregnant gilts

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Among the factors that influence litter size, the placenta shows an important component since it mediates the nutrition of the embryo/fetus after implantation until parturition. Some authors point out that the ratio of fetal weight with the weight of the placenta, known as placental efficiency, is heritability higher than observed for uterine capacity and litter size, thus showing a good feature for genetic selection. This study aimed to evaluate the placental efficiency of two genetic groups of pigs, a Brazilian native breed and a Commercial line, and the relations with prenatal survival and mortality. Twenty-four gilts (12 Piau breed and 12 of a Commercial line) were used, divided into two groups according to the type of crossing, Group 1: females and males from the Commercial line (n = 12) and Group 2: females and males from Piau breed (n = 12). The groups were divided into four subgroups based on gestational age at the time of slaughter (30, 45, 60 and 90 days). The placental efficiency was determined through the ratio fetus weight and placental weight (fetal weight / weight of the placenta). In the present study, placental efficiency did not differ in age in both groups evaluated (30d: 0.1 ± 0.0 vs 0.1 ± 0.0; 45d: 0.3 ± 0.0 vs 0.4 ± 0.1; 60d : 0.8 ± 0.3 vs 0.7 ± 0.1; 90d: 3.2 ± 0.1 vs 3.0 ± 0.6 in Commercial and Piau, respectively; P> 0.05), because of the proportional increase in fetus weight in relation to placental weight in both groups. However, placental efficiency increased during pregnancy, including placenta growth as a mechanism to supply the nutritional requirements of the fetuses until parturition, highlighted by the behavior observed in linear regression analysis of placental efficiency in relation to gestational age. The results show that Piau breed females show similar placental efficiency behavior to Commercial line females.

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Effect of microbial laboratory contamination in *in vitro* bovine embryo production

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The efficient *in vitro* production (IVP) of cattle embryos requires excellent laboratory conditions, as well as the safety of culture media. Not all chemicals used for disinfection are applicable to cell culture laboratories, since most chemical agents produce odor and residue (1), damaging the embryo development. Detergent removes organic matter that is important an environment for microbial proliferation (1, 3). The 0.1% sodium hypochlorite diffuses through the bacterial cell wall and disables enzymes that are very important for bacterial digestion of glucose (1, 3). The 70% alcohol acts on the denaturation of bacterial proteins and on the dissolution of the lipids of plasma membrane of bacteria or viral envelopes (1, 3). Despite 70% alcohol being considered having low or middle decontamination capability, it is normally used for this purpose because it has low residual potential. After successive contamination of the culture media and reduced embryo production in a laboratory, this study aimed to evaluate the efficacy of disinfection protocol of laboratory environment using 0.1% sodium hypochlorite and 70% ethanol in reducing environmental bioburden and subsequent embryonic development. Confirmation of microbial contamination of the Lab was checked by plating the swabs from the water, culture media, equipment and environment. Swabs were plated on nutrient agar to identify bacteria and on potato dextrose agar, to identify colonies of fungi and yeasts. Gram staining was performed (2). Decontamination protocol to disinfect the laboratory environment used the following steps: firstly, remove dirt using mild soap and water; secondly, surfaces were disinfected with a solution of sodium hypochlorite 0.1%; and thirdly, three disinfection steps using 70% alcohol, respecting 10 minute intervals between disinfections (3). *In vitro* embryo production was interrupted in the Lab during the next 30 days after cleaning and disinfection protocol. IVP (total number of viable embryos/total number of oocytes in culture) were statistically compared (Exact Fisher Test, 5% significance, Graphpad Instat 3.1) before and after disinfection of the laboratory environment. The result of embryo production before laboratory decontamination was 3.15% (268/8512), considering the *in vitro* embryo production by the total number of oocytes. After the decontamination protocol, it was observed that IVP was higher than the previous embryo production, whereas the embryo production/total number of oocytes was 29.88% (349/1168) ($P < 0.05$). Procedures for removal of contaminating microorganisms, as well as their vegetative forms, associated with the depopulation of 30 days were essential for resumption of routine IVP. The three disinfection steps were able to efficiently promote antisepsis and spore removal as verified by other authors (3). These results demonstrate that maintaining the safety of cell culture laboratories is essential to obtain satisfactory embryo yields and that the protocol used was effective in decontaminating the laboratory environment and resumption of IVP.

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Relationship between reproductive disorders and *Neospora caninum* in dairy cattle

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Neosporosis is a parasitic disease known worldwide, caused by *Neospora caninum*, which has as definitive host the dog, and as intermediate hosts cattle, among domestic animals, and wild animals. In cattle, abortion is characterized as a major cause of reproductive failure and may occur at any time during pregnancy. The present study aimed to investigate the presence of anti-*N. caninum* antibodies and assess its potential correlation with reproductive disorders in the studied population. Blood samples from 56 reproducing cows in of different ages from the Livestock Sector UFRRJ of milk were collected from the tail vein tube vacuntainer. These samples were for indirect immunofluorescence (IFAT) to select animals seropositive and seronegative for antibodies anti-*N. caninum*. From this total, 28 cows (14 seronegative and 14 seropositive, with or without reproductive disorders) were selected randomly and tested by statistical analysis (chi-square test at 95% confidence). The IFAT was performed according to the technique described by Yamane et al. (1997), with a cutoff of 1:200 used (Dubey et al., 1996). The reading was performed under a microscope (Carl Zeiss RFA) with epifluorescence system and 400X magnification system. Reactions were considered positive when the total peripheral fluorescence was observed in more than 50% of tachyzoites. When compared seropositive and negative animals regarding the manifestation of reproductive disorders (returning to estrus with longer periods of service and range of deliveries, abortions, retained placenta and uterine infections nonspecific), no statistically significant difference was observed, concluding that although more than 50% of seropositive animals show some kind of reproductive disorder, infection with *N. caninum* was not the main cause of these disorders in the population studied.

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Morphological characteristics of placentomes from crossbreed Holstein-zebu cows with different birth orders

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The placenta is a transient organ and has a vital role in the metabolic exchanges between mother and fetus. Understanding aspects of its morphology is of practical relevance in the retained placenta approach, a very common condition that decreases the reproductive efficiency of the herd. Several risk factors are associated with increased incidence of retained placenta, including the birth order. This study was conducted to evaluate the morphological characteristics of placentomes from crossbreed Holstein-zebu cows with different birth orders. To this end, 15 pregnant crossbreed cows, without retained placenta, were used and classified into three groups according to birth order: Group1 (G1) including first calf cows, Group2 (G2) with second calf cows and Group3 (G3) including cows that calved three or more times. Quantified variables include: number of caruncular epithelial cells, trophoblastic binucleated cell population and volumetric proportion (VP) of maternal and fetal collagen. An optical microscope was used to count the caruncular epithelial cells and the binucleated cells, in 40 random fields, using a 100 division's micrometric ocular aligned in parallel to the caruncular epithelium. These data were analyzed using a 400x optical zoom microscope. For the collagen analysis, the slides were stained with Masson's Trichrome. Under 400 X magnification, an integrating eyepiece with 25 equidistant points was attached to the eyepiece of a light microscope and 40 random fields were analyzed for each slide. With this technique, the collagen is stained in blue, so each point that reached the blue stained area was computed to calculate the volumetric proportion of collagen in maternal or fetal tissues. The total number of points incident in a particular structure was used to calculate its VP. Data were compared using Tukey's test at 5% significance level. The results indicated no statistical difference between the groups ($P > 0.05$) for number of caruncular epithelial cells or for binucleated cells. The VP of the maternal collagen was higher than the fetal one ($P < 0.05$) in placentomes, regardless of parity. The results for maternal collagen were 15.52±187, 15.22±1.99 and 15.84±2.85 for groups 1, 2 and 3 respectively and the results for fetal collagen were 6.46±2.01, 7.36±1.28, and 7.72±1.94 for the same groups. In this study, no differences were observed in the VP of maternal and fetal collagen among the three groups, showing no effect of birth order on this feature. Under the conditions in which this experiment was conducted and evaluating histologic features of placentome in Holstein-zebu crossbred cows of first, second and third or more deliveries, it can be concluded that the caruncular epithelial cells and binucleated cells do not differ according to the birth order. Nevertheless, there is a higher proportion of maternal collagen than fetal, but the difference was not found when we compare this feature among the birth order.

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Teratogenic effects on the offspring of mice exposed to Fluoxetine during pregnancy

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Fluoxetine (FL) is the most known and most prescribed drug nowadays for depression, a psychiatric disturbance that results from neurotransmitter deficiencies to the central nervous system, being characterized by humor alterations and lack of pleasure in activities in general. The aim of this study was to analyze the possible congenital malformations on the offspring of mice exposed to FL during pregnancy. For this purpose, Swiss mice were mated and, after the pregnancy confirmation, the females were divided into 4 experimental groups: Control group (G0), that received saline solution and the other groups, which received different doses of FL(Daforin®) – 10mg/kg (G1), 20mg/kg (G2) and 30mg/kg (G3). The solutions were administered by gavage from the 8th to the 17th day of pregnancy, this period corresponds to the organogenesis phase. On the 18th day of pregnancy the females were euthanized, following the laparotomy and hysterectomy procedures to remove the fetus and analyze the congenital malformations. The fetuses were evaluated under stereoscope microscope for external, visceral and skeletal malformations. The statistic analysis used was the exact Fischer test. Regarding the external malformations, we observed the occurrence of fetus with cleft palate and retroversal inferior members. However, these anomalies were not statistically different between groups. Through the visceral malformation found, we observed alteration on the ventricles that were dilated, presented more frequently in G2 6.3%, which was statistically different from G0 and G1, both with 0%; the trachea showed alteration on fetus from group G3 7.7%, statistically different from G0 1.5% and the kidneys presented statistically significant pelvis dilatation in G2 11.1% when compared to group G3 1.3%. Between the skeletal malformations found, significant statistical alterations were found on the supraoccipital bone on the fetus of treated groups G1 30.7%, G2 37.7% and G3 47.6% when compared to the control group G0 8.7%. Regarding the sternum, the sternebra were irregular, divided, reduced or even absent. These alterations showed statistical significance between G1 38.6 %, G2 39.8 % and G3 72.1% when compared to G0 24.1%. The metacarpus, metatarsus and phalanx showed lack of ossification or absence. On the superior members, these anomalies were more frequent in groups G3 22.1% and G2 14.5% which is statistically different from G0 5.4% and G1 7.6%. The FL during pregnancy caused visceral malformations on the concentrations of G2 and G3 and skeletal alterations on the three tested doses. The use of FL during pregnancy should be avoided, however, the specialist should analyze de cost-benefit for each patient.

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Teratological analysis of the offspring of mice exposed intraperitoneally to Elixir Paregórico

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Teratology is the area of science that aims to study organic deformities, whether genetic or not. It is known that maternal habits influence embryo development, and various properties of drugs may also cause problems in the normal development of the fetus. The Elixir Paregoric (Catarinense ®) is a medication prescribed casually as antidiarrheal and analgesic, being easily found in any drug store. The chemical composition of Elixir Paregórico (EP) is herbal being synthesized from the plant Papaver somniferum L., which is equivalent to 0.05% morphine, the major active substance in opium, when used in excess can lead to overdose. Thus, simply by changing the route of inoculation, the most harmless of drugs can become a potential problem, especially for young users of reproductive age. This study aimed to analyze and rank the malformations caused by EP when inoculated intraperitoneally. Female Swiss mice were used and after identifying pregnancy by vaginal plug were divided into four groups: G0 (control) received saline and G1, G2 and G3 groups received, respectively; 5, 20 and 35mg/kg of the drug. The intraperitoneal inoculation took place from the 5th to 17th day of gestation, corresponding to the period comprising post-implantation and late gestation. On the 18th day the females were euthanized and the fetuses were preserved in preparation solutions to further pass through a stereoscopic microscope analysis process, undergoing procedures that sought to find external, visceral and skeletal malformations. As for external malformations noticeable to the naked eye, it was observed the occurrence of fetal syndactyly, macroglossy, meromiely, oligodactyly, hydrops and brachycephaly. However, these abnormalities were not statistically different between groups. Skeletal malformations were revealed more frequently in G3, predominantly in the skull, jaw, axial skeleton and the upper and lower limbs. In visceral analysis there was no meaningful data. It is noted that the small dose of morphine present in the EP was enough to disrupt the development of tested fetuses. And the inappropriate use of a drug, however innocuous it may seem, can be disastrous, especially for pregnant women and their offspring.

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Osteopontin (OPN) and leukemia inhibitory factor (LIF) and their receptor (LIFR) genes in canine embryos

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The aim of the present study was to analyze the gene expression of the osteopontin (OPN), leukemia inhibitory factor (LIF) and their receptor (LIF-R), which are presumably involved in the signaling of embryo implantation in canine pregnancy. For that, ten healthy bitches were inseminated and the ovariohysterectomy was performed 8 days after the LH surge (n=5) and 12 d after he LH (n=5), for collected morulaes and blastocysts respectively. The oviduct and uterus were flushed and five embryos/female were frozen and stored in RNA at -80C. The RNA extraction was performed using the Quiagen RNeasy micro-kit and the DNase I Amplification Grade. For the reverse transcription we used the kit SuperScript II and SuperScript III. RT-qPCR was performed with the power SYBR Green PCR Master Mix. The constitutive gene used was HPRT. The relative gene expression was determined by student t- test. The LIF-R was expressed only in canine blastocysts (4.20± 0.02). There was no detection of mRNA LIF and OPN in morulae and blastocysts. The absence of the OPN mRNA expression in canine morulae and blastocysts are similar to results found in pigs and rabbits and the absence of LIF mRNA expression in all embryos is a different result from those obtained by Schafer-Somi et al (2008). That difference in results may be associated with low quantity of RNA extracted from embryonic structures in our study. Hou and Grouski (1993) reported that specific genes are expressed in the transition of morula to blastocyst, and there are changes in the synthesis and secretion of specific proteins from the early to late blastocyst.

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Global DNA methylation in different *Gallus gallus* tissues

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DNA methylation is a stable epigenetic modification found in most of the eukaryotes that plays a crucial role in many biological processes, including gene expression regulation, genomic imprinting (with considerable evidences that it does not occur in birds) and transposon silencing in mammals and plants. The chicken (*Gallus gallus*) is an important animal model that bridges the mammals and vertebrates in evolution and has long been used as a model species for the study of embryology, immunology, behavior and reproduction. In this study we aimed to evaluate the global DNA methylation percentage of tissues derived from different embryonic origins in one adult female and one adult male *Gallus gallus*. DNA was extracted from one ectodermal origin tissue (brain) and two mesodermal origin tissues (heart and gonad). Global DNA methylation was determined by ELISA [Imprint Methylated DNA Quantification Kit (Sigma-Aldrich)] and to obtain the percentage methylation samples (analyzed in duplicate), the following calculation was executed per sample: [(Sample-Blank)/(Methylated Control DNA-Blank)]X100, where the blank was a no template control sample (0% methylated) and methylated control DNA was a positive control (100% methylated DNA supplied by the kit). The statistical analysis was performed by variance analysis and Tukey's Multiple Comparisons test and 95% confidence interval. The average percentage of DNA methylation observed in the female was 26.47% in the brain, 21.97% in the heart and 27.76% in the gonad. In the male we observed 17.71% in the brain, 11.50% in the heart and 14.61% in the gonad. In both sexes, the brain and gonad showed a higher percentage of methylation compared to the heart. The male showed a different global DNA methylation percentage when compared to the female, not statistically significant, but with an overall tendency of being hypomethylated. This trend may be due to environmental and intrinsic factors to genomic DNA. The genome-wide DNA methylation maps of many organisms has been reported, such as human, *Arabidopsis*, rice and silkworm, but there is little knowledge about the DNA methylation patterns in birds.

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Effect of copaiba on defects caused by exposure to cyclophosphamide in the development of mice

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The Copaiba oil (OC) is an oil resin exudate from the trunk of Copaifera sp used as a natural medicine that has anti-inflammatory properties. Cyclophosphamide (CF) is an agent used in cancer treatment that has been shown to be teratogenic. This study was conducted to evaluate the possible protective effects of copaiba oil regarding malformations caused by CF. Mice of the Swiss strain were mated and females divided into 8 groups. Animals were treated with one of three concentrations of copaiba oil 0.3 ml/kg (OC0.3); 0.6 ml/kg (OC0.6); and 0.9 ml / kg OC0.9) from the 8th to 12th day of pregnancy, and on the 10th day animals from these three groups received either CF or phosphate buffer solution (PBS). The negative control group received medium-chain triacylglycerol, copaiba oil solvent in the same gestational days, and PBS on the 10th day. The positive control group received TCM medium from the 8th to 12th day, and CF (30mg/kg body weight) on the 10th gestational day. The OC and TCM were administered by gavage, whereas CF and PBS were administered intraperitoneally. On the 18th day of pregnancy, the females were euthanized and fetuses fixed and analyzed for verification of external, visceral and skeletal structural malformations. The Student's t test statistical analysis was performed, ANOVA followed by Tukey test and Fisher exact test. All CF-treated groups presented fetal alterations such as open eyes, exophthalmos, oligodactyly, polydactyly, cleft palate, hydrocephalus and skeletal malformations associated with head, vertebrae, ribs, sternum, scapula and pelvic girdles. There was a significant decrease in the incidence of fetal hydrocephalus in animals receiving a treatment with OC prior to CF administration (OC0, 3: 18.42% OC0, 6: 19.23% OC0, 9: 20%) compared to the positive control group (71.43%). Copaiba oil promoted a protective effect regarding the incidence of CF-induced hydrocephalus when administered at all doses tested. Therefore, copaiba oil is recommended for prevention of birth defects.

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Development of the female genital organs in cobaias *C. porcellus*

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The Guinea pig (*Cavia porcellus*) is a hystricomorph rodent, widely used as an animal model for development of research, especially those related to the reproductive biology, such as the classic study performed by Phoenix et al in 1959, which originated the model for sexual differentiation, widely used in many books of embryology and developmental biology. The aim of this study was to describe the morphological events of intrauterine sexual differentiation of the genital organs of female *C. porcellus*. We used 21 embryos and fetuses at 20, 25, 30, 45, 60 days and at term (n = 3 animals for each gestational period), from nine pregnancies. For this, the females were paired with males (n = 3 females and 1 male), distributed in different boxes of breeding. We conducted daily monitoring of copulation of animals by the method of vaginal cytology, proving copulation by the presence of spermatozoa in the vaginal smear, in the samples transferred to microscope slides and stained by Panoptic-fast method. The time of spermatozoa detection in the vaginal smear was determined as day 0 of gestation and then we wait the time required for collection of embryos and fetuses in determined gestational ages. Then the pregnant were anesthetized with xylazine 2% (40mg/kg/IM) and ketamine hydrochloride 1% (60mg/kg/IM) and euthanized by administration of thiopental sodium 2.5% (60mg/kg / IV). After euthanasia, the females were dissected and embryos and fetuses extracted from the uterus and dissected, and the genital organs processed for light microscopy. This research was authorized by the Bioethics Committee of the Faculty of Veterinary Medicine and Animal Science, University of São Paulo. Macroscopically the first anatomical structures on the external genitalia from still undifferentiated embryos at 20 days of gestation were genital lateral elevations (left and right), located caudal to the genital tubercle. After 25 days it was possible to observe the cloacal folds and a cloacal membrane. After 30 days it was possible to differentiate the female from the male due the formation of urogenital folds and urogenital sulcus positioned caudal to the clitorian genital tubercle. At 45 days, the urogenital folds already had characteristics of future labia, not yet fused urogenital sulcus covered by the urogenital membrane was evident and the perineum was not formed. From day 60 until the end of gestation occurred the formation of labia, fusion of the urogenital folds and formation of the perineum, dividing the anal ostium of the external vaginal ostium and the formation of the vaginal closure membrane. The urogenital sulcus was incorporated into the genital tubercle, developing in a penile clitoris. Regarding the internal genital organs, gonads was already differentiated into an ovary at 25 days. The uterine tubes, uterine horns, a short uterus and a flaccid vagina were present at 30 days. At 45 days was possible to observe a (partially double) septate uterus and a single cervix with a fornix dividing the uterus from the vagina. From 60 days until the end of pregnancy, the tubular organs (uterine tubes, uterine horns, uterus and vagina) lengthened and we observed a double uterus with a single cervix, divided from vagina by the fornix. The pelvic urethra was present from 25 days until the end of pregnancy. This organ extended ventrally to the vagina, from the urinary bladder to the clitoris, with the external urethral ostium opening at the top of the clitoris after 60 days of gestation. Microscopically we observed a pair of ovaries, lined by cubical epithelium and still undifferentiated tissue, where stromal cells and oogonias were observed inside the organ at 25 days of gestation. At 45 days of gestation, appears the first primordial follicle in the cortex region. After 60 days it was possible to note several clusters of oocytes and primordial follicles in the cortex. After 68 days, a large amount of primordial follicles was observed in the cortex. Large primordial follicles were found in the cortex and medulla of the organ during this period. The uterine tubes, uterine horns, uterus and vagina have epithelium and loose connective tissue externally lining the organ at 30 days of gestation. At the end of the pregnancy, characteristic tissue layers of tubular genital organs were already softly differentiated.

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Morphogenesis of Rhea (*Rhea americana*) embryos in different stages of development

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The knowledge of the embryonic development of wild species is very important for captivity and wild life animal handling. This information enables the identification of problems with feeding management. Behavioral alterations of animals during the incubation period of the eggs are connected to environmental conditions and hormonal changes. The aim of this study was to characterize biometrics and the external morphology of rhea embryos during different stages of development, important for determining the age of abandoned eggs and embryos of eggs undergoing artificial incubation. Forty-two embryos were sacrificed and fixed for the description of their external morphology through a stereoscopic microscope. The measurements were made using a digital caliper, ruler millimeter scale and cotton yarn, the measurements were crown-rump (CR), total length (TL), length cephalocaudal (CC), biparietal diameter (BPD), tibiotarsus, humerus and spout, the weight of the embryos was measured by digital scale. The embryos rhea at 5, 6 and 7 days of incubation is presented as "C". At 8, 9 and 10 days the eyes were large and pigmented. At 11, 12 and 13 days the eyelid was covering more than half of the eye resulting in an oval slot. On days 14 and 15 the rhea embryos showed thin skin, allowing the visualization of the ribs, but at 18 days the skin became thicker, disabling the visualization of internal structures. In embryos collected at 21 and 27 days of development closed eyelids were observed forming an eyelid closure, the eyeball being less pronounced at 27 days. Weight gain showed a curve of exponential growth, while consecutive measurements as CR, CT, DC, DBP, tibiotarsus, humerus and spout had a linear growth over time. When compared to other birds, differences were noted in the morphology of rhea embryos at the ages analyzed, the possible reason for this is the different methodologies and the incubation time used in this research. It was possible to characterize the rhea embryos at various ages' of incubation using external morphological and morphometric analyzes. The rhea morphological characteristics of embryos at various ages of incubation is an undescribed model, this research provide data to identify ontogenetic patterns of species, knowledge of their biology and promoting amelioration of production system in captivity. CEEA/ UFPI N°029/12, SISBIO Nº 35374-1).

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Histological morphogenesis of Rhea (*Rhea Americana*) embryos digestive system

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Rhea (Rhea americana) is the largest bird in South America that can be found in northeast, south and southeast of Brazil. Rhea has a seasonal reproduction which can be influenced by climatic conditions. The study of rhea embryonic development of digestive tract has fundamental importance for understanding the gastrointestinal biology of this species. This work aims to describe the histological features of the digestive tract from rhea developing embryos. Thirty-two rhea embryos undergoing euthanasia were analyzed. After fixation, fragments were collected from the gastrointestinal tract (tongue, esophagus, proventriculus, gizzard, small intestine and large intestine), dehydrated, sectioned and stained with hematoxylin-eosin (HE), alcian blue and periodic acid-Schiff [AB (pH 2,5%)-PAS] for analysis under light microscope. The tongue presented cubic stratified epithelium and muscle cell differentiation at 11 days of incubation, and early hyaline cartilage was evident at 13 days. Esophagus had a pleated mucosa with irregular lumen and after 27 days of incubation the epithelium showed lashes. After 9 days, invagination of the epithelium of proventriculus occurs, forming the beginnings of the proventricular glands that are present in only one pole of the organ. Gastric ventricle at 13 days showed stratified epithelium, alternating between cuboidal and columnar cells, and containing the largest number of mucous cells. At 31 days there was a large amount of glandular secretion into the lumen of gastric ventricle that will become the coelina membrane. Duodenum of embryos at 27, 30 and 31 days of incubation showed the coats well developed with longer villi lined by simple columnar epithelium and mucous cells with multifocal distribution, besides alcian blue and PAS positive; the submucosa had become thin. Mucous cells of the jejunum and ileum were positive for alcian blue on day 27. The cecum contained a columnar pseudostratified epithelium from 15 to 27 days. The colon-rectum at 15 days showed a mucous covered by stratified, with some areas of pseudostratified epithelium; at 27 days mucosa showed positive for alcian blue and PAS and at 30 and 31 days epithelium became simple columnar and some areas were pseudostratified. Histological aspects of the organs by digestive system of rhea embryos were similar when compared to other existing bird species data related to the dietary habits of the species and developmental stages. There are no reports in the literature on the rhea embryonic aspects. Histological characterization of the digestive tract of rhea, of fundamental importance for the understanding of gastrointestinal biology, is not described in current literature. The data from this study will provide a basis for further research related to the management, mechanisms of gene expression and for understanding of the digestive tract diseases in this species. (CEEA/ UFPI N°029/12, SISBIO Nº 35374-1).

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Echogenicity and biometric testicular evaluation in confined bulls

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Andrological examination and testicular ultrasonography are capable to detect early reproductive changes that may decrease (re)productive indices of the herd, including changes by intensive management (confinement-housed) of bulls, which routinely happens before exhibitions, auctions and other confined management situations. The aim of this study was to evaluate the echogenicity and testicular biometry in confined bulls, searching for possible changes in testicular stroma caused by nutritional management. Twenty bulls aged 14-16 months old were used, submitted to confined management based on corn silage (15 kg) and concentrate with 19% of protein concentration (5kg). The animals were weighed, and underwent ultrasonographic evaluation of testicular stroma and testicular size in four sessions every 21 days. Shenzhen Mindray bio medical device, using a 6.5 MHz linear array transducer was used. The testicular ultrasonographic evaluations were performed using acoustic gel on the scrotum, making images in sagittal and transverse plans, on the left and the right testicles by complete scan of all testicular stroma. The images were saved and analyzed comparatively by a trained evaluator. Data were expressed as mean and standard deviation, performing (nonparametric) one-way ANOVA using statistical package Prism 4 Graphpad. The study observed an increase in weight and testis size. Mean values were 331.6; 401.7; 432.4 and 480.1 kg for animal weight, 29.5; 31.71; 33.33 and 34.5 cm for scrotal circumference and 1281.89; 2163.85; 2963.89 and 3607.0 cm^3 for total testicular volume in the 1st, 2nd, 3rd and 4th assessments, respectively. Testicular stroma on the first evaluations were homogeneous, hypoechoic, certainly because of age and weight of the animals. Subsequent evaluations observed an increased echogenicity of the testicular stroma. From the 4th assessment there was a pattern change, observing hypoechoic points, making it heterogeneous. The study greatly contributes to the andrological findings, showing accurately and in a practical way the echogenicity evaluation of bovine testicular stroma, demonstrating that confinement management can bring harm to the reproductive function.

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Scrotal infrared thermography in Nellore bulls (*Bos taurus indicus*)

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Infrared thermography is a noninvasive method of assessing scrotal surface temperature (SST) by infrared emissions with an accuracy of 0.10ºC. Researchers have shown that the surface temperature of the scrotum is highly correlated with deep testicular temperature. There was no significant effect of diurnal variation on SST. The aim of the study was to determine the scrotal surface temperature measured with infrared thermometry in Nellore bulls under field conditions. Semen from eighty Nellore bulls, 24 to 35 months old, was collected by electroejaculation (Autoejac®, Neovet) at ambient temperature (31ºC) under field conditions. From each male a total of 1 sample was collected. Semen characteristics were evaluated according to the Brazilian College of Animal Reproduction. The infrared thermography was conducted before and after electroejaculation. A Camera Infrared Thermography (FLIR E-40®) was positioned 1m behind the scrotum to measure SST on spermatic cord (T1), at the top of the testicle (T2), at the middle of the testicle (T3), at the bottom of the testicle (T4) and at the cauda of the epididimides (T5). The means of variables: volume (5.48±3.08 mL), motility (73.44±15.28%), vigor (3.44±0.86), testicle volume (708.19±164.16 cm^3) and total spermatic defects (21.02±16.43%) showed a good semen quality. The testes in the scrotum must be maintained at a temperature 2 to 5ºC lower than the body temperature. The area of increased SST (T3 and T4) was attributed to a localized increase in SST over the cauda epididimides. Data were evaluated by analysis of variance and means compared by Tukey at 5%. The means from T1 to T5 before electroejaculation were different ($P<0.05$): T1 (36.91±1.56ºC), T2 (35.70 ±1.54ºC), T3 (34.74 ±1.57ºC), T4 (33.87 ±1.64ºC) and T5 (32.87±1.88ºC). The means from T1 to T5 were different ($P<0.05$): T1 (36.72±1.70ºC), T2 (35.65±1.57ºC), T3 (34.56±1.47ºC), T4 (33.61±1.52ºC) and T5 (32.77±1.57ºC). Comparing the condition before and after electroejaculation, there was no difference ($P>0.05$) between the temperatures on the same anatomical points of the testicles (T1 before – T1 after; T2 before – T2 after; T3 before – T3 after; T4 before – T4 after; T5 before – T5 after). In conclusion, infrared thermography can be performed before or after semen collection and can be done under field conditions. Infrared thermography should be used for evaluation differences with decreasing temperatures of the the spermatic cord (T1) in relation to the testicles (T2, T3 and T4), and these with the cauda of the epididimides (T5). (CEUA/UNOESTE/Protocol#1920).

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Possible effects of inbreeding on reproductive traits in male Beagle dogs

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Inbreeding increases the extinction risk of a population. At the individual level, deleterious effects of homozygosity have been associated with a decrease in both chance of survival and reproductive potential. In several species, there have been reports of inbreeding-associated reduction in semen quality, conception rates, and litter size. In the domestic dog, knowledge on the inbreeding effects on reproductive performance remains scarce. Therefore, the objective of this study was to compare semen quality between inbred and outbred Beagle dogs. Seven adult males (INB: inbred group, n=3; CON: control (outbred) group, n=4), with ages varying between 2 and 4 years (body weight, 14-18.5 kg), were used in this study. The three males in the INB group were siblings and were both offspring and a half-brother to an outbred male in the CON group. Three to five semen samples from each male were collected by digital manipulation, and the following parameters were assessed: ejaculate volume (mL), sperm motility (%), vigor (0-5), morphology (%), concentration (sperm count/mL of ejaculate), and total sperm output (sperm count/ejaculate). Additionally, testicular and prostatic dimensions (L x W x H) were measured using a caliper and an ultrasound machine, respectively, for calculation of the organs' volume and weight. Mean volume of ejaculate was smaller ($P<0.05$) in the INB group compared to the CON group. Mean sperm concentration in the INB group was higher ($P<0.05$) than that of the CON group; however, total sperm output tended ($P=0.06$) to be lower in the INB group. Sperm motility, vigor, and morphology were comparable ($P>0.05$) between the two groups. Although the two groups did not differ ($P>0.05$) in body weight or in testicular measurements (volume, weight), prostate volume and weight measurements were smaller ($P<0.05$) in the INB group. There was a positive correlation between prostate weight and ejaculate volume (r=0.87). The superiority of the INB group in terms of sperm concentration could potentially be explained by the lower ejaculate volume observed in this group. This finding might be related to the smaller prostatic volume within the INB versus the CON group. In conclusion, the results suggest that inbreeding in these dogs possibly caused a decrease in both prostate dimensions and function, as measured by ejaculate volume. Studying the effects of inbreeding on reproductive traits in the domestic dog could potentially contribute to our understanding about the consequences of inbreeding depression on reproductive function in endangered canids.

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Protein profile of rete testis fluid from Morada Nova rams

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Seminal plasma is a complex secretion composed by fluids from accessory sex glands, epididymis and testicles, and its molecular composition is capable of modulating sperm function. Proteins are the most abundant organic compounds in seminal plasma and play a crucial role in processes related to fertilizing capacity of sperm, and can be considered as potential molecular markers of fertility. The rete testis fluid consists of the secretions of the seminiferous tubules and epithelial cells of the rete testis and serves partly as a vehicle for movement of sperm in the epididymis. Few studies have been conducted on the protein profile of this fluid and its functional significance remains largely speculative. The aim of the study was to analyze the protein profile of rete testis fluid. The testes were obtained from five slaughtered Morada Nova rams. The testes were dissected and the head of the epididymis was separated to gain access to the efferent ducts. The fluid from the efferent ducts was obtained by testis massage and then collected using a pipette. Thereafter, the fluid was subjected to two centrifugations: 1500 x g for twenty minutes at 4°C and 5000 x g for thirty minutes at 4°C, respectively. The supernatant was collected and precipitated with acetone for two hours at -20°C. It was then centrifuged at 5000 x g for forty minutes at 5°C, and then, supernatant was discarded and the pellet was dried overnight in a freezer at 4°C. Posteriorly, the pellet was resuspended in sample buffer. Protein concentration of the rete testis fluid was determined by the Bradford assay. Four hundred micrograms of each sample were subjected to isoelectric focusing in strips of 13 cm (pH 4-7). The second dimension was conducted on SDS-PAGE 15%. The obtained gels were scanned with an ImageScanner II (GE Lifesciences, USA) and analyzed using the PDQuest® version 8.0.1 (Bio-Rad Laboratories, USA). In the gels 237 ± 46.4 spots (mean ± SD) were detected, where 66.2% of the proteins were found above 40 kDa, and 55.2% of the proteins in the pH range between 5.0 and 5.9. The fourteen more intense spots with a molecular mass between 66 and 70kDa and pH range between 5.4 and 6.6 corresponded to 25% of total proteins. Based on approximated molecular protein weights, those proteins could correspond to isoforms of albumin (68 kDa), which was also identified by Fritz et al. (1987) in the rete testis fluid in levels ranging from 11% to 17% of total protein. Our results show that the rete testis fluid from Morada Nova rams has a greater amount of high molecular weight proteins and albumin is probably the major protein in this fluid.

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Reproductive abnormalities in male sheep exposed to testoterone *in utero*

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Polycystic ovary syndrome (PCOS) is the most common reproductive abnormality in women. Animal models have been used to clarify mechanisms of development of PCOS through the developmental programing produced usually by fetal androgenization during gestation. Although a male phenotype for PCOS in humans is currently undetermined, reduction in volume of testis established by ultrasound, in sons of women with this disorder has been reported. Prenatally androgenized males also develop disruptions, especially related to the reproductive system (reduction in spermatic count and viability). To date, only a few studies in Suffolk have been conducted, and the observed changes in reproductive phenotype have not been fully confirmed or contradicted among different breeds of sheep. The present study aims to evaluate postnatal male sheep weight, scrotal diameter and wool quality during the first year of life in animals prenatally subjected to androgen administration. Our hypothesis was that prenatal androgenization in sheep could impair the development of normal reproductive features. In addition, our study investigated the possible impact of this treatment in the skin - an androgen sensitive tissue, through the analysis of the wool. This was an experimental study, approved by the local Animal Ethics Committee at UFSM. Briefly, 49 Corriedale sheep between 3-5 years were synchronized, artificially inseminated with fresh semen and administered equine chorionic gonadotropin 2 days before insemination. After 30 days and confirmation of gestation by ultrasound, the mothers either received testosterone propionate 100mg i.m. twice a week until 90 days of gestation (n=24) or nothing (n=25). All lambs were promptly identified with an ear tag after birth and the weight was recorded. Overall, 8 androgenized and 4 controls were studied, considering the fact that 7 males (androgenized and/or controls) died during the first week. No differences in weight were observed between the two groups (mean $\pm$ SD) in androgenized males (n=8) 4.17 $\pm$ 0.5Kg and controls (n=4) 4.40 $\pm$ 0.5Kg (NS). No changes in weight were detected at the age of four months between androgenized males. These similarities persisted at eight months of age where the weight of androgenized males was (mean $\pm$ SD) 28.9 $\pm$ 4.2Kg not different from controls 27.6 $\pm$ 5.86Kg (p=0.67). The characteristics of the wool regarding the diameter, length and modulation collected from the top of the shoulder were evaluated at 4 months of age. Curiously, an important decrease in hair diameter was observed in lambs sons of androgenized mothers (mean $\pm$ SD) 23.72 $\pm$ 0.2µm than in controls 26.2 $\pm$ 0.6µm (p=0.002). Similar results were seen in wool length between the androgenized group (mean $\pm$ SD) 3.84 $\pm$ 0.19cm and the control group 3.9 $\pm$ 0.3cm (p= 0.720). Finally, at 8 months of age, androgenized lambs presented a trend for the reduction in scrotal diameter (mean $\pm$ SD) of 18.8 $\pm$ 1.4cm against controls (mean $\pm$ SD) of 22.3 $\pm$ 2.3cm which has not reached statistical significance (p=0.22). To sum up, prenatal androgenization did not change the weight of male animals, although it produced some modifications in diameter of wool fiber before the first year of life. During the first 10 months, no changes in scrotal diameter could be clearly identified, despite a trend for reduction in prenatally androgenized animals.

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Doppler ultrasound of the normal testis in goat: B-mode, color and spectral Doppler imaging

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The goat is the most widespread species in the Northeast of the country due its characteristics of hardiness. Although reproductive studies on this species have grown widely, imaging methods are not fully elucidated, as well as the standardization of values related to reproductive function in these animals. In this context, five male Saanen goats between 2 to 3 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) testicular diameter=4.10±0.38; 2) rete testis diameter=0.53±0.02; 3) epididymis head length=1.78±0.39; 4) epididymis head Diameter= 2.39±0.19; 5) testicular artery flow velocity=11.66±1.21 cm/s; 6) resistivity index 0.45±0.02. 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 systolic peak with a deceleration slope similar to that described in humans. No citation was found for domestic species. The index showed little variation in resistivity value, characterized by a medium resistance flow slightly reduced from the evidenced in humans. 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 moderate average velocity, which we attribute, probably, to the 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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Oxytocin and androgen receptor immunolocalization in dog testis and epididymis

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Oxytocin (OT) plays a role in the male reproductive tract, and has been suggested to increas smooth muscle contractility, the induction of penile erection, the modulation of transport and maturation of sperm and sexual behavior. The present study aimed to correlate the immunostaining of androgen receptors and oxytocin receptors (OTR) in the testis of dogs. Materials and Methods: Testes of five dogs were collected by elective orchiectomy and fixed in 10% buffered formaldehyde and the samples were submitted to routine histological processing. Incubation was then performed with primary anti androgen receptor and anti-OTR human polyclonal antibody produced in rabbits and the polymer NOVOLINK (Nichirei ®). All experimental procedures were performed in accordance with the guidelines established by the Brazilian School of Animal Experimentation and were approved by the Ethics Committee for Animal Experimentation of the Paulista University. Our results showed that the immunostaining of androgen receptors of the testicle is correlated with immunostaining of OTR. The immunostaining was observed in myoepithelial cells, stromal smooth muscle cells and Leydig cells of the testes and stromal smooth muscle cells of the epididymis, possibly relating to the events of muscle contraction and sperm movement in the genital tract, essential to male reproductive success. This study corroborates with the compared study allowing the description of the role of androgen and oxytocin in the male reproductive system, since, so far, the basic descriptions in literature only mention their role in the female reproductive system. This study was supported by Paulista University and São Paulo University.

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Immunolocalization of enzymes: cytochrome P450 aromatase, and cytochrome P450c17 (17-α-hidroxilase/17, 20 lyase) in the epididymis of the freshwater turtle (*Kinosternon scorpioides*) in the dry and rainy seasons

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Currently, researches have studied the reproductive aspects in order to promote the conservation of freshwater turtle, *Kinosternon scorpioides* species in Baixada Maranhense by developing management strategies, as well as the creation of public policies to support the survival of this reptile in its natural habitat. Control of reproductive processes in wild animals cannot be understood only by classical endocrine approaches, such as hormone concentrations. The endocrinological approach for molecular biology can help to understand the process of steroidogenic metabolism by cytochrome P450 aromatase and cytochrome P450c17 enzymes. Some steroidogenic enzymes are membrane-bound and perform their function in intracellular specific compartments. Consequently, the steroidogenic reactions are spatially separated in steroidogenic cells. We aimed immunolocalize the cytochrome P450 aromatase and P450c17 (17-alfa-hidroxilase/17, 20 lyase) in the epididymis of *K. scorpioides* in rainy and dry seasons, by immunohistochemistry. In the rainy season, strong immunostaining of P450 aromatase was observed in interstitial cells, in the cells of efferent duct and semen. P450c17 enzyme was slightly immunolocalized in apical cells. In the dry period, the P450 aromatase enzyme had moderate immunolocalization in interstitial cells and cells of the efferent duct from the previous period. The P450c17 enzyme had moderate immunolocalization in interstitial cells and cells of the efferent duct and poor immunolocalization in basal cells. The presented data can be understood through the concept of cellular compartmentalization of the enzymes involved in the synthesis of sex steroids and in determined compartments may have effects on androgen and estrogen production. The epididymis showed immunostaining in the apical cells in the rainy season. At another time of the dry season, this marking was restricted to the basal cells for the cytochrome P450c17 enzyme. Perhaps this fact could influence the storage of sperm, a common aspect in the reproductive behavior of these animals. Steroidogenesis may be compartmentalized in organ, tissue and cellular levels. This may vary among species; however, in determined organizational mechanisms from extraordinary diversity of tissues, this fact suggests functional significance. In this work, the co-expression of enzymes P450aromatase and P450c17 in the rainy season have demonstrated influence in the spermatogenesis. (MMA/ICMBio/Sisbio - 33021-4/2014).

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Hydrogen peroxide promotes a dose-dependent decrease on motility parameters of bovine spermatozoa

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Oxidative status acts on sperm in different physiological pathways, from the capacitation process to fertilization. Reactive oxygen species can be beneficial or harmful depending of their content. Due to the reduced content of catalase and glutathione peroxidase, sperm following cryopreservation may be particularly susceptible to the hydrogen peroxide. The present study aimed to evaluate the pro-oxidative effect of different concentrations of hydrogen peroxide on sperm motility. Frozen semen batches of five Nelore bulls (*Bos indicus*) were thawed and submitted to Percoll® gradient (90/45%). The resulting sediment containing motile cells was adjusted to a final concentration of $25x10^6$ spermatozoa/ml with Fert-TALP (50 µg/ml gentamicin, 50 µg/ml pyruvate 0.2 mM and BSA FAF 0.006% m/v). Samples were allocated into five groups of treatments: control; 12.5; 25; 50 and 75µM of hydrogen peroxide and incubated during 1 hour at 38.5°C and 5% CO_2. After incubation, parameters related to motility were analyzed by Computer Assisted Sperm Analysis (CASA). The experiment was conducted in four replicates. The concentration of 75µM had a lethal effect on spermatozoa being removed from statistical analysis. Polynomial regression was used to evaluate the effect of hydrogen peroxide concentrations. A $p \leq 0.05$ was considered significant. Differences between treatments were found for all variables analyzed. By increasing hydrogen peroxide concentration, a decrease was observed for total motility (control = 62.15±3.41; 12.5 = 51.8±3.94; 25 = 44.7±5.19; 50 = 25.85±4.34) and progressive motility (control = 54.1±3.86; 12.5 = 41.2±4.48; 25 = 28.95±4.31; 50 = 9.2±2.67). For the variables indicative of sperm velocity, an interaction was observed between bull and treatment: VAP (average path velocity, p=0.02), VSL (straight line velocity; p=0.02), VCL (curvilinear velocity; p=0.02), and LIN (linearity, p=0.02). When analyzed separately, these variables indicate a positive effect of 12.5 µM, improving the motility parameter of one of the bulls. In conclusion, hydrogen peroxide, as an oxidative stress inducer, impaired sperm motility. However, individual variability in this response should be considered, suggesting an improvement of motility pattern in low doses.

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Evaluation of canine semen with the hypo-osmotic swelling test

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The hypo-osmotic swelling test (HOST) is used to evaluate the plasma membrane integrity of spermatozoa. Currently, it has been used as a routine test for assessing the quality of semen of domestic animals including dogs. In this test, a plasma membrane when submerged in a solution with a low osmolarity allows water entry into the intercellular space by osmosis, which elevates cell volume, and the folding tails of spermatozoa with intact plasma membrane. The HOST is correlated with male fertility, making it a good way to test the quality of semen. There are numerous protocols for testing, but there has been no standardization. The objective of this study was to evaluate the reactivity of the sperm cells of dogs to hypo-osmotic solutions with different osmolarity. Semen was collected from three mature dogs. All dogs were treated according the Ethics Committee for Animal Use of UFV College of Veterinary Medicine. Ejaculates (n = 18) were obtained by digital manipulation and collected into a plastic funnel connected to a 15-mL centrifuge tube. Immediately after collection, the ejaculate was processed for determination of concentration, morphology and motility parameters. For the assessment of the HOST, the semen was standardized to 100 x 10^6 sperm/mL. An aliquot of 100 µl of the thawed semen was incubated at 38°C for 45 minutes with 1 mL of two hypo-osmotic solutions: distilled water (0 mOsm/Kg) (1) and fructose and sodium citrate solution at 60 mOsm/Kg. A third solution was prepared incubating 20 µl of semen with 0.25 mL of fructose and sodium citrate solution at 150 mOsm/Kg. At the end of the incubation period, the samples were fixed with 0.5 mL of Karnovsky solution. Later an aliquot of the solution was placed between two glass slides for counting 100 sperm cells in contrast microscopy at 1000X magnification. The cells were classified by the presence or not of swollen or coiled tails and the result was determined using the formula: HOST (%) = (% of alterations in the caudal region after the HOST) - (% of alterations in the caudal region before the HOST by sperm morphology assessment) (2). The statistical analysis was done using the statistical program SAEG 9.1 (SAEG-UFV, 2007). The quantitative data were evaluated by analysis of variance (ANOVA), and when there was significance by the "F" test, the means were compared by the Duncan test, with a 5% error probability. The means for the samples were 0 mOsmol/Kg (36.7±19.6), 60 mOsmol/Kg (48.0±23.6) and 150 mOsmol/Kg (66.5±15.7). There was no difference between the mean values obtained for the reactive sperm cells in the studied hypo-osmotic solutions ($P > 0.05$). However, our results were still low compared with those of other authors. Garrido and Sanchez (2013) obtained 95.3±3.8 for a hypo-osmotic solution of distilled water and 77.0±10.5 for a solution containing 55 mOsmol/Kg. Kozink and Pinto (2008) using a solution of 100 mOsmol/Kg obtained in 1 minute and 60 minutes of incubation 86.2±1.4 and 85.6±1.3 respectively. However, it was possible to identify the reactivity of the sperm cells of dogs to hypo-osmotics solutions, with different osmolarity, showing that HOST must stimulate laboratory technicians and clinicians to incorporate this assay into standard canine semen analysis. References: 1. Sánchez RA, Garrido BD. 2013. Evaluación de una prueba hipoosmótica simplificada en semen canino fresco y refrigerado. Revista Científica, vol. XXIII, 6: 506-510. 2. Melo MIV, Henry M. 1999. Teste hiposmótico na avaliação de sêmen equino. Arq Bras Vet Zootec, 51:71-78. 3. Pinto CRF, Kozink DM. 2008. Simplified hypoosmotic swelling testing (HOST) of fresh and frozen-thawed canine spermatozoa. Animal Reproduction Science, 104: 450–455.

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Acute exposition of different chemical forms of arsenic on sperm parameters in rats

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Arsenic is a toxic metaloid considered by Brazilian and international environmental agencies as the most dangerous chemical element, and its contamination in drinking water is a major public health problem worldwide. Studies to assess the effects of chronic exposure of arsenic on sperm parameters showed higher percentage of spermatozoa with abnormalities and no motility. There is little information about the acute effects of arsenic on the sperm parameters, especially when animals are exposed to different chemical forms. Therefore, this work aimed to evaluate the effects of the acute exposure of arsenic in the pentavalent (arsenate, As^{+5}) and trivalent chemical forms (arsenite, As^{+3}) at different concentrations on sperm parameters. Thirty Wistar rats were randomly divided into five experimental groups (n = 6 animals/group). The control animals received saline, while the treated animals were orally exposed to sodium arsenate and sodium arsenite at concentrations of 0.01mg/L and 10mg/L (thousandfold 0.01mg/L), daily for 7 days (CEUA/ UFV nº 79/2013). On day 8 the animals were euthanized and the epididymis was collected. Sperm were obtained from the cauda epididymis and they were analyzed considering their morphology under light microscopy, and their membrane integrity under epifluorescence microscopy. The sperm morphological abnormalities were classified as head, midpiece and tail defects. The structural integrity of plasma and acrosomal membranes were assessed using PI and CFDA dyes. The results were submitted to ANOVA and Student Newman Keuls Tests, considering significant $P < 0.05$. Animals exposed to 10 mg/L of sodium arsenate presented higher percentage of head defects (8.25 ± 1.11) when compared to control animals (4.25 ± 1.11; $P < 0.05$). However, there are no significant differences among the groups when analyzed the percentage of sperm without abnormalities ($P > 0.05$). In addition, the assessment of the structural integrity of the sperm membranes showed that animals exposed to sodium arsenite 10 mg/L presented lower percentage of sperm with intact membranes (3.50 ± 1.76) when compared to the control animals (21.67 ± 4.18; $P < 0.05$). The damage of the sperm membranes can be explained by the arsenic action on phospholipids and proteins present in the plasma membrane. It is well known that arsenic can promote the lipidic peroxidation of these phospholipids, causing lesion in the sperm membrane. In conclusion, acute exposure to sodium arsenite at 10 mg/L in drinking water can reduce the percentage of sperm with integrity of membranes.

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Morphology of spermatozoa of Antarctic mollusks

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Spermatozoa morphology may indicate the pattern of fertilization and the type of larval development, important characteristics for the recognition of the reproductive strategies of mollusks in the Antarctic environment. The aim of this work was to describe the morphology of male gametes of 4 species of mollusk from the Antarctic region and to associate this morphology to the reproductive type. Samples of male gametes of *Nacella concinna* (Strebel, 1908), *Yoldia eightsi* (Couthouy, 1839), *Margarites refulgens* (Smith, 1907) and *Laternulla elliptica* (King, 1832) collected in the Admiralty Bay, King George Island were analyzed. Sexual mature individuals were mechanically opened and samples of male gametes removed. Male gametes were preserved in Glutaraldehyde and sodium Cacodylate used as buffer for photomicrography in Scanning Electronic Microscope. Morphological differences were observed in the heads of spermatozoa. *Nacella concinna* and *Yoldia eightsi* presents a spermatozoa with the round format of the head and diameters of 2.77 µm and 2.56 µm, respectively. The round format of the head and short acrosome indicates a primitive type of gamete with characteristics of external fertilization. *Nacella concinna* spermatozoa has a total length of 22.06 µm and *Yoldia eightsi* of 56.92 µm. The species *Laternulla elliptica* and *Margarites refulgens* present a prolonged head and acrosome that facilitates the penetration in the oocytes. For the spermatozoa of *Laternulla elliptica* a total length of 40.83 µm, with length and head diameter of 7.73 µm and 1.60 µm, respectively were obtained. Spermatozoa of *Margarites refulgens* have a head diameter of 3.04 µm and tail diameter of 0.344 µm. More studies in spermatozoa morphology of Antarctic mollusks can contribute to the identification of the patterns and/or reproductive strategies of mollusks in the area using Transmission Electronic Microscope techniques.

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Buffalo semen cryopreservation (*Bubalus bubalis*) using commercial extender containing low-density lipoproteins

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The aim of this study was to test the replacement of egg yolk by different concentrations of low-density lipoproteins (LDL) in commercial extender (Botubov®) for buffalo semen cryopreservation. The experiment was conducted at the Center for Biotechnology in Bubalinocultura in Pedro Leopoldo, MG, using six Murrah bulls, aged 29-36 months. Eighteen ejaculates were collected by artificial vagina. After semen collection analyzes of motility were performed in CASA (Sperm Class Analyzer - SCA ® v.4.0), force tourbillon, sperm concentration (Neubauer chamber), sperm morphology and hypoosmotic test (phase contrast microscopy) were performed. The ejaculates were separated and diluted according to the treatment until they reached a final concentration of $50x10^6$ sperm/mL. Five extenders were used: control (commercial extender) and 2, 4, 8 and 14% (v/v) LDL (in extender provided by the company, free from egg yolk). The diluted semen was packaged in 0.25 ml straws, and cooling was performed on a computerized machine (TK 4000), using a cooling rate of -0.25°C/min to 5°C. Semen was keep in balance at 5°C for 4 hours. The straws were frozen in an ice chest, kept at 5cm from the surface of liquid nitrogen for 20 minutes and then immersed in liquid nitrogen. The samples were keep bottled in nitrogen until thawing. After thawing (two straws each treatment) thermal resistance test (TTR) assisted by CASA (120 minutes, with assessments conducted every 30 minutes), hypo-osmotic test and membrane integrity by fluorescent probes (CFDA / Pi) were done. The average results of these three tests were compared by Anova followed by Tukey test ($P < 0.05$). The extender control were higher ($P<0,005$) than treatments containing LDL for motility in pre-cooling period (81.63%, 83.84%, 82.91%, 86.05% and 91.97% respectively for 2, 4, 8, 14% LDL and commercial) and post-thaw (25.14%, 27.19%, 28.56%, 24.41% and 50.66% respectively for 2, 4, 8, 14 % LDL and commercial). The superiority of the control extender was maintained for the remaining kinetic parameters analyzed by CASA (VCL, VSL, VAP, LIN and BCF) and during all readings of TTR. The concentration of 2% LDL had the worse effect, to preserve the integrity of membranes, than control extender in the fluorochromes test (22.67 ± 3.73% *versus* 37.89 ± 4.28% integrity of intact, respectively). There was no difference in membrane integrity in the hypoosmotic test ($P>0.005$). The replacement of egg yolk by low-density lipoprotein in commercial extender did not satisfactorily protect the buffalo sperm cryopreserved.

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Concentrations of sodium, potassium, calcium and alkaline phosphatase in sperm-rich fraction of dog semen

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The biochemical composition and enzymatic characteristics of canine seminal plasma have received little attention. The knowledge about these components can help the development of methods for sperm conservation. The seminal plasma mediates the chemical function of the ejaculate. The main cations in the seminal plasma are sodium, potassium and calcium, and their concentrations can affect sperm quality. There are few studies measuring the concentration of these components in the canine seminal plasma. This study aimed to measure some cations concentrations (sodium, potassium and calcium) and the enzyme alkaline phosphatase in the sperm-rich fraction of dog semen. Semen was collected from three mature dogs. All dogs were treated according to the Ethics Committee for Animal Use of the Universidade Federal de Viçosa. Ejaculates (n = 21) were obtained by manual stimulation of the penis and collected into a plastic funnel connected to a 15mL centrifuge tube. A maximum volume of 2 ml of the first and second fraction of semen corresponding to sperm-rich fraction (RF) was collected. RF collected was centrifuged (2400 rpm / 10 minutes) to use only the supernatant for analysis, which was placed into 1.5 ml plastic tubes. The samples of the sperm-rich fraction (FR) were referred to the Laboratório Clínico do Departamento de Veterinária of the Universidade Federal de Viçosa to measure the concentrations of Ca^{2+}(cresolphthalein complexone -In vitro Diagnóstica® LTDA) Na^{+} and K^{+} (flame photometry-Micronal®) and FA (IFCC method kinetic- In vitro Diagnóstica® LTDA); analyzes were conducted using a semi-automatic biochemical analyzer parameter (BIO-300 -BioClin®). The results obtained were: Ca^{2+} (mg/dL) 1.52 ± 0.62; K^{+} (mEq/L) 9.27 ± 1.78; Na^{+} (mEq/L) 137.83 ± 5.84 and FA (UI/L) 81,395.11 ± 65,318.46. Few studies have measured the concentration of these components in canine seminal plasma, among these there was similarity between the results obtained. The concentration of the enzyme alkaline phosphatase in sperm-rich fraction must be high, indicating that the discharge is coming from the epididymis. But no author described one as high as those found in this study. The levels of sodium, potassium and calcium behaved as described in the literature, high sodium and low potassium and calcium. The development of reproductive biotechnologies in dogs is necessary for the improvement of the breeds and the use of this species as an experimental model for wild canids. This can only be possible with a greater knowledge of the physiology of the species.

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Sperm parameters of adult rats treated with propiconazole

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Propiconazole (Prop) is a fungicide extensively used in agriculture for cereals, vegetables and fruits. There are evidences that this chemical may cause endocrine disrupting effects. In *in vitro* studies, it was demonstrated that Prop has the ability to inhibit the activity of CYP 19 (aromatase) responsible for converting androgens into estrogens, which have an important function in the development and maintenance of the reproductive system. Therefore, the present study aimed to evaluate the effect of Prop treatment in sperm parameters of adult male rats. Wistar rats were divided in three groups and were treated, by gavage, from postnatal day (PND) 50 to 120 with corn oil (control group: C), propiconazole 4mg/kg (Prop 4) and propiconazole 20mg/kg (Prop 20). After the treatment, all animals were weighted, euthanized and the reproductive organs were removed. Sperm count and spermatozoa morphology parameters were analyzed. Data ± SEM were compared by ANOVA; median (1^{st} -3^{rd} quartile) by Kruskal Wallis ($p<0.05$). No statistically significant difference between the groups was observed in sperm count. However, it was observed an alteration in sperm morphology shown by an increase in the percentage of tail abnormality in the Prop 4 compared to the control group [C: 5.37 (3.33-6.73)^{A}/ Prop 4: 11.65 (5.77-20.52)^{B}/ Prop 20: 9.39 (4.90-14.50)^{AB}]. These results suggest that Prop damaged the sperm quality, since the treatment covered the complete spermatic cycle, which takes around 48-53 days in rats. The absence of statistical significance in Prop 20 could be explained by nonmonotonic (biphasic) dose-response curve, where an increase in the response is observed at the lowest dose followed by a decrease at the highest dose. Besides, additional studies should be performed to fully determine the impairment of sperm quality.

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Relationship between enzymatic antioxidants in ram seminal plasma after stress induced by testicular insulation: immunodetection versus activity

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The ram sperm is considerably susceptible to damages induced during the application of reproductive biotechnologies. This sensitivity may be due to an imbalance between reactive oxygen species and the antioxidant protection present in the seminal plasma, leading to an event also known as oxidative stress. Testicular insulation is a technique known to induce oxidative stress in the testis due to increased temperatures within the organ. The aims of this study were to evaluate the effect of heat stress (i.e., testicular insulation) on the imunodetection and imunoquantification of the antioxidant enzymes catalase, superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR), on the activity of these enzymes also in the seminal plasma, and to further correlate these two approaches. Twelve adult rams were randomly allocated into two groups: control and testicular insulated animals (treated). After 288 consecutive hours of testicular insulation, semen samples were collected weekly with an artificial vagina for 9 weeks. To confirm the induction of oxidative stress by the testicular insulation, intracellular hydrogen peroxide analysis (dichlorofluorescein) was performed using flow citometry. Subsequently, aliquots of 500 µl of the ejaculate were centrifuged (6000g; at 5° C; 10 min); the supernatant (seminal plasma) was submitted to Western-Blot to detect catalase, SOD, GPX and GR enzymes. The enzymes were separated by standard SDS - PAGE and transferred to polyvinylidene fluoride membrane (PVDF) using a Trans-blot Turbo (Bio-Rad®). After blocking the nonspecific sites, each membrane was incubated overnight with the primary antibodies anti-catalase (H-300 - Santa Cruz Biotechnology), anti-SOD (H-90 - Santa Cruz Biotechnology), anti-GPX (H-45 - Santa Cruz Biotechnology) and anti-GR (GR24778-1 - Abcam) diluted 1/1000 in PBS - Tween with 1% BSA. Membranes were washed 3 times with PBS to remove the primary antibody solutions and subsequently incubated with secondary donkey anti-rabbit antibody (Li - COR Biosciences - 1/15000 in PBS - Tween with 1% BSA) for 75 min, at room temperature, in the dark. Visualization of the signal and band area was performed in Odyssey CLX (Li-COR® Biosciences). Colorimetrical evaluation of the antioxidant enzyme activity was performed using a spectrophotometer (Thermo Electron Corporation). Briefly, catalase was assessed through the measurement of hydrogen peroxide consumption for 3 minutes in λ=242nm; SOD was measured through the reduction of cytochrome C for 3 minutes in λ=470nm; and the activity of GPX and GR was based on the NADPH oxidation recorded for 3 minutes in λ=340nm. ANOVA, Spearman correlation and *post hoc* Least Squares Means tests were performed using *SAS System 9.3 for Windows*. Significance was considered when $p<0.05$. Increased percentage of cells stained for dichlorofluorescein was observed in the treated group when compared to the control group (5.60±2.09 vs 3.35±0.6, respectively). An increase on the activity (IU/mL) of glutathione peroxidase (1.20±0.07 vs 0.99±0.08) and glutathione reductase (0.057±0.004 vs 0.043±0.002) was observed in the heat-stressed animals when compared to the control group. No differences on the detection (signal) and the quantification (band areas) of the antioxidant enzymes (Western blot) were observed between groups. Furthermore, our findings showed a high negative correlation between GPX enzymatic activity and the immunodetection of SOD (r=-0.34) and GR (r=-0.42), indicating that GPX enzymatic activity increases concomitant to a decrease in SOD and GR immunodetection. A high negative correlation between SOD enzymatic activity and GPX immunodetection (r=-0.38) was also observed. Based on these results, we can conclude that despite the increase in antioxidant enzymatic activity caused by the testicular insulation, no differences were observed in the immunodetection of these same enzymes. A hypothesis to explain such results is that the antioxidant enzymes GPX and GR are present in the epididymis in an inactivated state; in case of necessity (heat stress) the activation would occur. In this context, a number of studies indicate the significant contribution of the epididymis on the enzymatic antioxidant capacity of the ejaculate. In fact, in the epididymal environment such mechanism (protein activation) would be essential for the protection of the spermatozoa, already not capable of protein synthesis. Furthermore, the activation of bioavailable enzymes would be faster and consequently more effective than the synthesis of new proteins, avoiding the beginning of the oxidative chain reaction.

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Does seminal plasma addition reduce tyrosine phosphorylation in frozen-thawed swine semen?

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Cryopreservation induces cryocapacitation (1) which has as essential element an increase in tyrosine phosphorylation (2). However, addition of seminal plasma (SP) in equine frozen-thawed semen reduces the tyrosine phosphorylation (3). Thus, we tested the addition of 10% SP in thawed boar semen. Four sperm-rich fractions were collected from six boars (n=24). Samples were divided in three aliquots, namely not centrifuged (NC), centrifuged resuspended (CR) and centrifuged added SP (CP). After centrifugation (500x*g*/10min) of CR and CP, the supernatant was removed from CP, passed to another centrifugation (2500x*g*/30min), filtered through membranes with 0.22µm and stored at -80°C for future use. CR was suspended in its own SP just after centrifugation. All treatments were extended in freezing extender (Botupharma®, Botucatu-SP, Brazil) to obtain a final concentration of $300x10^6$sperm/mL. The extended semen was stored in 0.5mL straws. The straws were frozen in an automatic system (TK Tecnologia em Congelação®, Uberaba-MG, Brazil) using a rate of -0.5°C/min until 5°C, -20°C/min until -120°C and immersed in liquid nitrogen (-196°C). Two straws were thawed in a water-bath at 37°C/30sec and extended 1:1 (semen : extender) in freezing extender. CS added 10% SP (v:v) in freezing medium originated treatment CP. The samples remained in a water-bath at 37°C until 60 min, and were evaluated at 5 and 60min. An aliquot was extended in TALP to 5 x 10^6sperm/mL, dyed with 2µL of Hoechst 33342, after 10min of incubation at 37°C 0.75µL of anti-phosphothyrosine antibody conjugated with fluorescein and 3µL of propidium iodide (PI) were added. After 5 min of incubation at 37°C the samples were analyzed in flow cytometer (BD FACSAria-Becton Dickinson, San Jose, CA, USA). The analysis were based on fluorescent intensity (a.u.) of viable cells (PI negative) captured in detector long pass 502 and band pass 530±15 nm. The data were analyzed by SAS program (SAS Institute Inc., 2010) and subjected to analysis of mixed models. Treatments were evaluated using orthogonal contrast to analyze treatment effects; contrasts (centrifugation effect NC x CR; Seminal plasma effect CS x CP; Without seminal plasma effect CS x NC + CP). Centrifugation, addition or absence of SP have no effect in tyrosine phosphorylation of swine sperm ($p > 0.05$; 1253.81 ± 56.83 a.u.; 1297.6 ± 51.45 a.u.; 1320.73 ± 67 a.u.; 1303.28 ± 63.88 a.u., mean ± SE of NC, CR, CS and CP respectively). Therefore, seminal plasma added in frozen-thawed boar semen does not reduce cryocapacitation. (1- Bailey J, Biloudeau J, Cormier N, 2000, Journal of Andrology, 21, 1-7; 2- Arcelay et al, 2008, The International Journal of Developmental Biology, 52, 463-472; 3 - Andrade et al, 2012, Theriogenology, 77, 1866-1872).

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Methods of DNA extraction for sperm sexing in dogs

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Several techniques have been developed for extraction of DNA from a wide variety of cells. However, although similar, the methods routinely used for isolating DNA from somatic cells have been ineffective for the isolation from mammalian spermatozoa. Based on this, the present study aimed to compare the efficacy of three DNA extraction techniques for application in quantitative PCR (qPCR). For the standardization of the technique three protocols have been tested, including the described by Taylor (Taylor TM. Comparing calf sex ratio and semen sex ratio determined by conventional PCR. Dissertation of Master, Louisiana State University and Agricultural and Mechanical College, Program in Animal and Dairy Sciences, Southern Arkansas University, 2005. p. 40) with modifications, called M1, and two commercial protocols that use mini-extraction columns denominated M2 (Illustra blood genomic Prep Mini Spin Kit, GE HealthCare, UK) and M3 (Wizard ® Genomic DNA Purification Kit, Promega Biotechnology in Brazil Ltda, Brazil). Four sperm concentrations (1, 10, 30 and $50x10^6$ cells) from three mixed breed dogs, from kennel FMVZ, UNESP, Botucatu, were used. After the harvest, the cells were divided in three aliquots, each of which was directed to a protocol. Prior to extraction, cells were washed twice in PBS pH 7.2 to 2.000xg for 10 minutes in order to remove seminal plasma. Lysis buffer was added to each aliquot in the group, for M1 group the buffer contained 10mM Tris, 10mM EDTA, 10mM NaCl, 2% mercaptoethanol, 0.5% SDS, pH 8.0 adjusted with 1M NaOH; for groups M2 and M3 the buffers used were the ones provided by the kit. Then the samples were frozen at -80°C overnight, thawed and sonicated in an ice bath using a 3.0mm probe, in a 20% amplitude, for 30 seconds, repeated 10 times, with 1 minute intervals between series. In M1 protocol, samples were heated to 50°C in a water bath for 50 minutes. Then, 5µL of proteinase K at 20mg/mL gently homogenized and incubated for 14 to 18 hours at 50°C in a water bath were added. After incubation, 250µL of phenol:chloroform:isoamyl alcohol (25:24:1) was added. The samples were homogenized by inverting the tube and centrifuged at 15.000xg for 10 minutes. The lowest density phase containing the DNA was removed and transferred to a new tube. The process was once more performed. The samples placed in the new tube were washed in 1.0mL of cold absolute ethanol and gently stirred. The tubes were centrifuged at 15.000xg for 1 minute, the ethanol was removed and the tubes remained open to dryness. Each DNA was resuspended in 25µL of elution buffer (10mM Tris, 1mM EDTA, pH 7.4) and stored at -20ºC. The M2 and M3 protocols followed the manufacturer's recommendations. The M1 protocol regained the highest concentration of DNA after extraction in any of sperm concentrations used, with a satisfactory ratio (above 1.7) for the absorbances 260/280. On the other hand, the other methods tested recovered a very low concentration of DNA (0 to 35.9 ng/mL) of lower quality (ratio 260/280 < 1.7), regardless of the sperm concentration. The extraction of DNA from sperm cells of dogs using the protocol with chloroform:phenol (M1) was efficient and allowed the quantification of X and Y chromosomes in qPCR, enabling their subsequent use for semen sexing and other biotechnologies.

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Determination of malondialdehyde (MDA) by high-performance liquid chromatography in ram semen as a biomarker for oxidative stress

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In vitro manipulation of gametes exposes them to large amounts of reactive oxygen species (ROS) and consequent cellular oxidative stress. Oxidative damage to membrane lipids is an example of ROS-induced sperm injuries responsible for initiating the lipid peroxidation cascade that results in a reduction in sperm membrane fluidity and reduced fertilizing capacity. During lipid peroxidation, lipid hydroperoxides accumulate in the plasma membrane of sperm and subsequently decompose to form malonaldehyde (MDA). The latter is one of the best-known secondary products of lipid peroxidation and is used as an indicator of damage to sperm membrane. Thus, the aim of this study was to assess the occurrence of lipid peroxidation in the spermatozoa of rams using high performance liquid chromatography coupled with diode array detector (HPLC-DAD). Six sexually mature rams with fertility histories were used. Ejaculates were obtained using an artificial vagina, with use of a female in heat as a dummy, collected on alternating days, totaling 36 ejaculates per ram. Samples from all six rams per repetition (n = 6) were pooled to eliminate individual differences. Each pool was diluted (egg yolk-Tris + 5% glycerol; 200 x 10^6 sperm/mL) and frozen in an automated system (TK 3000). Aliquots of fresh semen, thawed semen (37 ° C/30s) and the extender were evaluated for lipid peroxidation by HPLC-DAD. In brief, an aliquot (200 µL) of semen or extender was added 750 µL H_3PO_4 (440 mM) and 50µL of TBA (40 mM). This mixture was heated for 1 h at 100 ° C. After that, at an aliquot (500 µL) was added to 500 µL of MeOH: 1 M NaOH (91:9). Then the samples were centrifuged (13,000 rpm for 5 min) and the supernatant was removed, filtered through a membrane (0.22 mM) and analyzed on a filter wavelength of 532 nm using HPLC apparatus (Prominence Shimadzu model LC -20AT). For chromatographic separations the Rexchorm ODS column was used (150 mm x 4.6 mm x 5 µm) at 30 ° C. The mobile phase consisted of potassium phosphate (50 mM, pH = 6.8) buffer in the ratio of 40:60. The results were analyzed by ANOVA and Tukey-Kramer test, with significance set at $P < 0.05$. The MDA concentration in cryopreserved semen (2.57 ± 0.7 nmol/mL) was significantly higher ($P > 0.05$) than in fresh semen (0.51 ± 0.2 nmol/mL). Although significant differences were observed between fresh and cryopreserved semen, this result may be related to the type of extender used and not to the occurrence of peroxidation in the sperm cell. This hypothesis is supported by the fact that the Tris-egg yolk extender without the addition of sperm had a higher concentration of MDA (6.49 ± 1.1 nmol/mL) than samples containing semen. Components of the extender may have effects on lipid peroxidation in cryopreserved spermatozoa. In this study, the egg-yolk extender used has a large amount of polyunsaturated fatty acids, which are susceptible to lipid peroxidation, which may have contributed to the increased levels of MDA. Based on these results, we conclude that HPLC-DAD is an appropriate method for measuring MDA concentrations for the assessment of lipid peroxidation in the spermatozoa of rams. Moreover, the composition of the diluent used for freezing semen may influence the results of lipid peroxidation analysis.

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Membrane fluidity and cell viability of fresh, cooled and frozen/thawed dog semen

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The desire to utilize better quality sire dogs is increasing among dog breeders but that particular sire is not always available or near at the needed time. In that manner, frozen semen is an interesting tool once the breeder can have access to it at any time. Cryopreserved spermatozoa have a short lifespan and sperm quality and cryoresistence is an individual characteristic among males. These factors lead to variable fertility rates. Thus understanding the cryoinjury process is very import to achieve better cryopreservation and pregnancy results. The use of fluorescent dyes such as merocyanine 540 (M540) can be used to monitor the level of disorder of the phospholipids of the plasma membrane lipid bilayer. Such disorder is indicative of membrane destabilization, similar to that occurring during the first steps of capacitation. Despite capacitation being a necessary event for fertilization it also diminishes the sperm cell lifespan. The combination of M540 with the membrane-impermeable DNA-binding probe Yo-Pro 1 permits simultaneous analyzes of sperm cell viability and membrane integrity. Nevertheless the use of flow cytometry permits the analyses of thousands of cells in a short time (A.K.Alhaider, 2009, A. Rep. Sci. 110, 147-161). The aim of this study was to analyze the effects of refrigeration and cryopreservation on dog sperm membrane fluidity and cell viability. A total of 15 ejaculates, 1st and 2st fractions, were collected from 5 dogs. Semen was diluted (80 x 10^6 sperm/ml) on Tris-egg-yolk medium with 8% of glycerol (one step), filled into 0.5 ml French straws and refrigerated at 5^0 C for 1 h. After this, straws were suspended 6 cm above liquid nitrogen for 20 min and plunged. At each stage (fresh semen, cooled semen and frozen/thawed semen) sperm total motility (TM), progressive motility (PM) and % of rapids (RAP) were accessed by CASA, the membrane fluidity (M540) and cell viability (viable cell- VC) by flow cytometry using Merocyanine 540 in combination with Yo-Pro-1. For flow cytometry 1 ml of diluted semen, Yo-Pro-1 was added to a final concentration of 25 nM and incubated in the dark for 15 min. at 39^0 C, after M 540- concentration of 1.4 μM, were added 2 min before analysis. Statistics were performed using Kolmogorov–Smirnov, variance and Dunn's tests. The results were as follows: Fresh semen: TM 85% ± 7,PM 67% ± 9, RAP 80% ± 9; M540+ 4.0 ± 1,3; VC 78.7 ± 13.6. Cooled semen: TM 86% ± 5, PM 68% ± 9, RAP 78% ± 8; M540+ 2.1 ± 2.3; VC 75.7 ± 10.0. Frozen/thawed semen: TM 72% ± 12, PM 55% ± 11, RAP 63% ± 13; M540+ 6.4 ± 2.6; VC 23.0 ± 4.7. There were significant ($P<0.0001$) differences on frozen-thawed semen compared to fresh and cooled semen showing the deleterious effects of cryopreservation to the sperm membrane. We conclude that despite acceptable post thaw motility, cryopreservation causes membrane damage to most of the sperm population and this would impair spermatozoa life span and could be one of the causes for low pregnancy results using frozen/thawed dog semen.

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In vivo induced oxidative stress is efficiently detected by the CellROX Deep Red® probe in ram sperm

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New methods to detect the reactive oxygen species (ROS) in sperm samples are needed. The fluorescent probe CellROX Deep Red® is not yet used to detect oxidative stress in spermatozoa. We know that the disruption of testicular thermoregulation results in oxidative stress and cellular apoptosis characterizing the testicular degeneration. This disturbance can be induced *in vivo* by scrotal insulation. This way, in this present study we investigated the effectiveness of this method in detecting ROS in sperm samples of rams submitted to testicular degeneration. For that, sixteen White Dorper rams were submitted to scrotal insulation during 72 hours. Semen was collected by artificial vagina two times before the insulation period and two times after that. The sperm concentration was determined and the semen was diluted in TALP sperm to the final concentration of $25x10^6$ spermatozoa/mL and final volume of 200 µL. 0.5 µL of CellROX® (1 mM) and 2 µL of Hoechst 33342 (2.5 mg/mL, Life Technologies) were added to the final solution. The semen sample was incubated at 37° C/30 minutes. After incubation, the sample was centrifuged at 2.000g/5 minutes, the supernatant was removed and the pellet was resuspended in 200 µL of TALP sperm. Cells were classified as sperm under mild or no oxidative stress (unstained midpiece), sperm under moderate oxidative stress (midpiece stained pale red), and sperm under intense oxidative stress (midpiece stained strong red). Data obtained from the periods, before (control) and after insulation (testicular degeneration), were evaluated by analysis of variance (ANOVA) and the means were compared using Fisher's LSD test. The induced testicular degeneration caused a reduction in sperm with mild or no oxidative stress (control = $84.10\pm2.94\%^b$; testicular degeneration = $73.75\pm3.56\%^a$; p = 0.0287), and an increase in sperm showing moderate (control = $15.68\pm2.92\%^b$; testicular degeneration = $25.43\pm3.41\%^a$; p = 0.0339) and intense (control = $0.20\pm0.13\%^b$; testicular degeneration = $0.81\pm0.24\%^a$; p = 0.0343) oxidative stress. According to the results, it is possible to conclude that the CellROX® Deep Red fluorescent probe is efficient for detecting the production of ROS in sperm of rams induced to testicular degeneration.

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Seminiferous tubule parameters after isotretinoin dose dependent treatment in young male Wistar rats

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Isotretinoin is chemically known as 13-cis-retinoic acid and is widely used in the treatment of persistent and nodular acne. The disease develops with exacerbation of sebaceous glandular activity. Treatment with isotretinoin acts reducing the activity of sebaceous glands and has been associated with epithelial differentiation. It is known as a potent teratogenic substance and the treatment in fertile individuals depends on many exams and medical assistance during the entire treatment period. The Brazilian public health system offers the treatment and its regulation occurred in 2002. The daily dose is calculated according to the patient's weight, and ranges from 0.5 to 2mg/kg/day for 16 to 35 weeks. Although unusual, recurrences are prevented by a cumulative dose between 100 and 150mg/kg. The most commonly recommended dose is 120mg/kg. Spermatogenesis is very complex and involves proliferation and spermatogonia differentiation, meiosis and spermiogenesis. Thus, any change in one or more stages may lead to damage to spermatogenesis or even infertility. Despite having been widely prescribed, the effects of isotretinoin in the male reproductive system are still under investigation. The aim of this study was to evaluate the possible effects of 13-cis-retinoic acid in different doses on testicular morphology of young male Wistar rats. Two doses were used, one of them the recommended for human use (1mg/kg) and another as a super dosage (10mg/kg). The substance was diluted in soybean oil and offered by gavage daily for 60 days. 30 wistar male rats were divided in 4 groups: C: control with water; D0: control with soybean oil; D1: 1mg/kg of isotretinoin; D10: 10mg/kg of isotretinoin. After the treatments the animals were euthanized by application of xylazine and ketamine. The testicular weight was measured immediately after euthanasia. Testicular fragments were collected and treated with Karnovsky fixative solution. The fragments were routinely processed for light microscopy. The samples were embedded in glycol methacrylate medium and 2µm thickness sections were stained with hematoxilin and eosin and used for histological evaluation. Morphometry was employed to analyze the organ and the following parameters were determined: seminiferous epithelial height and diameter of seminiferous tubules. To complement these data, a proportion was observed counting a grid intersection overlying the seminiferous tubule and intertubular space so that the total volume of these components could be determined. No significant differences could be found in any of the treated groups (D1 and D10) compared to the control groups (C and D0), considering testicular weight and the morphometrical parameters. The total volume of tubular and inter-tubular space did not show any statistical difference. These seminiferous epithelium parameters were not altered with the different doses of 13-cis-retinoic acid. These are preliminary results and further evaluations are in process, studying sperm morphology, dosage of serum stress oxidative enzymes and ultrastructural observations. These initial results show that the medicine is secure and that the general testicular morphology may not be altered by the treatment. (CEUA/Unicamp/ protocol #2831-1).

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Comparative study between hematocytometric method and the use of spectrophotometry for measuring sperm concentration of young Nelore bulls

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Sperm concentration is an important physical aspect, since it allows for the measurement of the number of viable spermatozoa in the ejaculate from young bulls, with the purpose of determining the sexual maturity, being thus important in artificial insemination laboratories for all domestic species. The sperm concentration determination by Neubauer chamber (hematocytometric method) is a direct method for counting cells, and it is also the most reliable. However, the process is time-consuming, which turns it into the least practical method when the amount of ejaculates to be processed is very large. The spectrophotometer is a device that measures the sperm concentration through optical density, and its main advantages are its practicality and its speed. The aim of this paper was to compare the results between the evaluators in the hematocytometric method and the spectrophotometer in measuring sperm concentration in young Nelore bulls. A total of 73 ejaculations from 20 young Nelore bulls were collected by means of electroejaculation. After andrological examination, 10µL of the semen were diluted in 2mL saline formaldehyde for measuring the sperm concentration per mL using the hematocytometric method (measured by three different evaluators) and using the spectrophotometer method with 550nm wavelength. No significant differences were detected from the measurement of sperm concentration per mL among the evaluators when compared to the use of spectrophotometer ($p>0.05$). The intraclass correlation was high (0.9), showing a high replicability among the evaluator measurements. With the results from the present experiment, it can be stated that the measurement performed by the spectrophotometer is reliable, and it can, in the future, substitute the hematocytometric method for performing the sperm concentration measurement in young Nelore bulls, improving and standardizing the techniques used in andrology laboratories.

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Expression of estrogen receptor 1 isoform (ESR1-36) in rat Sertoli cells

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It is well known that the estrogenic activities are mediated by both genomic and rapid (nongenomic) signaling. This rapid signaling, initiated near the plasma membrane, may indirectly influence gene expression through the activation of signal transduction pathways that finally act on target transcription factors. In fact, our laboratory has shown that activation of estrogen receptor ESR1 by 17β-estradiol (E2) in Sertoli cells from 15-day-old rats increases Cyclin D1 expression and cell proliferation, and this effect is dependent on ERK1/2 and AKT phosphorylation and NF-kB activation. Furthermore, E2 increases, in a PI3K and CREB-dependent manner, the expression of proteins involved with cell differentiation, such as $p27^{Kip1}$, and the transcription factors GATA-1 and DMRT1. G protein-coupled estrogen receptor (GPER) also mediates rapid E2 signaling in Sertoli cells through activation of EGFR/ESR1/2 and plays a role in the regulation of apoptosis. Recently it was identified and cloned, in a breast cancer cell line, a novel 36 kDa isoform of the full-length ESR1, designated ESR1-36 (Wang et al. 2005, Biochem. Biophys. Res. Commun. 336:1023). This receptor is primarily located in the cytoplasm and the plasma membrane, responds to membrane-initiated estrogen and anti-estrogen signaling pathways, its expression may be regulated by GPER, and it is involved in the resistance of breast cancer to endocrine therapy. The presence of ESR1-36 in Sertoli cells has not been reported yet. Thus, this study was performed to characterize the expression, cellular localization and regulation of ESR1-36 in rat Sertoli cells. Primary cell culture of Sertoli cells was obtained from 15-day-old Wistar rats. Conventional RT-PCR and Western blot and immunofluorescence assays were performed for detection of ESR1-36 (mRNA and protein). A transcript of the expected size (290 bp) was detected in Sertoli cells and its identity was confirmed by direct nucleotide sequencing. MCF-7 cells were used as positive control. Western blot and immunofluorescence assays were performed, using rabbit polyclonal antibody raised against a synthetic peptide antigen corresponding to the unique C-terminal 20 aa of hESR1-36. Specific band of 36 kDa (ESR1-36) was detected in Sertoli cells. ESR1-36 immunostaining was found in the cytoplasm and plasma membrane. Sertoli cells were also incubated in the absence and presence of ESR1-selective agonist PPT, ESR2-selective agonist DPN or GPER-selective agonist G-1 (10 nM), for 24 h, and the expression of ESR1-36 was evaluated by Western Blot. The treatment with G-1 and DPN increased the expression of ESR1-36 (2.4- and 2.3-fold, respectively). PPT did not change the expression of ESR1-36. In conclusion, the estrogen receptor isoform ESR1-36 is present in a primary culture of rat Sertoli cells. The activation of ESR2 or GPER induces ESR1-36 expression. The identification of this novel extranuclear isoform of ESR1, involved in the activation of rapid (nongenomic) estrogen signaling may help us understand the estrogen-mediated functions in Sertoli cells and in testicular cancer. This study was approved by the Ethics Committee from the Universidade Federal de São Paulo (#1884/2008).
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Relaxin affects cell organization and contact in a short term coculture of testicular cells

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The knockout of relaxin decreases sperm maturation, and we have previously found that relaxin and its receptor RXFP1 are expressed in rat Sertoli cells, relaxin stimulates Sertoli cell proliferation, and influences early and late stages of spermatogenesis in a coculture of rat testicular cells. Cell–cell interactions are important to allow the appropriate development and function of the testis. The aim of this study was to analyze whether relaxin affects testicular cell organization and expression of proteins important for cell junctions. Cells were dispersed from the testis of immature 7 day old rats, and cell organization was analyzed in 2D and 3D cultures. For 2D cultures, cells were plated on dishes covered with washed Matrigel, and cultured for 48 h in the absence or presence of 0.5% fetal bovine serum (FBS) or 100 ng/mL H2 relaxin (RLN). Cells were then kept for 24 h in supplement-free DMEM/F12 medium, and cultured for additional 2 or 5 days in the absence of supplements. Cell organization was analyzed after 2, 5 or 8 days of culturing (D2, D5 or D8) by optical microscopy. The myoid cell marker α-SMA and the cell junction protein β-catenin were measured by immunofluorescence with an Operetta High Content Screening System (Perkin Elmer). Relaxin knockdown was performed by transfection with relaxin siRNA (Qiagen) on D2, and the effects were analyzed on D5 and D8. For 3D cultures, dispersed cells were mixed with 2 times diluted Matrigel, and then cultured for 2, 5 or 8 days in the absence or presence of FBS or RLN. Cultures were analyzed for the presence of spheres or spicules in a Nikon microscope. In 2D cultures, RLN favored the organization of cells in tubule-like structures, increased the number of myoid cells, and favored their arrangement around the tubule-like structures. Relaxin also increased the expression of β-catenin in the cell membrane region. The knockdown of relaxin with siRNA disorganized the cultures, impaired the arrangement of myoid cells around the tubule-like structures, reduced the expression of β-catenin, and shifted its localization from the periphery to the nucleus. However, so far we failed to find evidence for tubulogenesis in 3D cultures. These findings suggest that RLN plays a role in spermatogenesis by favoring the arrangement and contact of testicular cells. This study was approved by the Ethics Committee from the Universidade Federal de São Paulo (CEP: 0937/10).

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Cryopreservation effects on the electrophoretic protein profile in Sanmartinero bull sperm membranes

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Previous researches conducted into CORPOICA showed that Colombian Creole breed bulls have greater in-vitro fertility. Studies showed 49.7% and 38.9% of sperm-oocytes penetration by Sanmartinero and Brahman respectively. In this regard, in the present study we have addressed the effects of cryopreservation on the expression of the sperm membrane protein in Sanmartinero bulls. In order to prove the change in the protein profile, we performed two dimensional electrophoresis in polyacrylamide gels, as a biotechnological tool. Seminal sample was obtained by electroejaculation from nine adult Sanmartienro bulls, at the Centro de Investigación CORPOICA-La libertad, they are in the same maintaining conditions. The motility and concentration were determined by computerized system spermatic analysis (CASA, Hamilton), and membrane integrity by fluorescent double staining with carboxyfluorescein diacetate (CFDA) and propidium iodide (PI). Each ejaculate was divided in two aliquots of both fresh and frozen-thawed sample. The frozen cycle was in commercial bioxcell two-step (IMV). Protein extract from fresh and frozen-thawed semen were performed by the method described by Martinez-Heredia, using N-octyl-L-glucoperanoside. Proteins extracted from fresh and frozen-thawed semen were subjected to the 2DPAGE (pH 3-10, 8-16% polyacrylamide linear gradient gel) enhancing sample solubilization with a sample buffer containing two chaotropic agents (Urea and Thiourea), two surfactants (CHAPS and Sulphobetaine 3-10), and Tributyl phosphine as a reducing agent. The gels were stained with Oriole (Bio-Rad), and scanned, and the relative protein content of the spots was determined. Statistical differences in protein spots were established by student t test, and correlations between concentrations of protein spots with semen quality variables by Pearson's test. As a result, the presence of 336 protein spots, which showed changes in their relative concentrations between samples (fresh and frozen-thawed) was evidenced, as well as the absence and presence of new protein spots. On protein spots that appear after freezing-thawing, we especially highlight the 72.2 kDa and isoelectric point of 6.84, with its possible involvement in protecting the membrane, acting as a chaperone protein. Furthermore, the protein spot of 45.15 kDa and isoelectric point of 4.45, had a significant increase in their relative concentration after freezing, a positive correlation with viability in fresh samples (0.44972, 0, 0408) and negatively correlated with motility (-.49608, 0.0117) in frozen-thawed samples, possibly relating it to maintain the vitality of the cell during the process. In conclusion, the cryopreservation provokes change in the membrane protein profile and can affect its function.

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Morphological characterization of the spermatids of *Pachycondyla marginata* (Hymenoptera: Formicidae)

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The sperm morphology of Insecta has been used for 35 years as a promising instrument for phylogenetic studies. Some insect orders, such as Diptera, have their sperm morphology well studied, while others, as the Hymenoptera, have little information considering the high number of species and behavioral diversity. Moreover, the information about the spermiogenesis in Formicidae is very scarce and absent in the genus *Pachycondyla*. Thus, this study aims to describe the spermatids morphology of *Pachycondyla marginata* to provide data to understand the gamete formation in Formicidae and its phylogeny. Adult males were collected in natural nests in the conservation unit "Mata de Santa Genebra" in Campinas (São Paulo, Brazil), and had their testes processed for conventional transmission electron microscopy. The spermatids are constituted by head, nucleus-flagellum transition and flagellum regions, a common feature in the insect spermatids. In the head region, a young acrosome is constituted by a dense acrosomal vesicle overlying a perforatorium which has a base that penetrates in the nuclear anterior portion. These acrosomal components have different densities and a slightly spherical format, in cross section, and tapered in the logitudinal section. In cross sections, the nucleus is spherical in its anterior end and oval from the middle portion. In longitudinal sections the nucleus is elongated and conical. The chromatin filaments vary from granulated to filamentous, due to compaction of these filaments, so that in the center nuclear the chromatin is more condensed, in cross-sections. In the transition nucleus-flagellum region, a massive centriolar adjunct arises laterally to nuclear distal portion as a compact, dense and oval mass in cross sections. Next, a dense centriole appears as a dense and starry mass, from which the flagellum originates. The centriolar adjunct ends when the two mitochondrial derivatives develop, marking the flagellum beginning. This last region is characterized by a 9+9+2 axoneme, which arises from the transition region together with the adjunct centriole, and two symmetrical mitochondrial derivatives that present variations of densities along its extension and begin at different amplitudes. Throughout the spermatic extension there are microtubules surrounding their structures from the acrosome to the flagellum as it is commonly described for many insect groups already studied. In addition, the cytoplasmic volume of these spermatids is relatively smaller than in previous stages and tends to decrease as the process progresses, which is now described as a standard for the respective process in Formicidae. Such general spermatids morphology is similar to that observed for the majority of the Formicidae studied so far, although certain morphological changes that occurred in spermiogenesis are specific to *P. marginata*, such as the density variations exhibited by the mitochondrial derivatives and the complex arrangement of the transition region nucleus-flagellum. Therefore, considering such unique and peculiar characters of *P. marginata*, and so species-specific, with modifications accompanying the evolution of the species, they can contribute to the construction of a relevant matrix of data for the understanding of their phylogenetic relationships with other species of the genus *Pachycondyla*.

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GFRA1 and DAZL proteins are expressed in the germ cell line in prepubertal canine testis

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The spermatogonial stem cells (SSCs) are situated between Sertoli cells on the basement membrane of seminiferous tubules. The developmental dynamics are partially controlled by factors in the stem cell niche, such as Glial-cell-derived neurotrophic factor family receptor alpha-1 (GFRA1) that is a co-receptor for Glial cell-derived neurotrophic factor (GDNF). The latter is a Sertoli cell-derived factor essential for SSC self-renewal and proliferation. In SSCs, deleted in azoospermia-like (DAZL) is localized mainly to the nucleus and relocates into the cytoplasm during meiosis. DAZL-positive germ cells vary in their stage of spermatogenesis depending on the species examined and its developmental stage. In mouse testes, DAZL is observed in type-B spermatogonia, preleptotene, and zygotene spermatocytes. SSCs remain poorly characterized because of lack of specific molecular markers that permit us to distinguish them from other germ cells. The aim of this study was to identify GFRA1 and DAZL as markers for spermatogonia in canine prepubertal testis using immunostaining and flow cytometry. SSCs were enriched from testis of 3 prepubertal dogs (3-6 months old) by using a nylon strainer, percoll density gradient centrifugation, followed by differential plating. The cells were then cultured in DMEM F12 supplemented with bFGF on canine embryonic fibroblast cell layer. Expression of GFRA1 and DAZL proteins were examined using flow cytometry with 1-2 x 10^5 cells at 0, 5 and 15 days of culture with feeder cells. We also used immunohistochemistry to locate GFRA1 positive cells in canine testis. Cells enriched from canine testis were able to form non adherent clusters on the feeder layer of canine embryonic fibroblastic cells showing a characteristic of SSCs. Under flow cytometry, approximately 42% of SSCs expressed GFRA1 protein on days 0 and 5 of culture, but decreased to 21% on day 15. The expression of DAZL was smaller on day 0 of culture (14%), increased on day 5 (44%) and decreased again on day 15 (33%). By immunohistochemistry, GFRA1 was expressed only in type A spermatogonia membrane, that were located in the basal seminiferous epithelium. In conclusion, percoll density gradient together with differential plating are able to enrich SSCs. Otherwise, the expression of DAZL increased after culture, showing a differentiation of spermatogonia.

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Evidence of Sertoli cell progenitors and spermatogonial stem cell elements by label retaining cell approach in *Cyprinus carpio*

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In teleosts, spermatogenesis shows a cystic arrangement, which is formed when a single, undifferentiated, type A spermatogonium (Aund) is surrounded by one or more Sertoli cells. An important aspect of fish spermatogenesis is Sertoli cell proliferation which is responsible for generating new spermatogenic cysts and support the development of existing cysts. This adaptation determines the efficiency and amount of sperm produced. Among the single type A spermatogonia are the spermatogonial stem cell (SSC) candidates, which are located in a specific microenvironment called niche. Although the conditions in the niche are important for the fate of SSCs, the molecular and cellular composition of the spermatogonial niche remains unclear in many fish species. In this study, we start characterizing the spermatogonial niche in carp, evaluating also *amh* expression in testes with high or low proliferative activity. Amh (anti-Müllerian hormone) is a Tgfβ growth factor, which inhibits spermatogonial differentiation in zebrafish and could be involved in germ cell differentiation in carp testis as well. 20 adult carp received pulses of BrdU (5-Bromo-2'-deoxyuridine), during 3 consecutive days (2 intraperitoneal injection/day), and testes were sampled after 4 h, and 1, 2 and 3 weeks after the last pulse. Type Aund were the only germ cells able to retain BrdU after a long period of chase (3 weeks). Interestingly, as in zebrafish, BrdU disappeared more quickly from type Aund ("active" stem cell) progenitors while in type Aund* ("reserve" stem cell), it remained stable. Type Aund* label-retaining cells were located preferentially near the interstitium, while Aund were found in the intertubular area. Some Sertoli cells were BrdU-positive after 3 weeks of pulse. The percentage of BrdU-positive Sertoli cells decreased slowly during the period of chase. Among the slow-dividing, BrdU-positive Sertoli cells, ~30-40% are "free" (not associated to germ cells), and ~60-70% associated with germ cells (90% associated with Aund*). This suggests the existence of Sertoli cell progenitors (stem cells) which are located in the spermatogonial niche in association with Aund*, being responsible for supporting the development of new cysts. Regarding *amh* gene expression, testes with high proliferation activity among spermatogonia and Sertoli cells have decreased expression of *amh*, indicating that lower Amh levels might create a permissive condition for cellular proliferation and differentiation in the niche. Here we described the niche, where Aund* (reserve stem cells) are located near the interstitium. Sertoli cell progenitors (stem cell candidates) are associated with these cells to support the development of new cysts. To assure that, we suggest that *amh* has to be down-regulated to allow both germ and Sertoli cell proliferation and differentiation.

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Exosomal subunits in the primordial germ cells of male *Lithobates catesbeianus*: an immunohistochemistry and immunofluorescence study

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Spermatogenesis and steroidogenesis change according to seasonality in anurans. During winter, the testes of bullfrogs (*Lithobates catesbeianus*) show large seminiferous lobules containing numerous primordial germ cells (PGCs) with strong testosterone cytoplasm immunoexpression. In addtion, the citoplasmic presence of androgen receptors and sex hormone-binding globulin suggests that testosterone is maintained in PGCs by these proteins during this quiescent period. On the other hand, during summer, the weak or absence testosterone immulabelling observed in the PGCs cytoplasm was coupled to strong estrogen receptors immunolabeling in these cells, suggesting that testosterone may be converted into estrogen from winter to summer. Taking into account that protein expression levels can be controlled by RNA levels, the changes in the expression of different proteins and hormones may be related to the processing and degradation of RNAs. In human cells one of the most active RNases is the Exosome. However, until now, there is no information about this complex in anurans. Exosome is 3' – 5' exoribonuclease complex involved in RNA processing and degradation of different kinds of RNAs. In eukaryotes, this complex is formed by 11 subunits, including RRP6, RRP40 and RRP46. Because spermatogenic process requires a precise and coordinated control of gene expression, a frequent gene expression and RNAs processing changes are necessary during spermatognesis of bullfrogs due to its seasonal reproduction. Eight adult male bullfrogs were collected during summer (SG) and winter (WG). Some testes were removed, fixed, and embedded in paraffin; others were frozen for protein extraction. The paraffin sections were submitted to immunohistochemistry and immunofluorescence for detection of RRP6, RRP40 and RRP46 subunits. The protein extracts were used for western blot. The morphological results indicated that the cytoplasmic or nuclear immunoexpression of RRP6, RRP40 and RRP46 exosome subunits in PGCs from SG was higher than in WG. Western blot analyzes indicated that the antibodies used had affinity to the anurans proteins, confirming the presence of the exosome subunit proteins in the testes, as well as the high intensity of immunoexpression in SG. The results indicate that exosomal subunits take part of the seasonal spermatogenesis of bullfrogs, mainly in the period of intense spermatogenic activity, in summer. The seasonal immunoexpression of these subunits in PGCs may be related to the processing and degradation of RNAs. A possible participation of these subunits in the control of PGCs gene expression needs to be further investigated.

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Assessment of the effects trypanosomiasis on reproduction of experimentally infected bovine

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Trypanosoma vivax, a protozoan flagellate parasite belonging to the Trypanosomatidae family, can affect animal health and productivity leading to reduced production and infertility in male cattle. The aim of this study was to assess laboratory abnormalities, Clinic, Andrology of bovine experimentally infected with *Trypanosoma vivax*. The experiment was carried out in the corral at the Center for Reproduction of the Veterinary Hospital of Uberaba. Four mongrel animals with reproductive age (18-24 months) were used.The animals were evaluated by clinical (heart rate, respiratory rate and temperature) and laboratory parameters. The animals were subsequently subcutaneously inoculated with 2 ml of blood containing *Trypanosoma vivax*. The evaluated andrology was initiated externally, with inspection and palpation of the foreskin, penis, scrotum, and testicle. The rectal palpation was performed, in which the ampoules were evaluated for the vas deferens, prostate and seminal vesicle diameter. And finally, semen of animals was collected through electroejaculation. Clinical observations occurred daily for the first 20 days and then every 15 days, for two months. We evaluated the appearance of semen, volume, total sperm motility and vigor, fortnightly for two months. All these parameters were performed in fresh semen. The statistical analysis for the morphological characteristics of sperm and semen quality were performed by analysis of variance (one-way ANOVA) with a significance level of $P < 0.05$. Laboratory tests of animals 1 and 2 were positive at different times. Animal 1 was positive in 15, 16, 17 and 20 dpi, with a maximum of 48 x 105 parasites / ml of blood, the peak occurring at 16 dpi. Animal 2 was positive in 16, 17 and D 60 dpi. Its peak occurred at 17 dpi with 2.88 x 105 parasites / ml of blood.The other two animals, 3 and 4, were positive during the entire study. During the daily evaluations and subsequent fortnightly reviews, no changes were observed in heart and respiratory rates of evaluated animals ($p > 0.05$). In laboratory tests, in the percentage of pathologies it is observed that throughout the period of biweekly reviews, animal reviews here showed high amounts of pathologies, both major and minor defects. This high pathology may be correlated with the pathogenesis of the parasite. Males affected by trypanosomiasis by *T. vivax* have several reproductive disorders in either natural or experimental infections. However, animals exhibited acceptable values for scrotal circumference throughout the study period. The values for vigor were acceptable in D +15 and D +75, at the end of the experiment, being respectively 4.5 and 4. Motility was above 70% in three moments: D +15, D +45 and D +75 respectively 85%, 82.5% and 80%. The results suggest that the physiological effect of the presence of the parasite during spermatogenesis could somehow cause changes in morphology.

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Mitochondrial status during sperm maturation in dogs

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During the sperm transit through the epididymis, morphological and functional changes in sperm cells provide a gain of motility and ability to recognize and fertilize the oocyte (JERVIS et al., 2001. Biology of Reproduction, v65, p696-703). Among such modifications in sperm maturation, the mitochondria play a key role in the generation of ATP (CUMMINS et al., 1994. Molecular Reproduction and Development, v37, n3, p345-362, 1994). However, the mitochondrial status of epididymal sperm remains unclear in the canine species, which is considered an important experimental model for wild animals and humans (JERVIS et al., 2001. Biology of Reproduction, v65, p696-703). Thus, the aim of this study was to verify the sperm mitochondrial and motility changes of canine sperm along the epididymal caput, corpus and cauda. Twenty one healthy mature dogs (aged from one to six years) of distinct breeds and body weights were used. Epididymides were collected after bilateral orchiectomy and maintained at 5°C for 24 hours prior to processing. Spermatozoa was collected through incisions (<1 mm) of the caput (CAP), corpus (COR) and cauda (CAU), aspirated separately and maintained in 300 µl of PBS extender. Sperm samples were evaluated for motility and vigor through Computer Assisted Sperm Analysis (CASA). Sperm mitochondrial activity was evaluated by oxidation of 3.3' diaminobenzidine (DAB); and mitochondrial potential was determined by flow cytometry with a specific probe (JC1). Data were compared by ANOVA, Tukey test and Pearson correlation ($p<0.05$). For the analysis of sperm motility and vigor, statistical difference was noted between CAU samples (69.7±4.0% and 2.6±0.1, respectively) and COR samples (27.7±3.0% and 2±0.1), which were both higher than in CAP (0±0% and 0±0). The same result pattern was verified for CASA variables (MOTILE, RAPID, VAP, VSL, straight trajectory and linear). Therefore, acquisition of sperm motility occurred during the transit from epididymal caput to corpus. The highest mitochondrial activity (DAB I) was observed in CAU samples (75.9±3.4%), superior to COR (46.8±2.5%), which were both higher than CAP samples (26±2.4%). These results show an increase in mitochondria activity during sperm maturation, reaching maximum activity in the cauda epididymis. Additionally, CAU samples (65±4.2%) presented higher mitochondrial potential than both COR (38.3±3.6%) and CAP (43.6±4.1%). Mitochondria maintain a latent state in the epididymal caput, starting its activity in the epididymal body segment (YUAN et al., 2013. PlosOne, v8, n10, p1-12). In fact, our DAB results confirm the initial acquisition of mitochondrial activity at the corpus stage. On the other hand, JC-1 results show minimum electrical potential in the caput and corpus epididymides. During early sperm maturation, mitochondrial potential is low, increasing in the cauda segment, where spermatozoa start mitochondrial activity (YU et al., 2002. Theriogenology, v57, n3, p1179-1190). Nevertheless, we observed a positive correlation between percentage of low mitochondrial activity (DAB IV) and static spermatozoa ($r=0.53$, $p=0.01$) in the caput. Moreover, a positive correlation was found in the cauda between sperm motility and DAB I ($r=0.45$, $p=0.05$). This suggests that the acquisition of motility is related to changes in mitochondrial status. Thus, sperm mitochondrial activity ensures necessary energy for epididymal spermatozoa to acquire motility in the corpus epididymides in dogs.

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Anti-müllerian hormone (*amh*) in *Cyprinus carpio:* cloning, localization and endocrine regulation

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In teleosts the role of Amh in spermatogonial differentiation remains unclear. The aim of this study was to characterize full-length *amh* cDNA in carp, evaluate its gene expression and localization in testes. To obtain a full-length carp *amh* cDNA sequence, primers were designed based on the zebrafish *amh* cDNA sequence. Reverse transcription-polymerase chain reaction (RT-PCR) was performed on carp testis cDNA. Agarose-gel electrophoresis yielded PCR products of the expected lengths that were gel extracted, cloned, and sequenced. Based on the partial carp *amh* cDNA sequence obtained, new primers were designed and successfully used in 5'- and 3'-RACE to clone the full-length carp *amh* cDNA sequence (with an open-reading frame of 1704 bp that codes for 567 amino acids) was obtained. Further comparative analysis was done with Amh precursor sequences of other species. The evolutionary analyses were conducted by a method determining phylogenetic distance. Evolutionary hypothesis for *amh* showed that carp is in the same clade as *Carassius auratus*, and both are related to zebrafish, all species belonging to the order of Cypriniformes. Furthermore, specific carp *amh* primers were designed to evaluate by RT-PCR and q-PCR (Real-Time quantitative PCR) *amh* expression in different organs in both sexes. *amh* was detected in gonads; showing higher expression in the testis than in ovary. *In situ* hybridization revealed that the Sertoli cells expressed the *amh* gene in carp testes. To understand the role of Amh in the endocrine regulation, testis cultures were performed testing the following treatments at 18 hours and 7 days: estradiol (doses: 10, 100 nM and 1 µM) and recombinant zebrafish (rzf) Fsh (doses: 50, 100, 500 and 100 ng/ml). Rzf Fsh and estradiol only exhibited an inhibitory effect after 7 days. Amh has an important role in the SSC niche by inhibiting spermatogonial proliferation and differentiation, as shown in Japanese eel and zebrafish. Thus, rzf Fsh and estradiol can influence *amh* expression in Sertoli cells, thus decreasing the Amh production, to create a permissive environment for spermatogonial proliferation.

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Phenotypic characterization of rat Sertoli cells located in the transition region between the seminiferous tubules and the *rete testis*

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The region that connects the seminiferous tubules to the *rete testis*, known as the transition region (TR) or transitional zone, has not yet been well evaluated, so its morphological characterization and functional importance in spermatogenesis and testis function still deserve further investigation. Moreover, the fact that the TR presents peculiar characteristics, such as the presence of "morphologically modified" Sertoli cells (SCs), motivated us to investigate if these cells are indeed phenotypically different (i.e. express different factors or molecules) from those SCs located in the seminiferous tubules. Therefore, in the present study we investigated the SCs proliferation and differentiation status in three different regions: i) along the seminiferous tubules; ii) in the TR; and iii) in the connection between the seminiferous tubules and the TR. For this purpose, testes from six pre-pubertal rats (36 days) were fixed in Bouin's solution, and the testis fragments were collected considering the *rete testis* as a reference point. Through immunohistochemistry, BrdU and Ki-67 were used as SCs proliferation markers, while GATA-4 and androgen receptor (AR) were utilized in order to evaluate the SCs differentiation status. Unlike what it is established in the literature, in which it is considered that SCs stop proliferating *in vivo* in rats at 21 days of age, we found SCs positive for BrdU and Ki-67 at 36 days of age. Regarding the SCs maturation status, nearly 10% of SCs located in the TR do not express GATA-4, and we noticed a trend for these negative SCs to be located downstream the TR (i.e. closer to the *rete*) ($p < 0.05$). Furthermore, 20% of the SCs in the TR do not express AR, and those that are positive present a weaker expression (signal) when compared to the SCs located along the seminiferous tubules and in the connection between seminiferous tubules and the TR ($p < 0.05$). These data suggest a distinct behavior/function of SCs located in the TR, leading us to hypothesize the presence of transiently amplifying SC in this region. Further studies are now being performed aiming to evaluate other parameters and cell types which may provide important information regarding the Sertoli cell physiology in this particular testicular area.

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Expression of PDILT in swine testis and epididymis

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The epididymis is an essential organ for male fertility because it is where the post-testicular sperm maturation occurs. Sperm maturation is mediated by protein secretion in the lumen of epididymal tubules, and the result of this process is the transformation of an almost inert cell in a motile gamete with fertilizing capacity. The proteins required for sperm epididymal maturation have to be functional, being in the correct folding. The quality control of the proteins is done by molecular chaperones, a class of enzymes responsible for folding the protein properly right after its synthesis and when it is denatured. The present study investigates PDILT, a protein disulfide isomerase family member, described as a testis specific PDI. Our recent findings showed that this chaperone is found in sperm from caput and corpus epididymis, but interestingly found in cauda sperm only from immunocastrated boars. The aim of this study was to identify the gene expression of PDILT in the testis and epididymal tissues of boars surgically castrated and immunocastred with commercial vaccine Vivax (Pfizer). Testis and epididymis of 4 boars were used in this experiment. Tissues were dissected and approximately 100 mg tissue (testis, epididymal tubules from caput, corpus and cauda regions) excised, washed in cold PBS, and after the maceration, preceded to the protocol of total RNA extraction with a commercial kit. For the cDNA synthesis (RT-PCR), we used 1.5 μg of total RNA, 200 U of M-MLV, 10 mM dNTP, 10 mM random primers. For PCR we used degenerate primers based on the sequences described for PDILT mRNA from mouse, rat, human and bovine, once the sequence for swine is not yet available. Beta-actin gene was used as housekeeping control. The expression of PDILT mRNA was found in testis (4/4), as described previously in the literature. Bands with the expected molecular mass (200 bp) were also found in caput (2/3), corpus (2/3) and cauda (2/3) epididymis. The amplicon bands were excised from the agarose gel to be sequenced, aiming to confirm if the PDILT cDNA was successfully amplified. Once the expression of PDILT in the epididymis is confirmed, one can consider the hypothesis that PDILT content in spermatozoa is obtained from epithelial secretion inside the epididymal tubules lumen. More studies are being performed to understand the role of PDILT in swine sperm physiology.

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A 2D SDS-PAGE study of seminal plasma of discarded boars

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The working life of males in a boar stud is influenced by different factors, but mainly by its semen productivity doses. When a boar presents a significant reduction in doses produced per ejaculate per month, the Stud considers discarding it. Despite the efforts made to guarantee a good nutrition, housing and management, some boars show a reduction in productivity of unknown cause. This situation takes to a higher need of discarding and acquisition of more boars to the herd, creating a management problem, with severe oscillations in total semen doses production and economic losses. The study of seminal plasma composition brought a new comprehension of its contribution to semen quality and fertilizing capacity. The proteomic approach showed the complex relations between proteins, epididymis, spermatozoa, the female tract and oocytes. Recently, the proteomic profile of boar seminal plasma was described by González-Cadavid et al, 2014, and the spermadhesins accounted for 45.2 ± 8 % of the total intensity of all protein spots. The spermadhesin protein family exhibits the greatest diversity of members and contains five closely related genes encoding spermadhesins AQN-1, AQN-3, AWN, PSP-I, PSP-II and their glycosylated isoforms. And this glycosylation difference is responsible for wide distribution of these proteins in 2D gels. Swine spermadhesins are synthesized by the epididymis and accessory glands of the male genital tract, and interestingly, AWN is also expressed in the porcine oviduct (Töpfer-Petersen et al. 2008). The aim of this work was to describe the 2D SDS-PAGE profile spermadhesins of seminal plasma from boars discarded from a boar stud farm. Ejaculates from 4 discarded males were used for this study. Decision making for boar discard was based on the history of the last 10 ejaculates, presenting 59.8 ± 22.8 of total motility, 2.7 ± 0.9 of vigor, and a mean of 46.2 of doses produced (max: 31 and minimum 0 doses per ejaculate). Seminal plasma was obtained by two step centrifugation: semen samples were centrifuged at 3.000 x g for 10 min, then the supernatant was recentrifuged at 12.000 x g for 1h at 4°C. Protease inhibitor was added to the supernatant and samples were stored at -80°C until analysis. Four hundred mg were used for 2D electrophoresis, using 7 cm 3-10 IPG strips. Isoelectrofocusing was performed using: PROTEAN® i12™ IEF Cell. After equilibration with DTT and iodoacetamide, strips where placed in 15% SDS-PAGE slab gels and ran at 90V. Gels were stained with colloidal Coomassie Blue R-250, and after destaining gels were scanned and protein spots analyzed. The cluster of low molecular weight and acidic spots was the most abundant of all proteins visualized. The more abundant proteins were in the region of between 10 to 15 kDa and p*I* from 3 to 5, consisting of 19.3 ± 4.6 protein spots, with a maximum spot number of 27 and minimum of 16. The quantification of the protein spots is being undertaken. Spermadhesins is an important protein family of the porcine seminal plasma. Understanding their role on sperm physiology might shed a light in some problems with boar fertility and cryopreservation.

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Rhamdia quelen vasa transcripts: molecular characterization, tissue distribution and developmental expression profiles

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The *vasa* gene codes for a DEAD box helicase, essential for germ cell development. This gene shows specific expression in primordial germ cells (PGCs), germline stem cells (male and female), and in early stages of spermatogenesis. Germ cell transplantation is a new and powerful technique, with which spermatogonial stem cells are transplanted into spermatogenesis-depleted testis or into the peritoneal cavity of embryos at the time that the endogenous PGCs migrate towards the gonadal anlagen. In the last approach, transplanted cells colonize recipient gonads producing functional gametes with the donor genotype. To evaluate *vasa* expression during embryonic development (0 to 264 hours post-fertilization or 11 days post-fertilization) of Brazilian catfish, we isolated a full-length *vasa* cDNA sequence, using primers based on the African catfish (*Clarias gariepinus) vasa* cDNA sequence. This was followed by 5'- and 3'-RACE, cloning and sequencing approaches, from which a full-length Brazilian catfish *vasa* cDNA sequence (with an open-reading frame of 2016 bp that codes for 671 amino acids) was obtained. The amino acid sequence was compared with Vasa protein sequences from other fishes, indicating that the protein belongs to the DEXDc superfamily. Evolutionary analyses were conducted by the method of phylogenetic distance, Neighbor joining. A consensus cladogram was generated without rooting. The obtained sequence was compared with *vasa* gene sequences of several fish species, showing that *R. quelen* is a sister group of *Silurus meridionalis* and *C. gariepinus*, belonging to the Siluriformes group, and distant from other fish species. This phylogenetic relationship indicates that species of the Siluriformes are more appropriate for transplantation because of their lower genetic distance. Semi-quantitative RT-PCR demonstrates *vasa* expression throughout the evaluated period. The high expression of *vasa* from 0 to 7 hpf is attributed to maternal inheritance, as shown in other species such as *Oncorhynchus mykiss*. However, qPCR analyses suggest that expression in the embryo begins at 216 hpf or 9 days pf. Furthermore, specific *R. quelen vasa* primers were designated to evaluate *vasa* expression by RT-PCR and qPCR in different organs in both sexes. Expression was detected in gonads, being stronger in ovary than in testis. Here we presented *R. quelen* as a potential model for spermatogonial stem cell transplantation using newly hatched embryos.

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Presence of PDILT and PDIA1 in swine epididymal fluid

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Sperm maturation in the epididymis is an obligatory process that spermatozoa must suffer in order to acquire the ability to fertilize the egg. During epididymal transit both morphological and biochemical changes occur in the sperm cell and these modifications are tightly controlled by each segment of the epididymal tubule. Protein secretions by the epididymal epithelia are responsible for spermatozoa maturation, which includes the addition and removal of proteins from sperm membrane. A growing body of evidence suggests that chaperones are of great importance to control protein quality in spermatogenesis, i.e. the right conformation and function for a given purpose. Members of the protein disulfide isomerase (PDI) family are associated to the quality control of important proteins like ADAM3, calmegin and calsperin, necessary for intra-uterine transport and sperm-egg fusion. However, little is known about the possible endocrine regulation of the expression of these chaperones in the epididymis. Therefore, the aim of this work was to quantify the chaperones PDIA1 and PDILT in the epididymal fluid of immunocastrated boars. Immunocastration in boars leads to a depletion of serum testosterone within 15 days, and disrupts spermatogenesis. Epididymides from 8 boars were used: 4 were surgically castrated (control group) and 4 (treatment group) were obtained after 60 days of immunocastration protocol (Vivax, Pfizer). Epididymides were dissected and epididymal fluid samples were taken from caput, corpus and cauda, and analyzed by SDS-PAGE and Western blotting using anti-PDILT and anti-PDIA1 antibodies. Immunodetection was quantified by optical density of the bands formed using ImageJ software, and data was analyzed by means of ANOVA and Tukey test. Our results show the presence of both PDI in the fluid caput and corpus, but not in cauda epididymis in both groups studied ($P < 0.05$). These are intriguing results since our previous data on PDI content in epididymal spermatozoa showed the same PDI occurrence for the control group, but not for immunocastrated samples. Vaccinated boars present significant amount of PDIA1 and PDIL in cauda epididymis spermatozoa, and this points to a possible endocrine regulation on PDI chaperone expression in the epididymis. Studies on the expression of PDILT in boar epididymis are being done to check the hypothesis of PDI secretion by the epididymal epithelia.

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Is the chaperone ERp57 (PDIA3) secreted in the porcine epididymal lumen?

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Chaperones are enzymes responsible for protein quality control inside and outside the cell. The protein disulfide isomerase (PDI) is a family of molecular chaperones that control cell folding and functionality through isomerization, reduction and oxidation of disulfide bonds on cysteine residues. PDIs, especially ERp57 (also known as PDIA3) act mostly inside the endoplasmic reticulum, folding the recently synthetized proteins. However, our recent results demonstrate the presence of ER in spermatozoa collected from the epididymis, and since this cell doesn't have an ER, we questioned if this ERp57 content came with the cell from the testis via afferent ductules or if was added to the spermatozoa during epididymal transit. To answer this question, we checked the epididymal fluid from caput, corpus and cauda for the presence of ERp57. Epididymides from six boars were used: four were surgically castrated (control group) and two (immunocastrated group) were obtained after 60 days of immunocastration protocol (Vivax, Pfizer). Epididymides were dissected and epididymal fluid samples were taken from caput, corpus and cauda, and analyzed by SDS-PAGE and Western blotting using anti-ERp57 antibody. Immunodetection was quantified by optical density of the bands using ImageJ software. We observed that fluid from caput and corpus epididymis had similar ERp57 content within groups, but between groups immunocastrated boars had lower ERp57 in both regions ($P < 0.01$). Interestingly, ERp57 was not found in the fluid from cauda epididymis from castrated boars, and some immunocontent was detected in samples from this region obtained from immunocastrated pigs, however with no difference between groups. These results agree with our findings for ERp57 in sperm from caput, corpus and cauda epididymis, suggesting a possible secretion by the epididymal epithelia and incorporation by the sperm cell. Also, the androgen disruption caused by immunocastration impairs the possible secretion of ERp57 in the proximal and medial epididymal segments, and might induce the expression in the caudal segment.

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Peccary testicular cytoarchitecture seems to be controled by the seminiferous tubule components

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The xenograft of isolated testicular cells was recently developed and this approach successfully recapitulates testis development under the back skin of immunodeficient mice. In particular, it allows handling different testicular cell types in order to investigate their interactions during testis organization. Furthermore, this technique is useful for the preservation of the genetic stock and the male reproductive potential of different mammalian species. Different from the observed for pigs, the collared peccary (*Tayassu tajacu*), a species (Suborder Suiformes) related to the domestic pig, presents a unique testis cytoarchitecture in which Leydig cells (LC) are located almost exclusively surrounding the seminiferous tubules (ST) lobes. This particular characteristic observed for peccaries could represent an important clue related to the mechanisms that regulate testis development in mammals. The aim of this study was to investigate the capacity of collared peccary and pig testicular cells suspensions to functionally interact and resume testis development after enzymatic dissociation. Testes from six 3mo old collared peccary (C) and six 30d old pigs (P) were enzymatically digested. The obtained cell suspensions from each species were mixed and pelleted as follows: pig Leydig cell + collared peccary seminiferous tubules (P-LC + C-ST); collared peccary Leydig cell + pig seminiferous tubules (C-LC + P-ST); and the controls (P or C). The pellets were grafted under the back skin of castrated CB-17/SCID mice and evaluated at 10-16d, and 1, 2 and 4-8mo after grafting. Although the mice seminal vesicle weight has decreased after castration, it reached the control's weight at 2mo. In general, testes-like structures formed from C-LC + P-ST were heavier at all time points evaluated. The histological evaluation of the 4 groups showed that at 10-16d after grafting no recognizable testicular structure was observed; however, a central *rete testis* was well developed as early as 1mo. Seminiferous cords and the tubular lumen were respectively observed at 1 and 2mo. At 4mo C-LC + P-ST derived testes showed LC between ST resembling the pig testis organization. Nevertheless, at 5mo, in the P-LC + C-ST tissue, LC was located only surrounding ST, and round spermatids were the most advanced germ cell observed in these ST. At 6mo, spermatogenesis was already established in P- LC + C-ST and also in the control P tissue. The peccary typical arrangement of the seminiferous tubules and LC observed at 5mo was maintained over the subsequent time points evaluated; however, at 8mo this feature was easily distinguishable between P-LC + C-ST and P tissue. These findings indicate that LC and ST from two different species were able to interact and form testis *de novo*. Also, based on the histological arrangement of the neo-formed testis, the signals that would be controlling testicular organization may arise from ST. Further studies are being developed in order to mechanistically evaluate the central roles of Sertoli and Leydig cells during pig and peccaries testis development. (CEUA/UFMG nº64/2013; CEEA/UFPI nº001/13; IBAMA/SISBIO nº 37988-1).

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Domestic cat testicular aromatase activity as assessed by the tritiated water-release assay

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Testicular or serum androgen:estrogen ratio deregulation has been related to abnormalities in the reproductive tract of many species. Lower testicular tetosterone:17β-estradiol (T:E2) ratio was found in teratospermic domestic cats (<40% morphologically normal sperm). The aim of this study was to assess the reliability of the tritiated water-release assay (TWRA) to measure aromatase activity in domestic cat testes. Testicular T and E2 concentrations, measured by enzyme immunoassay, and sperm morphology were evaluated to verify the relationship between them. Aromatase activity was measured in microsomal fraction and in homogenates of cat testes. Rat ovaries and piglet testes were used for assay validation. Aromatase activity was not detected in cat testes microsomal fraction (n=8), not even when the protein amount added to the assay was increased from 50 to 200 µg. In homogenates, however, it was detected (3.5 ± 0.5 pmol.g^{-1}.h^{-1}; n=7), although in such low levels that no activity inhibition was detect when homogenates were incubated with increasing fadrazole concentrations. Although none of the cats in this study were classified as teratospermic, some sperm defects correlated with testicular T:E2 ratio (abnormal acrosome, r=-0.76) and with E2 concentration (proximal cytoplasmic droplet, r=0.77). However, we did not find any correlation between aromatase activity and hormonal or sperm morphology data. To our knowledge this is the first demonstration of testicular aromatase activity in domestic cats. However, due to the low aromatase activity measured and the lack of correlation with other reproductive data, we could not infer that TWRA is a reliable method to detect differences in testicular aromatase activity in normospermic cats. Perhaps, in teratospermic individuals, with an increased aromatase activity this method could be used. As an alternative we suggest that more sensitive techniques should be used to compare aromatase activity between normospermic and teratospermic cats. This would allow a better understanding of the relationship between the level of aromatase activity, the testicular hormonal concentrations and the sperm abnormalities in domestic cats.

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Sertoli cell phenotype in adult bullfrog (*Lithobates catesbeianus*)

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The bullfrog (*Lithobates catesbeianus*) is a North American anuran introduced in Brazil due to its substantial economic importance, and also because it represents an excellent experimental amphibian model for biological investigations, including the aspects related to reproductive biology. Spermatogenesis is a process that occurs in the seminiferous epithelium under the regulation of several factors, mainly those produced by Sertoli cells, and it is one of the most efficient cell-producing systems in sexually mature animals. Hence, in this epithelium, somatic Sertoli cells and germ cells interact both physically and biochemically, creating an ideal microenvironment for germ cell development, survival, self-renewal and differentiation. Although there are some species-specific particularities, spermatogenesis seems to be fairly conserved among vertebrates. However, the knowledge of testis structure and function in amphibians is still scarce. In this regard, our main aims in the present study were to investigate the structure and some potential molecular markers for Sertoli cells in mature bullfrog testes. For this particular purpose, testes from eight mature bullfrogs were collected and routinely processed for histological, morphometric, ultra-structural and molecular analyses. The expression of Androgen Receptor (AR) and Gata 4 in Sertoli cells was investigated using antibodies raised against rat or fish proteins and the eventual presence of these proteins was validated using the Western blot (WB) technique. Morphometrical results indicate that the volume of Sertoli cell nuclei changes according to the developmental stage of spermatogenic cysts (spermatogonial, spermatocytes, and spermatids) in association with this somatic element, being significantly higher in spermatids cysts where, in comparison to the spermatogonial cysts, an increase of 100% was observed ($300\mu m^3$ to $600\mu m^3$, respectively). This data suggests that Sertoli cells are functionally more active during spermiogenesis. Supporting this finding, the phenotypic evaluation of this key somatic element showed that during germ cell development Sertoli cells surrounding early and late cysts presented respectively nuclei and cytoplasmic characteristics of immature and mature cells. Moreover, transmission electronic microscopy (TEM) investigations revealed the presence of cytoplasmic projections surrounding partially or totally each germ cell in the inner cyst structure. This Sertoli cell-germ cell interaction is probably important for the development of the different stages of spermatogenesis. In association with lanthanum, TEM was also used to investigate the establishment of the blood-testis barrier (i.e. Sertoli cell maturation). Corroborating data previously found for teleost fish, this ultra-structural evaluation demonstrated that in bullfrogs tight junctions are first established in spermatid cysts. Regarding the molecular markers, we found Sertoli cells positive for AR and Gata 4 (mature Sertoli cells). Other markers for immature and mature Sertoli Cell are being tested. These important results were confirmed by WB. In summary, we expect that the important results herein found will be useful for comparative reproductive biology studies, being also valuable for future investigations using bullfrogs as an experimental model.

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Spermatogenic efficiency in the spiny-rat, *Proechimys guyannensis* (Rodentia: Echimyidae)

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The *Proechimys guyannensis*, also known as "casiragua" in Brazil, is a spiny-rat species that lives in the Amazonian region. Although this species has received some attention regarding ecology and evolution, as a natural host for infectious parasites and as a model for resistance to epilepsy, to our knowledge its reproduction is still poorly investigated. Therefore, our aims were to study for the first time the key parameters related to testis function and spermatogenesis in this rodent species, which will allow the determination of the duration of spermatogenesis, Sertoli cell number and Sertoli cells and spermatogenic efficiencies. Ten sexually mature spiny-rats from the Federal University of São João del-Rei vivarium were utilized. Testis were fixed by immersion in buffered glutaraldehyde and bouin´s solution, and routinely processed for histological, stereological and immunostaining analyses. Intraperitoneal injections of 5-bromo-2'-deoxyuridine (BrDU; 150mg/kg) were performed to determine the duration of spermatogenesis. All data are presented as mean ± SEM. The body weight and the testis weight in this species were respectively 288 ± 11g and 1.63 ± 0.2g, resulting in a gonadosomatic index (GSI; total testis mass divided by body weight) of 1.15 ± 0.1%. In comparison to other mammalian species already investigated, this GSI is very high. Based on the development of the acrosomic system, twelve stages of the seminiferous epithelium cycle were characterized and only one stage was found per seminiferous tubule cross section. Stages VI and VII presented the highest frequencies (~16%), while stages II, III, IV and V showed the lowest frequencies (~3 to ~4%). The most advanced germ cell types labeled at 1 hour and 20 days after BrDU injection were respectively preleptotene/leptotene spermatocytes at stage VII and elongated spermatids at stage III. Based on the stages frequencies and the most advanced BrDU labeled germ cells, each spermatogenic cycle and the entire spermatogenic process lasted respectively 7.5 ± 0.01 and 33.6 ± 0.06 days, being one of the shortest durations found up to date. The seminiferous tubules volume density in the testis parenchyma was very high (96 ± 1%), whereas Leydig cells comprised only 1.5 ± 0.4%. Sertoli cell efficiency and number per gram of testis were respectively 7.9 ± 1 and 78 ± 11x 10^6. Compared to most mammalian species already investigated the daily sperm production per gram testis (spermatogenic efficiency) in the spiny-rat was quite high, reaching 78 ± 8 million. Taken together, our data show that the elevated spermatogenic efficiency observed in *Proechimys guyannensis* is a result of the combination of the very high seminiferous tubule volume density and Sertoli cells number per gram of testis, and shorter duration of spermatogenesis.

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Influence of year periods on testis morphometry, semen characteristics and seminal plasma proteins by SDS-PAGE in zebu and taurine

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Seminal plasma is a set of secretions from accessory male reproduction organs which influence spermatic functions. Climate changes that affect gametogenesis may produce low reproduction efficiency in bulls. Electrophoresis is a great help in the diagnosis of reproduction pathologies and in animal differentiation regarding fertility within the context of climate changes. Current research aims at studying the influence of the rainy and dry periods in testis morphometry, semen characteristics and seminal plasma proteins by SDS-PAGE electrophoresis in Nelore and Simmental bulls. Five Nelore and two Simmental bulls, at mean age of four years, were utilized. Semen collection was undertaken during the rainy season (spring and summer) with mean temperature of 29ºC and dry season (autumn and winter) with mean temperature of 19ºC at 15-day intervals. One hundred and fifty-four ejaculations were analyzed and scrotal and testis measurement was provided. Samples of semen plasma were centrifuged and conditioned at -196°C till electrophoresis processing. Proteins were extracted from 200 µL from each sample in an extraction buffer composed of 0.625 M Tris – HCl, pH 6.8; 2% SDS, 5% β – mercaptoethanol and 20% glycerol. The concentration of proteins was measured using a spectrophotometer PF-901 (Chemistry Analyser Labsystems). Gels were submitted to a photodocumentation system (Bio Doc-IT and Visidoc-IT Gel Documentation systems, UVP) and analysed by Doc-IT-LS 6.0 software. GLM from SAS, version 6, was used in order to evaluate possible variations of seminal variables and protein molecular mass. Sixty samples for were used electrophoresis. Statistical significance was accepted from $P<0.05$. The expression TV=0.0396 x (average testicle length) x (scrotal perimeter)^2 was used for testicle volume (TV), with TV = 460.14 cm^3 for the Simmental breed during the rainy period and TV = 571.26 cm^3 during the dry period. Nelore bulls showed TV = 524.75 cm^3 during the rainy period and 515.13 cm^3 during the dry period. TV increased in Simmental bulls during the dry period, whereas Nelore breed increased during the rainy one. There was an increase ($P< 0.05$) in major spermatic defects during the rainy period for both breeds. Major defects for Simmental (18.75±7.48% rainy period; 10.33±3.72% dry period) and Nelore breed (10.94±6.95% rainy period; 5.86±2.15% dry period). Furthermore, 30 kDa in the seminal plasma occurred in all samples for both breeds and periods under analysis. Band 179 kDa occurred in the dry period in 43% and 14% of samples from Nelore and Simmental breeds respectively. Molecular weights of identified bands in the gels varied between 4 and 205 kDa. Testicle length axis varied significantly between breeds in specific seasons. Variation may be due to adaptation and anatomic plasticity of the testicles within specific periods of the year under analysis. TV rise in Simmental bulls during the dry period may have contributed towards the decrease of major spermatic defects with an improvement of semen quality. Total spermatic defects during the rainy period for Simmental breed provided a fall in semen quality. Morphological abnormalities of spermatozoa are lower in the dry period. Period affects testicle morphometry and protein composition of the bulls´ seminal plasma. Protein band 179 kDa occurred in Nelore bulls during the two analyzed periods, a fact that has not been described in the literature. (CEUA/UNOESTE/Protocol#909).

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Comparative analysis of reproductive aspects in female *Tropidurus torquatus* (Tropiduridae) in different periods in the municipality of Cuiabá, Mato Grosso, Brazil

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Lizards of the genus *Tropidurus* are widely distributed in the South American continent, with a reproductive pattern described for some species that is strongly influenced by seasonality. However, the lack of information for most localities where this genus occurs hinders a better understanding of the association between reproductive activity and environmental factors. This study was undertaken to evaluate the reproductive parameters of a population of *Tropidurus torquatus* females from the municipally of Cuiabá in Mato Grosso state in different periods and related to environmental factors (precipitation and temperature), and further compare these parameters with those of other populations already studied. The specimens were collected monthly with a noose during two periods: November/2005 to July/2007 and June/2012 to May/2013 at the Federal University of Mato Grosso in the municipality of Cuiabá, Mato Grosso state. Data of snout-vent length (SVL), clutch size, minimal length (SVL) for sexual maturity, presence or absence of follicles > 3mm and eggs in the oviductal were recorded. Climatic data was obtained from INPE and CPTEC. The relationship between snout-vent length (SVL) and clutch size was analyzed by linear regression using the R program (2.13.1). In the period from 2005-2007 a total of 23 females was collected, with a mean snout-vent length was 85.75 mm (±9.99). Reproductive females occurred from November/2005 to March/2006 and October/2006 to February/2007, coinciding with the period of highest precipitation 200mm – 250mm. The smallest reproductive female presented a snout-vent length of 73.11 mm, and four eggs in the oviducts and four vitellogenic follicles were observed simultaneously, showing that more than one clutch was produced per breeding season. Clutch size ranged from 4-6 oviductal eggs (4.57 ±1.60). For the period between 2012-2013, 53 females were collected with a mean snout-vent length of 90.4mm (±11.01). The smallest reproductive female presented a snout-vent length of 69.68mm and contained two vitellogenic follicles > 3mm. Reproduction occurred from August/2012 to March/2013, a period when the average precipitation ranged from 0mm to 234.2mm, indicating that reproduction occurs at the end of the dry season and during the rainy season. Clutch size ranged from 4 to 6 eggs (5.15 ±2.05). Considering all reproductive females from both periods (N=45), a positive relationship between snout-vent length (SVL) and clutch size was observed (F=29, DF=43 and p=0.000002). In other populations of *T. torquatus*, seasonal reproduction with a higher concentration of reproductive activity during the rainy season was verified, such as in the congenders *T. oreadicus*, *T. itambere* and *T. hipidus*. Seasonal reproduction has been attributed as the reproductive pattern of the genus *Tropidurus* and may vary slightly due to local environmental conditions. The number of eggs in the population of *T. torquatus* in Cuiabá was similar to the average number of eggs found for the same species in the Cerrado of Central Brazil, which was 5.7 ±0.3, but higher than that observed for the population of sandbanks, which ranged from two to four eggs, and these were only found in females with snout-vent length above 40mm. In conclusion, *T. torquatus* females in Cuiabá, Mato Grosso state showed a seasonal reproductive pattern, occurring from the late dry season (August) to the rainy season (March) with evidence of more than one clutch per breeding season. Clutch size is positively correlated with snout-vent length, but minimal length for sexual maturity was found in individuals larger than 60mm. The temperature in this region did not vary enough to influence the reproduction of females, so we consider rainfall as an important factor in reproductive aspects.

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Seasonal reproductive pattern in bucks isolated or permanently stimulated by estrous goats

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Goats are seasonal breeders, with their maximum reproductive activity during autumn, when light time length is decreasing. Contact with estrous females induces an increase of LH and testosterone secretion and greater testicular size in rams. The aim of this study was to compare the seasonal reproductive pattern in bucks isolated or continuously stimulated by estrous females. We used 16 Gabon bucks (3 to 5 years old, 29.2 ± 1.5 kg; mean ± SEM) located in pens at 35° S, and separated into two homogeneous groups in age and body weight. While 9 bucks were housed in complete isolation from females (IF group), another 7 bucks were housed in a pen separated by a wire fence from 3 females (FC group), allowing visual, chemical and auditory contact. One of those does was induced weekly into estrus by hormonal treatment, staying in estrus for at least three consecutive days. From April 2012 to May 2013, scrotal circumference was measured weekly and blood and semen samples were collected every two weeks. Semen samples were obtained by electroejaculation, and testosterone serum concentration was measured by radioimmunoassay. Scrotal circumference, testosterone concentration and all the seminal characteristics were compared by ANOVA for repeated measures. Scrotal circumference was maximum from November to April (21.7 ± 0.05, P< 0.0001), and testosterone concentration from February to April (26.9 ± 1.7 nmol/L, P< 0.0001). Spermatozoa concentration reached the greatest values from October to November and in January (1299 ± 295 spermatozoa/mL and 1713.4 ± 304.4 spermatozoa/mL, respectively, P< 0.0001), mass motility in July and from January to May (3.1 ± 0.3 and 2.2 ± 0.3, respectively, P< 0.0001), and total motile spermatozoa from May to July and from October to April (545.3 ± 130.8 x 10^6 and 586.6 ±169.8 x 10^6, respectively, P< 0.0001). Percentage of abnormal spermatozoa had the lowest values in September, January, March and April (49.6 ± 4.9, 42.0 ± 3.6, 41.5 ± 4.0 and 45.5 ± 1.6, respectively, P< 0.0001). Scrotal circumference did not differ between IF and FC males. Testosterone concentration was greater in IF than in FC bucks (13.9 ± 1.0 nmol/L and 9.6 ± 0.9 nmol/L, respectively, P= 0.04). On the other hand, spermatozoa concentration and mass motility were greater in FC than in IF (1118.2 ± 377.6 x 10^6 spermatozoa/mL and 828.3 ± 295.9 x 10^6 spermatozoa/mL, respectively, P= 0.05; and 2.5 ± 0.6 and 1.6 ± 0.4, P= 0.05). Total motile spermatozoa tended to be great in FC than in IF (595.0 ± 246.0 x 10^6 and 368.9 ± 163.7 x 10^6, respectively, P= 0.1). Percentage of abnormal spermatozoa was not affected by treatment. In conclusion, general reproductive activity was greater from November to April. Permanent stimulus of estrous females did not modify the general seasonal reproductive pattern in bucks. Stimulation with estrous goats induced better seminal parameters. Therefore, considering that those males had lower testosterone concentration, stimulation with estrous goats induced a greater impact of testosterone on semen production.

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Reproductive parameters after puberty in lambs reared artificially or with their mothers

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It has been observed in rodents that the presence and the bond with the mother during the lactation period affects the reproductive development of the offspring, and has consequences on the sexual performance during adult life. Preliminary results from our group have shown that male lambs reared with their mothers have a faster reproductive development and display greater sexual behavior towards ewes in estrus than male lambs weaned 24-36 h after birth and artificially reared. The aim of this work was to compare rams' sexual behavior towards estrous ewes and reproductive parameters of those males after puberty. We used 27 Polwarth lambs that were born in September 2011: 14 that were separated from their mothers 24-36 h after birth and were artificially reared (AR) with sheep milk, and 13 that were maintained with their mothers until 2.5 mo old (MR). The experiment was carried out when the animals had 42 to 90 weeks of age. Animals were weighed, blood samples were collected weekly, and serum testosterone concentrations were analyzed by RIA. The scrotal circumference was measured every 7-15 days and immediately semen was collected by electroejaculation. The seminal parameters determined were: volume, mass motility, percentage of individual motile and of progressive spermatozoa, spermatozoa concentration the percentage of live spermatozoa, morphologically abnormal spermatozoa and spermatozoa with acrosome integrity, and the total number of spermatozoa in the ejaculate was calculated. Sexual behavior was evaluated every 2 weeks in individual pen tests with estrous ewes. The frequency of ano-genital sniffing (OAG), lateral approaches, flehmen, mounting attempts, mounts, and mounts with ejaculation (ME) were recorded during 20 min, and total mounts were calculated as: mounts + mounts with ejaculation. All the variables were compared by ANOVA for repeated measures, including the effect of the group (AR vs MR), time (week) and the interaction between them. Group did not affect body weight, scrotal circumference, serum testosterone concentrations, or any seminal parameter. In all these variables there was the effect of time ($p<0.0001$), and there was no interaction between group and time. MR males had greater frequency of OAG (MR: 5.5±0.4 vs AR: 4.2±0.4, respectively, $p=0.003$), and tended to show higher frequency of ME (MR: 1.68±0.13 vs AR: 1.32±0.12, respectively, $p=0.056$) than AR rams. There was a significant interaction between group and time in the frequency of ME ($p=0.002$): MR males had greater frequency of mounts with ejaculation on weeks 50, 52, 57 (spring 2012) and 76 (autumn 2013) than AR rams. There were no effects of group on the other sexual behaviors. The presence and bond with the mother during the lactation period of lambs generated long-term effects, which implied greater displays of some sexual behaviors even one year later.

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Dose dependent effect of cadmium on peroxidation and epididymis sperm profile

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The controversial effects of cadmium on reproduction are very worrying. Nowadays an important part of the population is chronically exposed to this metal, either by inhalation or food and water intake. In most cases its toxicity is related to oxidative stress induction, triggering lipid peroxidation. Considering that the sperm are rich in polyunsaturated fatty acids, this peroxidative action can lead to severe damage. Its accumulation in the testis also reduces sperm viability. Cadmium can interact with germ cell DNA, reducing its tight condensation, causing, for example, sperm morphologic alterations. It can also lead to cell loss, altering signalization pathways and disrupting the body's antioxidant defense. Taking into account all these compromising actions we considered it pertinent to evaluate the dose dependent effect of cadmium on epididymis relative weight, sperm count and transit time. Thus, 18 *Wistar* rats were distributed into 3 groups: CT (without treatment), Cd1 (1.2 mg/Kg $CdCl_2$) and Cd2 (1.9 mg/Kg $CdCl_2$). At an age of 80 days, the animals received an intraperitoneally injection of the metal. After a completion of the first round of spermatogenesis in adult life (136 days old) the rats were euthanized under xylazine and cetamine anesthesia (10 and 80mg/kg, respectively). The thoracic cavity was opened to collect blood in heparinized tubes by puncture of the left ventricle. The left epididymis was removed, weighted, and the sperm count of the caput/corpus and cauda was made, using a Neubauer chamber. Based on this count the transit time was calculated. In order to evaluate the peroxidation level, Malondialdehyde (MDA) was measured in the serum using TBARS Assay Kit from Cayman Chemical®. These procedures were approved by the ethics committee of the State University of Campinas (2900-1(A)). The statistical test considered was Kruskal-Wallis followed by the Dunn's post-test, with 5% significance. Cadmium did not alter epididymis weight. On the other hand, a significant increase in the MDA level was observed in group Cd2 when compared to Cd1. Corroborating this observation, it was possible to note a reduction in the sperm per portion of the epididymis and per gram of caput/corpus and cauda in Cd2 when compared to CT. The sperm number per cauda also showed differences between groups Cd1 and Cd2. Moreover, the transit time in caput/corpus and cauda was reduced in Cd2 when compared to CT and Cd1. The relation of cadmium induced peroxidation and sperm alterations could be registered in Cd2. This process can lead to an increase in reactive oxygen species which triggers epithelial disruption and alterations in sperm morphology and viability, considering that this cell has many polyunsaturated fatty acids. Modifications in epididymis morphology, including reduced epithelium height have already been described for cadmium intoxication, and are directly related to transit time acceleration. The first dosage of cadmium could not significantly alter the parameters analyzed; nevertheless the mean values of sperm count in Cd1 were at least 30% less than in CT. In conclusion we could verify the dose-dependent toxic effect of cadmium, demonstrating that the increase in dosage can rise lipid peroxidation as well as reduce sperm count in epididymis portions.

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Sodium arsenite effects on testicular parameters of Wistar rats

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Metals occur naturally in the earth's crust and can be found in air, soils and water in various concentrations, moving through biogeochemical pathways. However, due to their extensive use in industrial and commercial applications, these concentrations have been increasing in the environment and can be harmful to all kinds of life forms. Arsenic is considered one of the most dangerous metals and its effects on reproduction must be studied. This study investigated arsenic effects on testicular parameters of Wistar rats, in its chemical forms sodium arsenite (As^{+3}) and sodium arsenate (As^{+5}). Thirty Wistar rats were randomly divided into five experimental groups (n = 6 animals/group). The control group received saline (G1; NaCl0.9%), while the treated animals were orally exposed ad libitum to sodium arsenate (G2=0.01mg/L; G3=10mg/L) or sodium arsenite solution (G4=0.01mg/L; G5=10mg/L), daily for 56 days. The rats were euthanized (CEUA/UFV nº 19/2011) and their left testis was removed. Testicular fragments were immersed in Karnovsky's fixative solution and routinely processed for light microscopy. Digital images of the testicular parenchyma were taken and analyzed with the software Image-Pro Plus. Volumetric proportion of epithelium, tunica propria, lumen, seminiferous tubules and intertubular compartment were obtained using a grid with 266 intersections over 10 digital images of histological random fields per animal. Absolute volume of tubular and intertubular compartment and tubular somatic index were also calculated. Diameter of seminiferous tubules and luminal diameter were obtained from 20 circular tubules in each animal. In the same tubules, seminiferous epithelium height was also measured. The length of the seminiferous tubule was calculated using the volume formula for a cylinder, where the length is the volume divided by the area of the base. Thus, the total volume of seminiferous tubules divided by the average area corresponded to the total length of seminiferous tubules per testis, which can also be converted to meters per gram of testis. Results were submitted to ANOVA and Student Newman-Keuls tests and considered significant for $P < 0.05$. Animals treated with sodium arsenite at 0.01mg/L (2.40±0.07) and 10mg/L (2.67±0.22) showed an increase in the percentage of tunica propria when compared to the control animals (1.75±0.12). Significant decrease in epithelium height was observed in animals treated with sodium arsenite at 10mg/l (65.81±3.46 µm) when compared to control animals (76.26±2.67µm). The results obtained for the other parameters did not present differences among treatments ($P > 0.05$). Tunica propria thickness may be a physiological protective response of the tissue due to the presence of a toxic element causing a change in the proportion of collagen fibres and contractile myofibroblastic cells, which may prevent the appropriate release of spermatozoa from the germinal epithelium into the lumen. Futhermore, quantitative parameters related to the seminiferous tubules, such as epithelium height, have a relationship with spermatogenic activity, and the alteration observed in epithelium height may be related to low sperm production. In addition, there were no significant differences among the animals treated with sodium arsenate ($P > 0.05$) when compared to the control and sodium arsenite group. Thus, it can be inferred that ingestion of sodium arsenite at the concentrations tested can alter some testicular morphometric parameters.

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The environmental enrichment using coffee husk does not influence the acrosome integrity and osmotic resistance of boar cooled semen

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The development of methods to improve the ambience of confined animals has been recommended to refine the welfare, improving not only the life quality but also the reproductive performance. In coffee husk, substances such as caffeine and chlorogenic acid can interact with the semen, improving its quality. The aim was to evaluate the effect of coffee husk used as environmental enrichment on the semen quality represented by osmotic resistance and acrosome integrity of boar semen stored until 96 hours. The experiment was conducted on a commercial farm ("Fazenda São Paulo") located in Oliveira-MG, Brazil. Sixteen boars were housed in individual stalls and divided into two groups (coffe husk and control groups). The control group had eight boars kept in conventional system with concrete floor, while the coffee husk group had eight boars in the same conditions but with coffee husk covering the floor in 3.0 to 4.0 cm. The experimental design was a randomized block with two treatments and eight repetitions. On days 0 and 60 of the experiment, semen was collected, diluted at 3 x 109 spermatozoa in doses of 100 ml and cooled at 17 ºC for 96 hours. At the beginning and after 240 minutes aliquots of 10 mL of semen were incubated in test tubes in a water bath at 37 °C for 240 minutes. At the beginning and after 240 minutes of incubation, the acrosome integrity was evaluated by POPE and the osmotic resistance by the hypoosmotic test. The data were submitted to the analysis of variance and Scott-Knott test at 5%. There was no difference ($P>0.05$) between the treatments, regardless of the storage time. It was concluded that environmental enrichment using coffee husk did not influence the resistance to osmosis and acrosome integrity of boar semen cooled and stored for 96 hours.

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Characteristics of fresh semen of breeding pigs submitted to environmental enrichment using coffee husk

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In modern systems of animal production, pigs are often confined within simple housing systems that offer little potential to accommodate their highly motivated species-specific behaviors. The stress generated by these conditions can decrease the reproductive performance of the animals. Environmental enrichment is the modification of the environment to improve the housing conditions for animals, contributing to their welfare and reducing the stress. In addition, coffee husk contains coffee compounds, caffeine and chlorogenic acid, which can modulate the production and sperm maturation, improving semen quality. Thus, the aim was to investigate the influence of coffee husk as an environmental enrichment on the quality of boar semen used for artificial insemination. The experiment was conducted on a commercial farm (*Fazenda São Paulo*) in Oliveira-MG, Brazil. Sixteen boars were housed in individual stalls and divided into two groups (coffee husk and control groups). The control group had eight boars kept in the conventional system of concrete floor, while the coffee husk group had eight boars kept in a concrete floor covered with coffee husk (3.0 to 4.0 cm). The experimental design was a randomized block, in which the experimental plot was represented by the animal, with split plot in time (before and after implementation of the coverage). On days 0 and 60 of the experiment, semen was collected and forwarded to the reproduction laboratory at the farm, where the initial assessments of volume and concentration, sperm motility and intensity of movements were performed, since these are the parameters evaluated in the routine of the farm. Seminal plasma samples were collected for the purpose of dosing the caffeine and chlorogenic acid in this material, analyzing if the voluntary intake of the husk could alter the composition of this secretion. The data were subjected to analysis of variance and Scott-Knott test at 5%. The seminal plasma of animals kept in pens containing coffee husk as floor covering showed 2.545 ± 0.868 mg of caffeine/100 mL of semen without significant amounts of chlorogenic acid. None of these substances were found in the seminal plasma of boars from the control group. The use of coffee husk as a floor covering did not influence ($P>0.05$) the volume, concentration of ejaculate and intensity of movements, but negatively influenced ($P<0.01$) the motility of semen. Although the addition of caffeine in seminal storage media has been linked to higher sperm motility, the present study found a reduction of that characteristic in the in fresh semen when animals were kept on the coffee husk. This result may be attributed to the presence of the caffeine in these animals' ejaculate. During ejaculation and seminal activation, there may have been an exhaustion of energy reserves contained in the semen, due to a higher rate of glycolysis induced by caffeine. It was concluded that the use of coffee husk as covering of breeding pigs' pens impairs sperm motility of boar semen *in natura*, but does not influence the volume, concentration of ejaculate and intensity of movements. Regarding the presence of caffeine in the seminal plasma of breeding housed in pens containing coffee husk, we conclude that the intake of this product changes the composition of this fluid.

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Uterine inflammatory reaction caused by artificial insemination results in decreased serum concentrations of negative acute-phase protein

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An acute inflammatory response is characterized by the production of differentiated acute phase proteins (APP). In an inflammatory process, as there is an increase or decrease of the APPs, these are considered positive or negative, respectively. Among the negative APPs, the albumin can also be cited. This study aimed to verify if the inflammatory reaction that occurs after semen deposition in the female cattle reproductive tract may be reflected on changes in the APPs concentrations. 20 Nellore calved cows (between 50 and 90 days postpartum) were selected. Females were previously examined for reproductive tract soundness and health status, and body condition score. Animals were homogeneously divided into two groups: Inseminated Group (AIG, n = 10) and non-inseminated group (CG, n = 10). All the animals underwent the same estrus synchronization and ovulation protocol, but only the AIG had semen deposited in the body of the uterus. All the animals had blood samples collected at four moments : 30 hours before IA (immediately before ovulation-inducing hormone administration), 4, 24 and 48 hours after AI. Albumin was measured in the blood serum of animals by the biochemical analyzer (RX Daytona) using specific kits (Randox ®). Data were analyzed by SAS PROC GLM (SAS, version 9.2, 2010) to verify the residual normality and variance homogeneity. Afterwards, data were analyzed by PROC MIXED using a linear mixed model for repeated measures. Statistical differences were considered when $P<0.05$. No group effect was observed at any moment ($P>0.05$). A time effect for GC ($P>0.05$) was not observed, in other words, albumin concentration was similar between the times 30 hours before AI (3.366±0.062), 4 (3.344±0.066), 24 (3.188±0.114) and 48 (3.155±0.072) hours after AI. For AIG a significant decrease ($P<0.0001$) in albumin concentrations 24 (3.044±0.0603) and 48 (3.044±0.0689) hours after AI was noticed, compared to those observed 30 hours before AI (3.466±0.068) and 4 hours after AI (3.466±0.068). Since albumin is a negative acute phase protein, the sharp drop in albumin concentrations that occurred in AIG 24 hours after AI may be due to inflammatory reaction caused by AI. It can be concluded that the uterine inflammatory reaction has a systemic reflection and it is evidenced by the decrease of albumin concentrations in blood serum.

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Methodology for the measurement of the endometrial thickness and uterine diameter in female cattle

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In all eutherian species, an ideal uterine environment is essential to prepare the uterus for embryonic implantation and growth. Thus, some studies in cattle have shown the endometrial thickness as an alternative to determine the optimal time of insemination (1). Other studies claim the diameter of the uterine horns as an alternative to evaluate the development of heifers as a predictor of fertility in heifers (2). However, they do not detail the methodology used. The aim of this work was to evaluate different regions of sexually mature female bovine uterus through transrectal ultrasound exams. Five Holstein females destined for slaughter were used. Approximately 12 hours before slaughter, examination was performed through transrectal ultrasound to measure endometrial thickness and diameter of the uterine horns in different regions. The uterine horns were divided into three regions, modifying the segments claimed by Ginther (1998), the first region considered the internal bifurcation of the uterine horns until the beginning of the greater curvature, the second one was from that last point, until the beginning of the horn tip, and finally the horn tip was considered as the third region. Right after slaughter reproductive organs were collected and endometrial thickness and diameter of the uterus in these same regions were evaluated. Analysis of variance and comparison of means by F or Wilcoxons test were performed to evaluate the measurements pre and post slaughter, as well as different regions of the horns. The comparison of measurements made to before and after slaughter by ultrasonography and measurement with calipers in the endometrial layer in parts post-mortem showed no differences in any of the regions of the female reproductive organ. This indicates that the measurement by ultrasonographic endometrial thickness and uterine diameter is reliable when pictures are done in transverse sections from different regions of the uterus *in vivo* animal. However, *in vivo*, there was no difference in uterine diameter between regions two and three ($P < 0.05$), and in region one the values were intermediate to two and three ($P < 0.05$). There was no difference in endometrial thickness in different regions of the uterine horns ($P < 0.05$). Therefore, the transverse ultrasound images of the uterus in region one (uterine bifurcation) was efficient to measure endometrial thickness and uterine diameter of the uterus horn in female Holstein cows.

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Morphofunctional remodeling and DNA damage in rat testes under single exposure to cadmium: individuality at pathological response levels

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It has been known for decades that cadmium (Cd) must enter the cell to cause damage, but the response pattern and pathway that cause damage at low doses in the male reproductive system remain unknown or are sometimes controversial. Herein, we have explored the application of different doses of Cd and analyzed the response pattern in long term and short term after single Cd exposure as a novel system to solve the controversial outcomes in some experiments that use the same doses under the same conditions. Adult male Wistar rats were randomized into 5 groups with 12 animals in each. The control group was injected with saline solution (NaCl 0.9% IP) and the four other groups received a single IP dose of 0.67; 0.74; 0.86 and 1.1 mg of Cd per kg body weight. Testes from six animals per group were collected and weighted 7 days after the Cd exposition (acute) and the six animals per group remained 56 days after the exposition (chronic). Fragments were fixed in Karnovsky for histological analysis and processed in resin or paraffin, and stained with toluidine blue, Von Kossa method (for calcification analysis), Picro Sirius method to identify collagen fibers and immunohistochemistry TUNEL to identify apoptotic cells. Random fields were photographed for analysis and quantification of testicular changes. Fresh fragments from animals euthanized seven days after exposition were used for DNA extraction and electrophoresis to determine DNA integrity. Severe injuries showing high rate of apoptosis and reduced gonado-somatic index (GSI) were shown in 0, 2, 3 and 3 animals respectively in the acute groups with doses of 0.67; 0.74; 0.86 and 1.1 mg of Cd per kg body weight. Severe injuries such as calcification, high rate of fibrosis and reduction of GSI were observed at 1, 2, 3 and 4 animals, respectively in doses of 0.67; 0.74; 0.86 and 1.1 mg of Cd per kg body weight of chronic groups. The remaining animals in each group had all outcomes similar to the control group. By analyzing the DNA integrity it was observed that the animals with tissue remodeling were the same that had DNA damage, regardless of which dose they were exposed to. Thus we conclude that a very small difference in the dose of Cd causes an increase in the number of animals with tissue remodeling. There is an individuality of response, some animals remaining with normal morphology in the highest dose. The exact number of animals showing severe damage was not constant from one experiment to another. This can be the cause of controversial results in experiments using low doses of Cd, because the individuality in animal response increases the biological variation.

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Ultrastructure of epididymis caput and cauda epithelium of aged rats

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Although many studies have described the histology of young and adult epididymis in both humans and experimental animals (rats, humans, monkeys), very few have focused on how the epididymis is affected during aging (Serre V et al. 1998. Biol Reprod 58:497-513). The most frequently employed animal models for studying basic mechanisms of aging are rodents (Roth GS et al. 1993. J Gerontol 48:213-230), especially the Brown Norway rat. In this study we described the ultrastructure of epididymis caput and cauda epithelium in male Wistar rats at 18 months of age. Eight animals (18 months old) were studied. The animals submitted to Ketamine (80 mg⁄BW) and Xylazine (5 mg⁄BW) anesthesia were fixed by whole body perfusion. Briefly, after a saline wash to clear the vascular bed of the testis, they were perfused with glutaraldehyde 5% in sodium caccodylate buffer 0.105 M (pH 7.2) for 20 min. The epididymes were removed and post fixed in the same solution overnight. The tissues were postfixed with 1% osmium tetroxide in the same buffer at 4°C, dehydrated in acetone, and embedded in epoxy resin. Ultrathin sections of caput and cauda were cut with a diamond knife and stained with 2% uranyl acetate and 2% lead citrate prior to observation with a transmission electron microscope (Zeiss, Leo 906). Caput epididymis. The basal cells are flat and elongated. They had an elongated nucleus enclosed by a small amount of cytoplasm with a few organelles. The halo cells had large nuclei and pale cytoplasm. These cells were filled with lysossomes which content was heterogeneous, as well as lipid droplets. These cells are located at the base of the epithelium or more apically. The clear cells had an irregular nucleus and the apical area was rich in vesicles of different sizes and multi-vesicular bodies. A large amount of heterogeneous lysossomes were found in the median region and lipid droplets are observed in the basal region. The principal cells have a single irregular nucleus, numerous cisternae of rough endoplasmic reticulum, a very prominent Golgi apparatus and a free surface bearing long stereocilia. Numerous coated vesicles and multivesicular bodies were seen in the apical cytoplasm. Large irregular dense bodies varying in size were most frequently situated in the supranuclear region. They contained a very heterogenous material characteristic of lipofucsin pigment. Myelin figures and irregular membrane arrays were frequent. These cells also had lipid droplets in the perinuclear region and electron-lucent vacuoles. Many lysosomes have round, parallel membranous electron-dense profiles. There were a great number of lipofuscin deposits. Cauda epididymis. The basal and halo cells were similar to the caput. The principal cells showed the same morphology of caput except for the reduced number of large irregular dense bodies and the presence of mitochondria with irregular cristae. Some principal cells had large vacuoles containing a pale-staining homogeneous material occupying a large area of the basal cytoplasm. The clear cells are larger and had a infranuclear region rich in lipid droplets while the supranuclear region comprised a few lysossomes of moderate and high density, as well as endosomes. The results presented above show that the ultrastructure of Wistar rat epididymis epithelium is drastically transformed by aging. The morphological alterations were characteristics of the aging process and were observed in other species such as brown Norway rats (Serre V et al. 1998. Biol Reprod 58:497-513), in hamsters (Calvo A et al. 1999. Anat Rec 256:335–346) and rabbits (Cran D G et al. 1980. Exp Gerontol 15: 93–101). (CEUA/UNICAMP/ Protocol 2177-1)

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Effects of chronic exposure to Propiconazole in sexual behavior of F1 generation in female rats

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Propiconazole (Prop) is a fungicide largely used in agriculture. *In vitro* studies showed that Prop has an antiestrogenic effect by inhibiting the activity of CYP 19 (aromatase), which converts androgens into estrogens. These hormones are important for hypothalamic sexual differentiation during the perinatal period, and for development and maintenance of the reproductive system function. The aim of this study was to evaluate the effects of chronic exposure to Prop in sexual behavior in female rats. Female and male Wistar rats, parenteral generation, were treated daily by gavage with Prop 4mg/Kg/day or Prop 20mg/Kg/day or corn oil (control group). Each female was mated with a male from the same dosage group (n=12-13/group), and the treatment was continued throughout the mating, gestation and lactation periods. The female pups (F1 generation) were treated with the same dosage (n=12-13/group) from postnatal day 22 to 90. At the end of treatment, the sexual behavior was evaluated. The lordosis quotient (LQ: number of lordosis/ten mounts x 100) was calculated and the magnitude of the lordosis reflex was observed. LQ: median (1st -3rd quartile) by Kruskal-Wallis and lordosis reflex magnitude by Fisher's exact test ($p<0.05$). It was observed an increased in lordosis reflex magnitude 2 (C: 6,15% $[8/130]^{A}$/ Prop 4: 21,66% $[26/120]^{B}$, / Prop 20: 23,07% $[30/130]^{B}$) and a decreased in lordosis reflex magnitude 3 (C: 83,84% $[109/130]^{A}$/ Prop 4: 63,33% $[76/120]^{B}$/ Prop 20: 69,23% $[90/130]^{B}$) in both Prop groups. It is well established that the female receptive behavior (lordosis) is dependent on both estrogen and progesterone levels. The results of this study suggest that Prop decreased estrogen levels, since a decrease in lordosis reflex magnitude 3 was observed. However, additional studies and plasma hormone level measurements are necessary to elucidate the results.

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Estradiol and progesterone serum levels during the estrous cycle in Pelibuey ewes under Colombian tropical conditions

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The environmental conditions of the tropics can have an effect on ovine reproduction. Therefore, it is necessary to establish physiological reference values of clinical and productive importance for sheep living under these conditions. The aim of this study was to evaluate the concentrations of Estradiol (E2) and Progesterone (P4) in Pelibuey ewes during the estrous cycle (EC). Twenty-two ewes located under tropical conditions were synchronized with two doses of Prostaglandin F2 alpha (PGF2α). Ewes were identified in estrus by epididectomized rams and blood samples taken on that same day (day 0). A sample was taken every three days (days 0, 3, 6, 9, 12 and 15) during two consecutive EC. The samples were centrifuged and the serum was stored at -20°C to measure the hormonal concentrations by competitive chemiluminescent enzyme immunoassay. The data was analyzed with ANOVA to determine the differences between the EC. Due to no statistical differences found between the ECs, the gathered data was presented as media ± SEM of both EC. For day zero E2 concentrations were 73.08±11.94 pg/ml and P4 were 0.04 ±0.02 ng/ml. In the early luteal stage a P4 increase was detected, with 0.72±0.13 and 1.96±0.18 ng/ml on days 3 and 6. In the late luteal stage the P4 was 3.26±0.30 and 3.56±0.35 ng/ml on days 9 and 12. On day 15 the P4 concentrations declined to 0.78±0.18 ng/ml. The E2 level was as expected, with transitory elevations that might be related with the follicular waves. The levels were 7.16±1.80 pg/ml on day 3, 5.15±1.70 pg/ml on day 6, 5.40±1.75 pg/ml on day 9, 9.58± 4.53 pg/ml on day 12 and 5.68±1.66 pg/ml on day 15. We concluded that Pelibuey sheep in Colombian tropical conditions presented similar levels of E2 and P4 as reported by other authors. The data reported here and obtained in future studies can be employed as reference values for clinical work and the practice of assisted reproduction programs.

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Post-mating progesterone and estradiol serum levels in Pelibuey ewes housed under tropical conditions

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Uterine receptivity depends on a precise synchrony between the endometrium, embryo and corpus luteum, all governed by a proper estradiol (E2) and the progesterone (P4) relationship. The correct hormonal balance enables the establishment of an uterine environment that favors an early development and implantation of the embryo. The aim of this study was to evaluate the serum concentrations of E2 and P4 in Pelibuey ewes after mating. Ewes were identified in estrus by epididectomized rams and a blood sample was taken (day 0). Immediately after the heat detection, ewes (n=22) were exposed to males of proven fertility and breeding was monitored allowing two successful mattings. Then, blood samples were taken every three days (day 3, 6, 9, 12 and 15) from the jugular vein. Serum was obtained by centrifugation and stored at−20°C until further analysis. The P4 and E2 concentrations were measured using competitive chemiluminescent enzyme immunoassay. Repeated measures of ANOVA were carried out; the data is presented as media ± SEM. Pregnancy rate was 63.63% and the duration of the estrus cycle in the animals that re-entered heat was 16.14 ±0.70 days. P4 concentrations were 0.02±0.01 ng/ml and 0.00±0ng/ml on day zero and 0.77±0.11 ng7ml and 0.86±0.12 ng/ml on day 3 for the pregnant and the non-pregnant group, respectively. On that day, no significant differences were found between the groups. However, for days 6, 9 and 12 differences ($P<0.05$) were found between the groups. The P4 levels in the pregnant group were 2.39±0.27, 3.79±0.35 and 4.43±0.26 ng/ml, whereas the P4 levels for the non-pregnant group were 1.78±0.26, 2.65 ± 0.33 and 2.52 ± 0.37 ng/ml. On day 15 the concentrations of the groups were 3.96±0.45 and 0.00±0ng/ml ($P<0.01$). The E2 levels did not present differences between the two groups. On day zero the E2 was 77.66±20.66 and 80.83±20.73 pg/ml. It is possible that the early increase of the P4 in pregnant sheep is related with the establishment of a favorable uterine environment, which promoted the implantation and the development of the embryo.

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Ultrastructural changes in the Leydig cells and plasma testosterone concentration of rats exposed to lead

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Although it is well known that lead exerts toxic effects on the testes, resulting in decreased reproductive capacity, few studies have qualified the ultrastructural changes in the intertubular environment from exposure to this metal. Therefore, the mechanisms that lead to changes in the testicular parenchyma, especially in the Leydig cells that produce testosterone, are of utmost importance to search for alternatives in the prevention or treatment of such injuries. Thus, the aim of this study was to evaluate by ultrastructural analysis and measurement of serum testosterone levels, changes in intertubular environment of adult Wistar rats exposed to lead. Twenty-five adult Wistar rats were used and divided into five groups: Group I (control) received distilled water and treated groups (II, III, IV and V) received lead acetate solution at concentrations of 16, 32, 64 and 128 mg Pb / kg, by gavage for 30 consecutive days. Testicular sections were fixed in glutaraldehyde (5%) for 24 hours and post-fixed in osmium tetroxide (1%) for 2 hours, dehydrated in acetone and embedded in Epon resin. Ultrathin sections (20-60 nm) were obtained with a diamond knife and contrasted with uranyl acetate (2%) and lead citrate (2%) before observation in a transmission electron microscope (Zeiss ®, Leo 906). The plasma concentration of testosterone was measured by the chemiluminescence method, using Access ® Testosterone kit (Beckman, USA). Readings were performed on apparatus Access II (Beckman, USA) and the results were expressed in ng / dl. Data were submitted to Univariate one-way analysis of variance followed by the Student-Newman-Keuls post hoc test for multiple comparisons ($p < 0.05$). The Leydig cells of the control group were normal, and containing an eccentric spherical nucleus with shallow recess. The perinuclear cistern had a normal appearance. In the cytoplasm the presence of smooth and rough endoplasmic reticulum and mitochondria was observed. The Leydig cells of the treated groups were pleomorphic, usually presenting irregular nuclei with deep indentations and prominent nucleoli. The perinuclear cistern was found to be dilated. The cytoplasm was dense and disorganized. Still evident were many projections on the surface of the cytoplasm of Leydig cells. A significant reduction in serum testosterone levels in the groups receiving lead acetate compared to control. Treatment with lead acetate promoted a series of ultrastructural changes in the Leydig cells, and the same percentage reduction in intertubular space, which was also observed in studies with other metals such as mercury, nickel and cadmium. Furthermore, rats intoxicated with lead have shown disturbances in spermatogenesis with degeneration and atrophy of Leydig cells. The ultrastructural changes evident in Leydig cells, as well as a reduction in serum testosterone levels found in this study indicate that these cells are the target of the toxic action of lead. Compromised Leydig cell reduces the steroidogenic activity and then it can induce failures in the reproductive process.

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Effects of exposure to di-(2-ethylhexyl) phthalate (DEHP) during lactation and puberty on sexual maturation and glycemic homeostasis in rats

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Some environmental chemicals can disrupt the endocrine control of the metabolism. Among these chemicals, special attention has been given to phthalate esters, substances used as plasticizers or additives in a variety of products used by people on a daily basis. Evidences indicate that some phthalates, such as di (2-ethylhexyl) phthalate (DEHP), may lead to metabolic and / or hormonal changes in critical physiological periods of sensitivity. This study aimed to evaluate the effects of DEHP during lactation and puberty in relation to possible changes in the glucose homeostasis, weight gain and the concentration of fecal metabolites of steroid hormones. In the first experiment, female Wistar rats were exposed orally to DEHP (7.5 mg / kg / day and 75 mg / kg / day) from day 1 to day 21 of lactation. The dams and their male offspring were evaluated regarding weight gain, food ingestion, serum concentrations of glucose and lipids, and insulin tolerance test (ITT). In addition, the male offspring was submitted to the quantification of insulin secretion in pancreatic islets isolated *in vitro*, concentration of fecal metabolites of androgens and determination of the age of preputial separation. In a second experimental phase, peripubertal male rats were exposed to DEHP (7.5 mg / kg / day and 75 mg / kg / day) for 30 days, starting at weaning (22 to 52 days of age). These animals were again evaluated for weight gain and concentration of fecal metabolites of androgens, glucocorticoids and preputial separation throughout the treatment period. Evaluation of ITT, fat deposit and biochemical analysis were performed at the end of the treatment. The dams which were orally exposed to DEHP were not affected by the treatment, indicating the absence of maternal toxicity or metabolic effects. The male offspring exposed during lactation showed higher vulnerability to DEHP, presenting alterations in glucose homeostasis in the adult phase, characterized by increased fasting glucose, reduced insulin sensitivity and lower insulin secretion in isolated pancreatic islets. The doses of DEHP used in this study did not induce changes in the androgenic profile and concentrations of fecal glucocorticoid metabolites. The age at preputial separation, external marker of puberty onset, was not affected in male rats exposed during lactation (experiment 1) or during puberty (experiment 2). A decrease in concentrations of cholesterol and triglycerides was found in male offspring treated during lactation. Animals exposed during the pubertal period (evaluated at 52 days of age) presented changes in fasting plasma glucose, but no changes in the androgenic profile. The use of DEHP alters insulin sensitivity and secretory control of insulin in isolated pancreatic islets and more substantial responses in male offspring. The dosage of DEHP used in this study did not induce classic anti-androgenic changes.

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Oxidative status of *Bos taurus* bull semen undergoing testicular heat stress

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Previous reports revealed that *Bos taurus* bulls are significantly more susceptible to heat stress than *Bos indicus* due to an increase in testicular oxidative stress with consequent impairment of sperm quality (NICHI et al., 2006. Theriogenology, v66, p822-828). Spermatic cells are particularly vulnerable to the harmful effects of oxidative stress due to the high amount of polyunsaturated fatty acids (PUFA) in the plasma membranes and the extremely reduced cytoplasm. While the double bounds that characterize the PUFAs are more easily oxidized, the diminished cytoplasm limits the content of antioxidant enzymes (VERNET et al., 2004. Molecular and Cellular Endocrinology, v216, p31-39). In such context, much research has been done aiming to evaluate the effect of antioxidant therapy to prevent the oxidative stress in the sperm. However, the effectiveness of an antioxidant treatment is highly dependent on which reactive oxygen specie (ROS) is causing the damages to the spermatozoa. In the present experiment four *Bos taurus* bulls were submitted to testicular heat stress by scrotal insulation over a period of 4 days. Sixty days after insulation, semen was collected by electroejaculation. Semen samples were divided into four aliquots and incubated for 30 min with different reactive oxygen species (superoxide anion - O_2^-, hydrogen peroxide - H_2O_2 and hydroxyl radical - OH^-) and malondialdehyde (MDA, product of lipid peroxidation). After inductions, samples were evaluated for motility patterns by computer assisted sperm analysis (CASA), membrane integrity (eosin - nigrosin), acrosome integrity (Fast - green /Bengal - rose), mitochondrial activity (3'3 diaminobenzidine) and susceptibility to lipid peroxidation (TBARS). Results demonstrated that incubation with malondialdehyde promoted lower percentage sperm showing intact plasma membrane (O_2^-, 51.00 ± 9.00^{b}; H_2O_2, 71.50 ± 5.50 ^{ab}; OH^-, 73.50 ± 2.50 ^{a}; MDA, 4.00 ± 2.00 ^{c}), lower percentage of cells with high mitochondrial activity – DAB I (O_2^-, 45.50 ± 2.50 ^{a}; H_2O_2, 45.00 ± 1.00 ^{a}; OH^-, 49,50 ± 0,50 ^{a}; MDA, 0.00 ± 0.00 ^{b}) and increased susceptibility to oxidative stress (O_2^-, 7.63 ± 0.00 ^{a}; H_2O_2, 311.00 ± 0.00 ^{a}; OH^-, 113.09 ± 102.73^{a}; MDA, 83002.99 ± 0.00^{b}). The higher susceptibility of sperm from heat stressed bulls to the MDA, product of the attack of ROS to lipids, highlighted the importance of preventing oxidative stress, since, once started, the oxidative chain reaction may lead to the production of even more dangerous substances. These results suggest that antioxidant therapy with α-tocopherol in heat stressed *Bos taurus* bulls would be the treatment of choice due to this vitamin´s lipophilic properties that would allow an interaction with the sperm membrane, inhibiting lipid peroxidation and, consequently, preventing MDA formation (BRADFORD et al., 2003. Journal of Lipid Research, v44, p1940-1945; SIES, H., 1993. European Journal of Biochemistry, v. 215, n. 2, p. 213-219, 1993).

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Establishment of ewe-lamb link: differences in Corriedale X Corriedale and Texel X Corriedale lambs

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To improve productive results, in many countries of South America terminal crossbreeding with rams from meat producing breeds have developed great interest among sheep producers. Therefore, since dams have greater energetic costs as lambs are heavier at birth, the establishment of ewe-lamb link may be affected. Maternal Behaviour Score (MBS) is a standardized test used to assess the maternal behaviour quality. The aim of this study was to compare the lambing birth weight and the MBS in Corriedale X Corriedale and Texel X Corriedale lambs. The study was performed at an experimental farm located in Uruguay (32º S) with 77 multiparous Corriedale ewes. Forty-seven ewes were inseminated with fresh semen from two Texel rams (TC group) and thirty ewes with fresh semen from two Corriedale rams (CC group). Since 31 days before mating until 103 days of pregnancy all the ewes grazed on native pasture with 10 to 12 kg of dry matter/100 kg of body weight. From then until 1 to 5 days before parturition all ewes grazed on fescue (14 kg of dry matter/100 kg body weight), and then on native pastures with *ad libitum* allowance until parturition. During these last periods, ewes received daily rice bran (200 g/kg body weight) and 50 mL of crude glycerine /animal. Births were continuously recorded, and 12 to 24 h after, while the lamb was weighed, an observer recorded the MBS (scale 1 to 5; 1 = minimum, 5 = maximum) (Everett-Hincks et al., 2005. Livestock Production Sci 93:51-61) based on the distance a ewe retreats from her lamb. Body weight and MBS were compared with the ANOVA and Mann-Whitney tests respectively. The lamb birth weight tended to be greater in the TC group than in the CC group (CC: 5.4 ± 0.1 vs. TC: 5.7 ± 0.1; P=0.08), but there were no differences in MBS between treatments (CC: 4.6 ± 0.1 vs. TC: 4.7 ± 0.1). In conclusion, at least under the conditions used in this experiment, the ewe-lamb bond immediately after birth assessed by MBS was not affected by crossbreed lamb.

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Reproductive characteristics and proteins of seminal plasma and sperm membrane of male goats supplemented for long periods with de-oiled castor cake

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The present study was conducted to evaluate the effect of de-oiled castor cake on reproductive parameters of cross-breed goats. Fourteen males were grouped into two lots (n = 7), as described: group without de-oiled castor cake (WCC; 21.3 ± 1.7 kg and 282.7 ± 2 days of age) and group fed with de-oiled castor cake (CC; 21.3 ± 1.2 kg and 284.5 ± 2.1 days). Goats received two diets composed of a mixture of bermudagrass hay and isoenergetic (73 % of TDN) and isonitrogenous (15 % of CP on DM basis) concentrates, during 150 days. Formulation of diets was based on the nutritional requirements for young male goats and presented the same concentrate:roughage ratio (40:60). In the first group, the diet included Bermudagrass hay and concentrate (80 % corn, 15 % soy meal and 5 % minerals). For the second group, the diet included Bermudagrass hay and concentrate with de-oiled castor cake instead of soy meal (80 % corn, 15 % de-oiled castor cake and 5 % minerals). The study lasted 150 days, corresponding to ages from 40 weeks (puberty) to 60 weeks. Blood plasma concentrations of urea, albumin, lactate dehydrogenase (LDH), creatinine, alanine aminotransferase (ALT) and testosterone were determined by commercial kits. Also, we evaluated scrotal circumference, sperm concentration, motility and morphology parameters, as well as seminal plasma and sperm membrane proteins during the reproductive development of the male goats. Seminal plasma and sperm proteins were evaluated by 2-D electrophoresis and mass spectrometry. After 150 days of feeding there were no changes in the biochemical composition of the blood plasma of the animals, suggesting an absence of intoxication by ingestion of ricin. Scrotal circumference (WCC = 22.8 ± 0.5 cm; CC = 22.6 ± 0.6 cm), testosterone concentrations (WCC = 4.6 ± 2.1 ng/ml; CC = 4.5 ± 2.4 ng/ml), sperm concentration (WCC = 1.6 ± 0.1 x 10^9 cells/ml; CC = 1.6 ± 0.1 x 10^9 cells/ml), motility (WCC = 85.8 ± 3.5 %; CC = 81.4 ± 4.1 %) and morphology (WCC = 97.3 ± 1.2 %; CC = 98.5 ± 0.5 %) were not affected by the diet containing de-oiled castor cake ($p > 0.05$). One possible explanation for this result probably relates to the low concentration of ricin (50 mg/kg coproduct) present in the de-oiled castor cake used in our study. Moreover, it is possible that this toxic protein was inactivated by rumen microbial proteases because previous studies have reported some degree of tolerance in ruminants fed with ricin. Despite the fact that some sperm parameters remained unchanged after de-oiled castor cake feeding, the expression of certain seminal plasma and sperm membrane were altered. The proteins identified in the seminal plasma as binder of sperm 1 precursor, relaxin, clusterin isoform 1 and phosphoglycolate phosphatase had lower expression in the animals fed de-oiled castor cake, whereas a protein identified as pancreatic lipase-related protein 2 showed a higher expression in this same group ($p < 0.05$). Sperm membrane proteins identified as binder of sperm 1 and 5 precursor and bodhesin-2 were found with greater expression in the de-oiled castor cake group and an albumin spot showed lower expression in this same group ($p < 0.05$). We can conclude that the use of de-oiled castor cake in substitution for soy meal given for prolonged periods has no toxic effect on animals and does not affect reproductive development of male goats, despite the observed changes in the expression of a few proteins of the seminal plasma and sperm membrane.

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The presence of corpus luteum and their correlations with laparoscopic ovum pick-up yield of Anglo Nubian goats fed with different fat sources

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The aim of the present study was to evaluate the fat source effect on the presence and number of corpus luteum (CL) and its correlation with total number of aspirated follicles (AF) and oocyte recovered (OR). Eighteen goats were divided into 3 groups, on diets of 40% concentrate 60% corn silage with 4% dry matter of ether extract from different fat sources [soy oil (SG, n=6), linseed (LG, n=6) and Megalac® (MG, n=6)]. The goats were submitted to an adaptation period of 15 days and then an experimental period of 70 days. The Laparoscopic Ovum Pick-up (LOPU) was performed on Day 42 (T1) and Day 70 (T2) of the trial, after 36 hours of an ovarian superstimulation protocol [FSH (80mg) + eCG (300IU) after 36 hours of the last shot of GnRH from the Ovsynch protocol].The presence and number of CL were evaluated during the LOPU. These data were then analyzed by ANOVA with Tukey test ($p \leq 0.05$) and correlations between variables with Pearson and prevalence of CL by Chi -square using software R®. There was no interaction between groups and time, and the results are expressed by principal effects (means ± sem). The percentage of goats with CL during LOPU in T1 were 50%, 83% and 50% and in T2 were 83%, 100% and 66% for SG, LG and MG respectively. The number of CLs were similar between groups and times (SG: 1.42 ± 0.40; LG: 2.50 ± 0.48 and MG: 1.67 ± 0.43; T1: 1.72 ± 0.40 and T2: 2.00 ± 0.33). The percentage of goats with CL from the LG group were higher both in T1 and in T2, and all treatments had CLs number increased from T1 to T2, however it was not significant. None of the correlations performed were statically significant. In conclusion, linseed did not increase the number and presence of CL, and the presence of those does not interfere with or enhance AF and OR.

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Evaluation of cashew nut meal inclusion in the diet of male goats: preliminary data

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Goat producers from the Brazilian Northeast need to supplement the animals in order to maintain herd production along the year. Therefore, there is a demand of low-cost food sources to reduce the use of corn and soybean, such as cashew nut meal. However, the effects of cashew nut meal on goat reproduction are still poorly studied. As any alternative feed source for goats, effects from cashew nut meal on male reproduction must be analyzed. Thus, the aims of this current study were to evaluate the effect of 11% cashew nut meal inclusion in the diet of crossbred male goats on sperm parameters (motility and concentration). The study was conducted in Fortaleza-CE for 90 days. Twenty healthy one year old male goats were equally distributed in two groups: control group (COG) which was fed with 50.0 % tyfton hay, 38.9% corn meal, 8.6% soybean meal and 2.5% of mineral mix, and cashew nut group (COG) which was fed with 50.0 % tyfton hay, 11% cashew nut meal, 30.9% corn meal, 5.4% soybean meal and 2.7% of mineral mix. However, both diets were calculated to be iso-proteic (protein crude) and isocaloric (total digestible nutrients). The cashew nut meal was composed by 92.2% of dry matter (DM), 19.6%DM of crude protein and 33.6%DM of ether extract. All animals were housed in individual stalls, where they were fed two times a day and received water *ad libitum.* The amount of ration provided was adjusted daily according to the previous consumption, in order to maintain 10% of leftover feed. The dry matter intake as percentage of live weight (%LW) was measured daily. The live weight (kg) was measured using a scale. Semen was collected weekly from all animals by electroejaculation during 90 days. The sperm parameters evaluated were motility (%), individual motility score (0-5) and sperm concentration (10^6 sptz/mL). The percentage of motile spermatozoa (%) and individual motility score (0-5) were analyzed in a drop of semen diluted with 0.9% NaCl on a warm (37^oC) slide-coverslip under a microscope at 400x magnification. Sperm concentration was determined with the aid of a Neubauer cell counting chamber after dilution (1:400) in 0.1% formolsaline. The experimental design was completely randomized, with two groups: control group (COG, n=10) and cashew nut group (CNG, n=10). The statistical analysis was performed with SYSTAT12® software. Differences between groups (COG and CNG) until 90 days were evaluated by GLM for repeated measures method and the data were expressed as mean ± SE. There were not any significant differences in dry matter intake (COG: 1.0 ± 0.1% vs CNG: 0.9 ± 0.1%) and live weight (COG: 33.6 ± 1.7 kg vs CNG: 33.1 ± 1.8 kg) between control group and cashew nut group after 90 days ($p > 0.05$). Besides, the sperm parameters: motility (COG: 76.7 ± 3.3% vs CNG: 77.8 ± 2.8%), individual motility score (0-5) (COG: 3.5 ± 0.3 vs CNG: 3.8 ± 0.2) and sperm concentration (COG: 1.8 ± 0.1 10^9 sptz/mL vs CNG: 1.7 ± 0.2 $.10^9$ sptz/mL) also did not differ between groups ($p > 0.05$) after 90 days. Considering the lack of negative impacts from cashew nut on sperm production and motility, cashew nut meal can be used as an ingredient in buck nutrition.

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In vitro maturation of agouti's oocytes (*Dasyprocta prymnolopha*, Wagler 1831)

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The in vitro maturation (IVM) is an assisted reproduction technique used in different animal species, domestic and wild, which allows, under artificial conditions, to maximize the use of cumulus oocyte complexes (COCs), preparing them for subsequent fertilization and embryo development allowing us to obtain a greater number of oocytes then obtained by the natural process of ovulation. In this regard, the aim of this study was to test protocols for *in vitro* maturation of agouti's oocytes. Seventeen female agoutis from the Study and Preservation of Wild Animals of the Center for Agricultural Sciences UFPI, with average age and weight of 3.9 years and 2.2 kg, respectively, were submitted to ovariohysterectomy. After dissection, the ovaries were weighed on a precision scale and sliced individually. COCs were selected and classified under stereomicroscope. The COCs were subjected to washing in maturation medium with 10% fetal bovine serum and transferred to the maturation medium. The viable COCs were divided into three groups: MAT - 16 (16 hours of maturation), MAT - 20 (20 hours of maturation) and MAT - 24 (24 hours of maturation). After the maturation periods the COCs were denuded by exposure to hyaluronidase enzyme (0.1%) and maturation was evaluated by the presence of polar body. Groups of matured COCs were analyzed using the chi-square test to compare the proportion of cells that reached the metaphase II stage, using the "Statistical Analysis System" (SAS - 2002). The group MAT - 24 (27.4%; 17/43) presented similar percentage of matured oocytes to the group MAT-20 (22.6%; 12/53), but both were greater than the group MAT-16 (8.8%; 04/52) ($P<0.05$). Although the rates of maturation presented were low, these results prove to be promising, and this is the first study to use these procedures in agoutis.

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Identification and molecular characterization of CXCR4-positive bovine spermatogonial cells

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Spermatogonial stem cells (SSC) are responsible for continued spermatogenesis throughout the adult life of an individual. These cells may be isolated and have the ability to repopulate the testicle after transplantation. Due to this property, SSC have the potential to generate transgenic sperm and consequently, transgenic animals. However, molecular markers such as C-X-C chemokine receptor 4 (CXCR4), used to separate SSC in the mouse are not well studied in bovine. The aim of this study was to verify the expression of CXCR4 in the testis of prepubertal bulls and to select and characterize CXCR4-positive (CXCR4+) cells in isolated testicular cells using magnetic activated cell sorting (MACS). We surgically removed the testicles of 8 prepubertal bulls. Samples were fixed in 4% paraformaldehyde for 24h and embedded in paraffin prior to mounting slides for immunohistochemistry. Testicular cells were isolated and cryopreserved after sequential enzymatic digestion with collagenase and trypsin. Isolated cells were thawed and submitted to magnetic activated cell sorting (MACS) using CXCR4 antibody. MACS separated cells were further analyzed by flow cytometry and quantitative RT-PCR. Immunohistochemistry of testicular fragments revealed that few spermatogonia were positive for CXCR4. Flow cytometry analysis after MACS revealed that 68.51% of cells were positive for CXCR4, a 6.42 fold increase when compared to non-sorted cells. Quantitative RT-PCR analysis revealed that CXCR4+ cells had reduced expression of *CXCR4* mRNA, increased expression of *OCT4* and unchanged expression of *GFRa1*, *THY1, ITGA6* and *SOX2* when compared to CXCR4 negative cells. These results indicate that CXCR4 could be potentially used to enrich spermatogonia in prepubertal bulls. More studies are in course to further characterize the protein expression and testicular repopulation potential of the CXCR4+ cells. (CEUA VET – 2809-2012)

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Effect of catalase, superoxide dismutase, and reduced glutathione diluted in LDL extender on ovine sperm viability after freezing thawing process

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Many antioxidants have been applied for improving the sperm viability after freezing and thawing process in mammalian. The aim of this study was to evaluate the motility, kinetics and membrane integrity of ovine sperm cryopreserved in extenders containing 8% LDL with enzymatic antioxidants at different concentrations. Four Santa Inês rams were used to form four pools of semen (four rams/one ejaculate per ram/pool). Each seminal pool was divided into eight aliquots for the following treatments: Tris-glucose-glycerol (TGG) + (16%) egg yolk (control 1); TGG + 8% (w/v) LDL (control 2); TGG + 8% LDL + Catalase 100 U/mL; TGG + 8% LDL + Catalase 200 U/mL; TGG + 8% LDL + superoxide dismutase 100 U/mL; TGG + 8% LDL + superoxide dismutase 200 U/mL; TGG + 8% LDL + reduced glutathione 5 mM; and TGG + 8% LDL + reduced glutathione 10 mM. The samples were packed into 0.25 mL straws, cooled (-0.25 °C/ min), maintained at 5 °C for 2 h and then frozen (-25 °C/ min) using a TK4000®. Immediately after thawing (38 °C/ 30 s), sperm motility and movement characteristics were assessed by computer sperm analysis (CASA). The functional integrity of membranes was assessed using a hypoosmotic swelling test. The structural integrity of the plasma and acrosomal membranes was analyzed using fluorescent dyes. As assessed by ANOVA, significant differences among treatments were only observed for VCL, VSL and VAP. The VCL values were similar ($P>0.05$) for the samples frozen in the control extender with egg yolk (32.2µm/s) and the extender containing LDL with 10 mM reduced glutathione (31.1µm/s). However, both values were lower than those obtained with the other extenders (38.1 – 44.4µm/s; $P<0.05$). The VSL values for samples frozen in the control extender with egg yolk (12.8µm/s), the control with LDL (12.6µm/s) and in the extender containing LDL with 10 mM of reduced glutathione (10.4µm/s) were all similar and lower than those found for other extenders (13.4 – 15.4µm/s; $P<0.05$). The VAP values for samples frozen in the control extender with egg yolk (17.8µm/s), the control extender with LDL(19.7µm/s), the extender containing LDL with 100 U/ml of superoxide dismutase (20.2µm/s) and the extender containing LDL with either 5 mM (19.9µm/s) or 10 mM of reduced glutathione (15.6µm/s) were similar, and all were below the VAP values found for samples frozen in the other extenders studied (22.5 - 23.4µm/s; $P<0.05$). Sperm motility varied from 18.5 to 33.3%. There were no differences among the extenders in preserving the functional integrity (average variation between extenders from 9.0% ± 6.1 to 18.5 ± 14.6%) or structural integrity of the sperm membranes (average variation between extenders from 10.3% ± 6.9 to 22.8% ± 23.3), as assessed with the hypoosmotic tests and fluorescent probes (CFDA/IP), respectively ($P>0.05$). In conclusion, enzymatic antioxidants did not improve the protective activity of extenders containing LDL on frozen ovine sperm.

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Epigallocatechin gallate: hero or villain on frozen goat semen?

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Although semen cryopreservation is a reproductive bio-technique with an important role in the animal industry, this process exposes the sperm to injuries, particularly linked to increased production of reactive oxygen species (ROS). On the other hand, the flavonoid (-)-Epigallocatechin gallate (EGCG) is a powerful antioxidant. Thus, the aim of this study was to evaluate the effect of different concentrations of EGCG on goat semen freezability. Ejaculates from six mature male goats were collected at 48h intervals with an artificial vagina and those approved were pooled, totalling six *pools*. Aliquots of semen were processed for the plasma removal and diluted with a skim milk-based extender (7% glycerol) containing different concentrations of EGCG (G1=0, G2=15 and G3=100 µM), according to experimental groups. Extended semen samples were packed into straws (0.25 mL; $200x10^6$ spt/mL) and frozen. After thawing (37 ° C/30 s), aliquots of semen were evaluated at 0, 1 and 2 hours for: total (TM) and progressive motility (PM) in a computerized system (CASA), plasma membrane integrity (PMi) by double staining with carboxyfluorescein diacetate and propidium iodide, and oxidative stress (OS) by nitroblue tetrazolium test. For statistical analysis one-way ANOVA and Tukey-Kramer were used, with 5% significance. It was observed that EGCG did not affect (P>0.05) the TM (T0: G1=68.8±9.0, G2=66.4±11.5 and G3=69.1±8.3; T1: G1=59.5±7.4, G2=49.8±16.1 and G3=54.4±17.4; T2: G1=32.6±14.2, G2=30.7±12.5 and G3=31.6±14.4) and PM (T0: G1=27.1±2.6, G2=27.22±4.3 and G3=24.6±6.6; T1: G1=24.2±9.1, G2=19.7±6.2 and G3=19.8±5.9; T2: G1=13.0±6.5, G2=11.9±5.2 and G3=12.8±7.3) of frozen goat semen, regardless of the antioxidant concentration and evaluation time. In opposition, 100 µM EGCG showed a tendency to better preserve the PMi (P=0.06) at 0h (T0: G1=39.3±4.7, G2=44.5±6.8 and G3=51.3±8.3), and the cells without OE (P=0.07) at 2 h (T2: G1=71.5±2.9, G2=75.3±4.4 and G3=76.8±3.9). In the other times no statistic difference or tendency was observed for PMi (T1: G1=28.1±6.7, G2=32.8±8.7 and G3=33.6±7.4; T: G1=20.4±7.4, G2=18.3±9.0 and G3=20.8±5.2) or -OE (T0: G1=78.8±4.0, G2=81.2±4.9 and G3=82.3±4.3; T1: G1=76.3±4.8, G2=76.5±3.3 and G3=80.2±5.9). Thus, EGCG at higher concentrations has a promising positive effect on the plasma membrane and oxidative stress of frozen goat sperm.

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Effect of cyclodextrin-loaded cholesterol conjugates on plasma membrane viability of Piau swine breed frozen/thawed spermatozoa

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Cholesterol, one of the main components of the sperm membrane, interferes with lipid behavior, enlarging its phase transition, preventing sudden changes and minimizing phase separation. The swine sperm plasma membrane presents lower cholesterol/phospholipids ratio than that of spermatozoa of other species (0.26 over 0.36 in ovine and 0.38 in bovine), one possible responsible factor for higher thawing sensibility. The aim of this study was to investigate the effect of cyclodextrin-loaded cholesterol conjugates addition to freezing extenders on plasma membrane viability of frozen-thawed spermatozoa of the Piau swine breed. Twenty semen samples were used from five males. The freezing extender was based on lactose-egg yolk extender, added to 2% glycerol, 3% dimethylacetamide. The addition of cyclodextrin-loaded cholesterol conjugates was performed after centrifugation, when semen was diluted with the cooling extender. Four groups were subjected to the following treatment: without addition (group 1); 1.5 mg of cyclodextrin-loaded cholesterol /120x10^6 sperm (group 2); 1.5 mg of cyclodextrin-loaded cholestanol /120x10^6 sperm (group 3); 1.5 mg of cyclodextrin-loaded desmosterol /120x10^6 sperm (group 4). To check post-thawing sperm quality, sperm motility and sperm morphology evaluation were used. Additionally, to check sperm viability, the hypoosmotic swelling test, supravital staining, and fluorescent assay were used. The mean values recorded for total sperm motility of semen immediately after thawing were 54.5±5.8, 55.5±5.3, 53.7±6.7, and 52.5±6.6% respectively for groups one to four, without differences between them ($p>0.05$). Regarding fluorescent assay, the results were 28.3±13.2, 26.9±12.2, 22.2±11.4, and 32.0±15.3% respectively for groups one to four, also without differences between groups ($p>0,05$). Similarly, complementary tests for evaluating the integrity and functionality of the plasma membrane showed no difference between treatments ($p>0.05$). Apparently, the improvement in post-thawing sperm quality after cholesterol addition is not as evident in swine and seems to be inferior (6 to 8%) than that observed in other species (10 to 20%). However, this difference can be explained by swine sperm sensitivity to cold shock, due to membrane phospholipids' differences and its relations with cholesterol (inferior) and proteins (superior) comparing to other species. CLC addition did not improve or decrease post-thawing sperm quality. The present study demonstrated that the use of cyclodextrin-loaded cholesterol conjugates added to the plasma membrane of sperm did not demonstrate any additive effect on increasing and/or maintaining sperm motility. Thus, there was no improvement in post-thaw sperm viability in Piau swine Breed.

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Kinetics of sperm recovered from the cauda epididymis of sheep fed with *Tifton-85* and alfalfa hay

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Forages represent the most abundant ingredients in diets for ruminants. The genus Cynodon aroused great interest and has gained popularity due to its ease of cultivation and high forage yield of good nutritional value such as the *Tifton* -85. For ruminant feed, alfalfa forage is considered to gather a large number of desirable characteristics. Regarding grasses, it is worth noting the contents of soluble carbohydrates and cell wall and high content of true protein. Sheep fed diets high in protein show a significant increase in testicular size and body weight. Results of studies show that testicular volume is most sensitive to changes in diet and body weight indicating the preference of allocation of nutrients in the reproductive system. The study aimed to evaluate the kinetic parameters of sperm sheep confined and fed different amounts of *Tifton*-85 hay and alfalfa. Twenty mongrel sheep, eight months old, housed individually, were divided into four groups of diets with roughage: G1 = 60 % *Tifton*-85 ; G2 = 40% *Tifton*-85 + 20% alfalfa ; G3 = 20% *Tifton-85* + 40% alfalfa and G4 = 60 % alfalfa , all with 40% concentrate (soybean - Glycine max and Corn bran - Zea mays). Sixty days after confinement castration was performed and testis-epididymis complex referred to ANDROLAB into isothermal container. The cauda epididymis of each animal was dissected and placed in petri dishes containing 5 ml of Tris buffer (3,605g Tris, 2,024g citric acid, 1,488g of fructose, pH = 6-8) at 37° C and then the sperm was removed by flotation. Samples containing the sperm remained on board for five minutes at 37° C for subsequent computerized assessment of sperm kinetics (Sperm Class Analyzer , Microptics , SL , Version 3.2.0 , Barcelona , Spain). Data were subjected to analysis of variance (ANOVA) and analyzed using the Statistical Analysis System computer program. Significance level of $P < 0.05$ was adopted. No significant differences between groups were observed in this experiment diets: MT (%)(P=0.6813): G1: 49.38± 19.74; G2: 45.52± 26.68; G3: 42.78±21.56; G4: 37.86±17.08; VCL (µm/s) (P=0.6062): G1: 32.27±10.73; G2: 36.62±14.06; G3: 31.80±17.44; G4: 28.62±8.95; VSL (µm/s) (P= 0.8896): G1: 8.25±3.90; G2: 8.68±4.86; G3: 7.38±5.16; G4: 7.46±3.22; VAP (µm/s) (P=0.8552): G1: 14.86± 6.10; G2: 16.05±9.37; G3: 14.05±9.02; G4: 13.19±4.84; LIN (%) (P=0.4914): G1: 24.66±6.72; G2: 23.13±6.81; G3: 21.41±5.29; G4: 25.90±8.02; STR (%) (P=0.2500): G1: 56.78±13.58; G2: 50.24±9.21; G3: 49.37±6.61; G4: 55.73±9.13; WOB (%) (P=0.7420): G1: 45.01±6.38; G2: 45.31±6.39; G3: 42.75±6.30; G4: 45.74±7.07, collaborating with other researchers who also found no differences in semen quality in animals treated with different protein levels. Although alfalfa has a high content of true protein, using the diet with *Tifton-85* or alfalfa, there was no difference in the kinetic parameters of ovine sperm recovered from the cauda epididymis under the conditions of this experiment. Alfalfa enters as an excellent alternative, as it highlights the other characteristics for good productivity, nutritional value, palatability, digestibility and low seasonality of forage production.

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Time course study of inhibitory action of roscovitine in sheep oocytes

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The majority of complex *cumulus*-oocytes (COC) destined for *in vitro* embryo production spontaneously resume the meiosis without, however, undergoing all ultra-structural and molecular changes necessary to support the maturation, fertilization and early embryogenesis. In this context, the temporary meiotic arrest has been proposed as a strategy to restore the synchrony of oocyte development in several animal species (Ponderato, 2001, Mol Reprod Dev, 60, 579-585). This study aimed to evaluate the potential of meiotic inhibition of roscovitine, a cyclin-dependent kinase inhibitor, at different times of *in vitro* culture in sheep oocytes. For this, sheep COCs grade 1 and 2 were cultured for 6, 12 and 20 hours, in maturation medium consisting of TCM199, cysteamine, pyruvate, penicillin and 10% fetal bovine serum (control) plus 75μM roscovitine (treatment). The culture was performed in 100μL droplets of maturation medium in a 96 well plate without mineral oil, at 38.5°C and 5% CO_2. At the end of each culture time, COCs were denuded, stained with Hoechst 33342 and evaluated under fluorescence microscope. The experimental design was completely randomized with five replicates for each culture time and 100 oocytes for each experimental group. A total of 600 oocytes were evaluated. Besides, an additional group of 100 oocytes was stained immediately after removal from follicle (0 h). The results were submitted to analysis of variance and means were compared by the Tukey test at 5% probability. At 0h, 90% of oocytes were at germinal vesicle (GV), which demonstrates the meiosis had not resumed before *in vitro* culture. After 6h, the rate of oocytes at GV in roscovitine treatment (87%) was similar to that observed at 0h and significantly higher compared to the control group (47%). In this same time, while similar rate of oocytes from treatment and control groups (13% and 24%, respectively) were at germinal vesicle breakdown (GVBD), a significantly higher amount of oocytes from control group (29%) were at metaphase I (MI) in comparison with 0% in the treatment group ($p<0.05$). At the end of 12h, while the rate of oocytes at GV in the control group was significantly lower (26%) compared to the treatment group (66%), the quantity of oocytes at MI and metaphase II (MII) (55% and 11%, respectively) in the control group was higher than the rates of 17% and 4% observed in the treatment group for these same stages, respectively ($p<0.05$). Besides, the rate of oocytes at GVBD and degenerate in the treatment (11%, and 2%, respectively) and control (8% and 0%, respectively) groups were similar ($p<0.05$). At 20h, the rate of oocytes at GV (66%) for roscovitine treatment was the same observed at 12h for this same group, which was significantly higher than that observed in the control group (13%). In this same time, while the quantity of oocytes at GVBD, MI and degenerated did not differ between roscovitine (11%, 10% and 12%) and control (9%, 10% and 5%) groups, the rate of oocytes at MII was significantly higher in the control group (63%) compared to 1% in the treatment group ($p<0.05$). So, the results obtained demonstrate that roscovitine, at studied concentration, was effective to keep sheep oocytes at vesicle germinal stage, mainly during the first 6 hours of *in vitro* culture. The subsequent decline of the potential of meiotic inhibition is probably due to the competition with ATP for its binding site in the maturation promoting factor. However, even this way, a significant rate of oocytes was kept at GV. Besides, the progression of nuclear maturation observed in control indicates that the culture condition was adequate.

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Enrichment of bovine spermatogonial cells by differential plating

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Spermatogonial stem cells (SSC) have important application in animal reproduction, from establishment of treatments for infertility to more advanced biotechnologies, such as animal transgenesis. Differential plating is one of the most common methods used for SSC and spermatogonial enrichment. The aim of this study was to identify the most efficient differential plating method for the isolation of bovine spermatogonial cells. In this matter, 10 g of testicle parenchyma (n=5) from prepuberal Nelore bulls (3-5 months) were minced with scissors and consecutively digested with two solutions: colagenase (1 mg/ml) for 30 min at 37 ºC and trypsin (2.5 mg/ml) for 5 min at 37 ºC. After the enzymatic isolation, testicular cells were cryopreserved ($20x10^6$ viable cells/vial). We tested three methods of differential plating: laminin (20 ng/ml), BSA (0.05 mg/ml) or PBS. Cells were thawed and resuspended in BSA medium and incubated at 37 °C in 5 % CO2 in air for 15 min on laminin coated dishes (Laminin group) or 2 hours on BSA or PBS coated dishes (BSA and PBS groups). The adherent cells from the laminin group were recovered by trypsinization (0.01% Trypsin) for 1 min at 37 °C and non-adherent cells from BSA and PBS groups were recovered for analysis. Recovered cells were analyzed for spermatogonial molecular markers (GFRA1, CXCR4 and ITGA6) expression by quantitative RT-PCR and flow cytometry. Testicular cells before differential plating were assumed as control group. The effect of differential plating on flow cytometry results were analyzed by ANOVA (SAS®) and Tukey's Studentized Range (SAS®) was performed as post hoc test. RT-PCR results were analyzed by %QPCR_MIXED (SAS®). A significance level of 5% was assumed for all statistical analyzes. *ITGA6* was more expressed in all treatments than the control (P=0.007) but *GFRA1* (P=0.0156) and *CXCR4* (P<0.001) were highly expressed on non-attached cells from the PBS group. However, the same expression profile was not observed in cytometry results. ITGA6 was more expressed only in cells adherent on laminin (P<0.001), that also had lowest expression of CXCR4 (P=0.001). GFRA1 was more expressed in non-adherent cells from BSA and PBS treatments (P<0.001). In conclusion, PBS differential platting is the most efficient method to enrich bovine spermatogonial cells based on RT-PCR and flow cytometry results. More studies are fundamental to improve the efficiency of the spermatogonial enrichment technique (CEUA/FMVZ/USP 2509/2011).

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Microfilament organization and meiosis kinetics in bovine oocytes treated with meiotic inhibitors during transportation

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Meiosis delay with meiotic inhibitors can be a strategy to preserve oocyte quality and integrity during transport. In this regard, the aim of this study was to evaluate the effects of transport of bovine oocytes treated with different meiotic inhibitors [physiological: follicular fluid (FF); or chemicals: butirolactone-I (BL), milrinone (MR) or forskolin associated with IBMX followed by cilostamide (CL)] in meiosis kinetics and microfilament organization through oocyte maturation. Oocytes (n=3246) were transported in 100µl of IVM medium that was TCM199 supplemented with 0.6% BSA, antibiotic and hormones (Control) or in the same medium supplemented with 0.3% BSA for treated groups and one of the following inhibitors: 100µM BL; 100µM Forskolin + 500µM IBMX; 100mM MR; or FF (100%). The transport was done in a portable incubator (Minitub) for 6h at 38.5°C. There were two control groups: C1 (oocytes were cultured in 5% CO_2 in air) and C2 (cultured in the portable incubator). After transport, oocytes of all groups were cultured in the CO_2 incubator for another 18h (until completing 24h of IVM), in IVM medium at 38.5°C in 5% CO_2 in air. During the 18h-period of incubation, oocytes from the CL group were washed from transport medium and were cultured in IVM medium supplemented with 20µM cilostamide. The meiotic stage and microfilament distribution were evaluated after follicular removal (0h), after pre-maturation (6h) and at 18h, 20h, 22h and 24h of IVM, when oocytes were stained with 1µg/mL phalloidin and 1µg/mL Hoechst 33342. Stained oocytes were evaluated under an epifluorescence inverted microscope (excitation 495nm and emission 517nm) and classified by nuclear status as germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), anaphase I or telophase I (A/T) or metaphase II (MII). Microfilament distribution was classified as normal (N), discontinuous (DC), diffuse (DF) or absent (A), according to Albarracín, J. L. *et al*., 2005 (Molecular Reproduction and Development, 72, 239-249). The nuclear status was analyzed by Chi-square test and microfilament distribution data were transformed in an arc sen$\sqrt{}$% and analyzed by ANOVA followed by Tukey's test ($P<0.05$). The percentage of oocytes that were in GV stage at 0h was 84.9%. Oocytes that reached GVBD at 6h for C2 (67.6%^{a}) were higher ($P<0.05$) than C1 (47.1%^{b}), BL (45.0%^{b}), CL (46.1%^{b}), MR (49.4%^{b}) and FF (52.7%^{b}), which did not differ ($P>0.05$). At 18h, the percentage of oocytes that were at A/T stage was 44.59%^{a} (C1), 49.25%^{ab} (C2), 49.43%^{ab} (BL), 63.64%^{bc} (CL), 66.22%^{c} (MR) and 65.52%^{c} (FF). At 20h, the percentage of oocytes that reached MII stage in BL (48%^{b}) and CL (44.3%^{b}) was lower ($P<0.05$) than C2 (64.86%^{a}), and the other groups did not differ (55.88%^{ab} to 56.72%^{ab}; $P>0.05$). At 22h, MII percentage was lower ($P<0.05$) in C2 (60%^{b}) and CL (62.3%^{b}) compared to C1 (79.7%^{a}), but they were similar ($P>0.05$) to the other groups (67.9%^{ab} to 75.5%^{ab}). At 24h of IVM there were no differences ($P>0.05$) in the percentage of oocytes that reached MII between groups (62.1% to 75.0%). Microfilament distribution after transport (6h) was similar ($P>0.05$) between groups for the classification N (12.8±10.4 to 46.1±7.2), DC (45.0±8.8 to 61.8±8.2) and A (1.8±1.8 to 7.9±6.2). For DF classification, MR (37.4±4.6^{b}) was higher ($P<0.05$) than 0h (6.4±3.5^{a}) and FF (8.1±4.6^{a}), but did not differ ($P>0.05$) from C1 (16.7±5.1^{ab}), C2 (8.9±5.3^{ab}), BL (19.8±4.4^{ab}) and CL (27.2±10.8^{ab}), these latter being similar ($P>0.05$). Through maturation there were no differences ($P>0.05$) in microfilament distribution among groups at 18, 20, 22 and 24h for all categories and the majority was classified as N (18h: 45.7±15.6 to 79.0±7.9; 20h: 66.4±7.3 to 83.5±5.7; 22h: 65.8±1.8 to 88.1±9.9; 24h: 65.5±5.4 to 84.2±2.6). The results indicate that oocytes were able to mature in the proposed conditions of transport, since the supplementation of medium with meiotic inhibitors did not promote detrimental alterations in microfilament organization that could not allow the completion of meiosis. Further studies will be developed to evaluate the effects of these drugs on the acquisition of oocyte competence for embryo development.

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Semen extraction in anaesthetized mouflons (*Ovis musimon*): comparison of electroejaculation and transrectal ultrasound-guided massage of the accessory sex glands

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In non-domestic ruminants, semen is traditionally obtained by electroejaculation (EE) under general anesthesia. It has been reported that even under general anesthesia, EE provokes physiological changes indicative of a stress response (Fumagalli et al., 2012. Reprod. Dom. Anim. 47:308-312). We have recently described the use of transrectal ultrasound-guided massage of the accessory sex glands (TUMASG) to obtain semen in aoudad (*Ammotragus lervia*) (Santiago-Moreno et al., 2013. Theriogenology 15:383-391), which may be a less invasive technique. Therefore, our aim was to compare the physiological responses and semen characteristics obtained in mouflon with EE or TUMASG under general anesthesia. Seven males allocated in the facilities for animal management in INIA (Madrid, Spain), were subjected to both techniques inverting the order in 4 and 3 animals. The animals were anesthetized using i.m. detomidine (100 μg/kg) plus ketamine hydrochloride (1 mg/kg) and tiletamine-zolazepam (1 mg/kg); anaesthesia was maintained with isoflurane. We recorded the time length of the procedures, the heart and respiratory rates at the beginning, mid, and end of the procedures, and rectal temperature at the begging and end of the procedures. Oxygen saturation was monitored through the experimental procedures. Ejaculated volume, sperm concentration, sperm motility, semen score (range 1 to 5), percentage of alive spermatozoa, of spermatozoa with progressive motility, and of sperm with an intact acrosome was determined in all semen samples. The TUMASG procedure was shorter than EE (12.1 ± 1.2 vs 21.7 ± 1.4 min, P=0.0002). Heart frequency increased during both procedures from 77.1 ± 6.2 to 101.1 ± 3.6 beats/min (mid-treatment, P=0.0003), and was still elevated at the end of the treatments (97.7 ± 3.5 beats/min, P=0.002). There were no differences between treatments (P=0.99). Respiratory rate was not affected by the treatments. Rectal temperature decreased from 38.2 ± 0.2ºC to 35.3 ± 0.2ºC after the procedures (P<0.0001). This decrease tended to be greater in TUMASG animals (34.9 ± 0.1 vs 35.7 ± 0.3ºC, P=0.08). Oximetry was similar in both treatments (99.0 ± 0.4 %). Semen score was greater in EE animals (3.4 ± 0.3 vs 2.6 ± 0.2, P=0.01), and the total number of spermatozoa ejaculated (volume X spermatozoa concentration) tended to be greater in EE animals (982 ± 299 vs 710 ± 542 millions, P=0.06). There were no other differences in semen patterns. Spermatozoa concentration (1735.5 ± 562.9X 10^6/mL), percentage of live (47.2 ± 5.2 %) and motile spermatozoa (72.0 ± 4.1 %) were not affected by type of semen collection. Overall, although TUMASG is a shorter procedure, slight differences in semen parameters were obtained with EE.

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TUNEL assay for apoptosis assessment of bovine oocytes *in vitro* matured with PVA, IGF-1, LongR3-IGF-1 or BFS

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Studies involving addition of growth-promoting substances such as the family of insulin-like factors (IGFs) in maturation media have been highlighted. The insulin-like-1 recombinant 3 (LongR3-IGF-1) factor is an analog of IGF-1 widely used in follicles and granulosa cell culture showing a thousand fold lower affinity for IGF binding proteins (IGFBPs) which ensures greater bioavailability. However, there are no studies about the effects of LongR3-IGF-1 on bovine oocytes *in vitro* maturation. Therefore, this summary aimed to evaluate apoptosis rate of bovine oocytes after *in vitro* maturation with PVA, IGF-1, LongR3-IGF-1 or BFS by TUNEL technique. Cow ovaries were obtained in slaughterhouses, with aspiration of 2-8 mm follicles. Only grade 1 and 2 oocytes were selected for maturation. Four different maturation media [TCM199, 0.1% PVA, 0.011g/ml Piruvate, 1000µg/mL FSH, 5µg/mL LH and 100µg/mL Penicillin and Streptomycin (1); TCM 199, 100ng/mL IGF-I, 0.1% PVA, 0.011g/ml Piruvate, 1000µg/mL FSH, 5µg/mL LH and 100µg/mL Penicillin and Streptomycin (2); TCM199, 100ng/mL LongR3-IGF-1, 0.1% PVA, 0.011g/ml Piruvate, 1000µg/mL FSH, 5µg/mL LH and 100µg/mL Penicillin and Streptomycin (3); TCM199, 10% BFS, 0.011g/ml Piruvato, 1000µg/mL FSH, 5µg/mL LH and 100µg/mL Penicillin and Streptomycin (4)] were prepared for each experimental group. Twenty three oocytes were matured in 90µl drop of each maturation media covered by sterilized mineral oil, in nine replicates, for 22-24 hours at 38.5ºC in a humidified atmosphere of 5% CO_2. After maturation period oocytes were denuded, subjected to TUNEL technique and evaluated with fluorescence microscope at FITC filter for the presence of apoptosis. The effects of treatments on incidence of apoptosis were tested by ANOVA. The rates of oocytes under apoptosis were 5/122, 12/134, 7/121, 8/119 for oocytes from mediums 1, 2, 3, and 4, respectively. No statistical difference for the presence of apoptosis was observed between groups. Compared to PVA, IGF-1 and BFS, LongR3-IGF-1 did not increase apoptosis rate when added to oocyte *in vitro* maturation medium. Further studies are now being performed to evaluate meiotic progression *in vitro* and embryo production.

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Cooled semen with skimmed milk, yolk-glycine and modified yolk-glycine extenders in Nelore bulls (*Bos taurus indicus*)

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Biotechnical bovine semen cooling is an alternative that can be used for artificial insemination purposes. Extender formulators for cooled semen are studied in order to maintain the metabolism of spermatozoa between 5 and 15ºC in the range from one to 12 hours. The survival of spermatozoa in the seminal plasma is limited to a few hours in the absence of extenders. The sperm viability can be maintained for longer periods with the addition of extenders in refrigeration temperature. The aim of this work was to study the cooling of semen with the extenders: TRIS-skimmed milk (TSM), yolk-glycine (YG) and modified yolk-glycine (MYG) in Nelore bulls. Forty bulls aged between 24 and 36 months were used for semen collection with electroejaculation (Autoejac®, Neovet), evaluating the characteristics of fresh semen: motility (75±6%), vigor (3±1), major defects (7±2%) and minor defects (9±3%). The ejaculates were separated and diluted in the proportion of two parts of extender to one part semen (2:1) at 37°C with extenders TSM, YG (Botu-Semen ®) and MYG (Botu-Special ®) with subsequent storage at 14ºC for 8 hours according to the cooling curve of the transportation box (BotuBox®) according to the manufacturer's information (BotuPharma®). The analysis was performed using the Biostat software 5.3, adopting a 5% significance level. The sperm motility (%) and vigor (0 to 5 scale) were greater in the MYG (28±12% and 2±1) and YG (23±11% and 2±1) extenders than the TSM extender (10±5% and 2±1). The minor defects were lower ($P<0.05$) for the TSM extender (6.47±6.29%) compared to the MYG extender (10.84±6.75%) and YG (8.6±5.8%). There were no differences ($P>0.05$) among the three extenders for major defects (5.38±3.41% to 5.97±2.73%) and total defects (12.52±4.02% to 13.72±3.25%). We conclude that the YG and MYG extenders were superior to keep the sperm metabolism of the semen cooled in bred Nelore bulls. (CEUA/UNOESTE/Protocol#1607).

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Cryopreservation of Pampas deer semen with Andromed or Triladyl extenders

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Pampas deer is a native deer of the southern cone of South America that is included in the Red Book of Endangered Species as near threatened (IUCN). In the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (ECFA), Uruguay, there is a semicaptive population with approximately 70 animals. Since the Andromed extender does not need the addition of egg yolk, its use decreases the risk of transmitting diseases. Andromed also provides a clearer media for sample assessment under the microscope than samples diluted with egg yolk extenders, being a more defined standard extender for research purposes. The aim of this experiment was to compare the effectiveness of two different commercial semen extenders: one with and one without the need of egg yolk (Andromed and Triladyl). Semen was obtained by electroejaculation, under general anesthesia from 10 adult males, during the breeding season. The semen collected (T_0) was split in two aliquots, one diluted with Andromed (Minitube, Tiefenbach, Germany) extender and the other with Triladyl (Minitube, Tiefenbach, Germany) with the addition of 20% of egg yolk. Semen was evaluated again immediately after the dilution (t_1), cooling to 5 °C (t_2), after thawing (t_3) it, and at one and two h post thaw (t_4 and t_5, respectively). The evaluation included the determination of motility score, percentage of motile spermatozoa, and percentage of progressive motile spermatozoa. We also evaluated the percentage of abnormal spermatozoa, spermatozoa with intact acrosome, and the percentage of spermatozoa with membrane integrity (determined by hypoosmotic swelling test; HOS). The data were compared using ANOVA for repeated measures, with treatments (extenders) and time (t_0; t_1; t_2; t_3; t_4 and t_5) as main factors, and the animal as a random effect. Samples with Triladyl had greater percentage of motile spermatozoa ($p = 0.03$), progressively motile spermatozoa ($p = 0.05$), and tended to have greater motility score ($p = 0.07$) and greater percentage of intact membrane ($p = 0.07$) than samples diluted with Andromed. No effect was found between treatment and time in the percentage of motile spermatozoa, progressive motile spermatozoa, in the motility score and in the percentage of spermatozoa with intact membrane. The percentage of abnormal spermatozoa and of spermatozoa with intact acrosome was not affected by the extender. Although Triladyl has egg yolk, it seems to be a better extender for cryopreserving pampas deer semen than Andromed.

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The role of IGF-I on developmental competence of oocytes recovered from Holstein and Nelore cows undergoing heat stress

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Summer heat stress compromises oocyte competence and preimplantation embryonic development in cattle. The deleterious effect of heat stress on the reproductive function is greater for *Bos taurus taurus* than *Bos taurus indicus* animals. There is evidence that heat-induced cellular damage in bovine oocytes can be manipulated by a wide range of biological factors, such as insulin-like growth factor-I (IGF-I). Therefore, the aim of this study was to evaluate the role of IGF-I on developmental competence of oocytes recovered from *Bos taurus indicus* (Nelore - NEL) and *Bos taurus taurus* (Holstein - HPB) cows undergoing heat stress. Non-lactating Holstein (n=6) and Nelore (n=6) cows were housed together and synchronized (pre-experimental phase: Day - 90 to 0). Animals were randomly distributed into Thermoneutral (TN, day 0 to 28: free stall dry bulb temperature of 25°C and relative humidity (RH) of 69%; n=3 HPB and n= 3 NEL) and Heat Stress [HS, Day 0 to 14: 37°C with 70% relative humidity (RH) during the day and 30°C with 90% RH during the night followed by Day 14 to 28: 38°C with 75% RH during the day and 30°C with 90% RH during the night; n=3 HPB and n=3 NEL] in an environmental chamber. During this period, animals were submitted to follicular aspiration (OPU) every 3 to 4 days. Cumulus-oocyte complexes (COCs) were morphologically classified and in vitro matured in Tissue Culture Medium 199 [TCM 199 – bicarbonate supplemented with 50 μg/mL gentamicin, 0.2 mM pyruvate, 2 μg/mL estradiol, 20 μg/mL FSH, 10 μg/mL LH, 6 mg/mL essentially fatty-acid free BSA] containing 0 or 25 ng/mL IGF-I. Then, COCs were fertilized and cultured in vitro. Cleavage and blastocyst rates were determined on days 3 and 7-9 after fertilization, respectively. Parametric data were analyzed by least-squares analysis of variance using the PROC-GLM and PROC MIXED procedures of SAS. The mathematical model included main effects and all interactions. The effect of cow nested within breed was considered random and all the other main effects were fixed. Non parametric data was analyzed by Kruskal- Wallis and Wilcoxon procedures of SAS. Exposure of NEL and HPB cows to *in vivo* HS reduced ($p< 0.05$) the percentage of grade I COCs regardless of breed. Similarly, there was a negative effect of temperature ($p< 0.05$) on the percentage of grade II HPB oocytes compared to NEL HS. Heat stress tended to reduce ($p= 0.07$) the percentage of grade II HPB HS COCs when compared to HPB TN. There was a negative effect of HS ($p< 0.05$) on cleavage rate regardless of breed. There was no effect of HS and IGF-I on developmental competence of NEL oocytes, while IGF-I modulated the ability of HPB oocytes to survive after HS. IGF-I increased ($p< 0.05$) the percentage of heat-shocked HPB oocytes that developed to blastocyst stage on day 9 after fertilization. The addition of 25 ng/ml IGF-I to IVM medium did not affect oocyte development to blastocyst stage in the TN group. In conclusion, HS reduced oocyte quality as well as cleavage rate in both breeds. Nevertheless, NEL oocytes proved to be more resistant to elevated temperature. In addition, IGF–I acted as a thermoprotector molecule rescuing heat-shocked oocytes from the deleterious effects of elevated temperature on developmental competence.

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Viability of spermatozoa recovered from equine epididymis after refrigeration at 4°C for 48 h

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In many occasions, horses suffering from bone fractures are euthanized. In the case of genetically or emotionally valuable stallions, it is desirable to recover gametes for preservation of the individual's genetic potential. Spermatozoa can be recovered from the epididymis immediately postmortem or after castration. However, the period of time between the harvest of the epididymitis and the arrival at the laboratory for gamete recovery might require refrigeration of the organs. Therefore, the aim of this study was to assess the viability of the sperm cells recovered from the epididymis of horses after 48 h at 4°C. Ten pairs of epididymis/testicle complexes were harvested from privately owned, adult horses. After orchiectomy, each complex was immersed in Lactated Ringer's solution and kept on ice for transport to the laboratory, which happened within 30 minutes, on average (up to two h maximum). One epididymis/testicle complex was kept at 4°C for 48 h (T48); the contralateral complex (T0) was dissected to isolate the epididymis tail, and spermatozoa were recovered via retrograde flushing. Semen characteristics (sperm motility, vigor, concentration, morphology, and membrane integrity) were analyzed. Testicles were longitudinally sectioned and imprints were prepared and stained for testicular cytology evaluation. Additionally, a testicular fragment was removed for histological analysis. After 48 h of refrigeration, all the procedures previously described were applied to the other epididymis/testicle complex. There was a decrease ($P < 0.05$) in sperm motility and vigor and an increase ($P < 0.05$) in percentage of spermatozoa with strongly coiled tails after 48 h of refrigeration. Testicular cytology revealed a decrease ($P < 0.05$) in the number of early spermatids at T48 and the number of Sertoli cells tended ($P = 0.06$) to increase at T48. No difference ($P > 0.05$) was observed between T0 and T48 in terms of histological findings (hydropic degeneration, number of interstitial macrophages and eosinophils, and number of spermatozoa). Results from this study indicate that, despite a decrease in sperm motility and vigor, semen samples recovered from the epididymis of stallions after 48 h under refrigeration could potentially be used in reproductive technologies such as intracytoplasmic sperm injection.

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Influence of caffeine and chlorogenic acid on the integrity of the sperm membrane and the rate of lipid peroxidation in boar semen stored at 15ºC for 72 h

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In commercial production, boar semen used for artificial insemination is normally stored at 15 to 18 ºC. Under these conditions, the preservation along time gradually reduces their fertility, being the lipid peroxidation induced by reactive oxygen species (ROS) one of the causes. Therefore, supplementation with antioxidants such as chlorogenic acid and metabolic stimulants like caffeine can improve the quality of semen. Thus, the aim was to evaluate the influence of caffeine and chlorogenic acid on the integrity of the sperm membrane and the peroxidation degree of boar semen stored at 15 °C until 72 hours. Twelve ejaculates were processed in randomized block design in 2 x 2 factorial schemes with 12 repetitions of one ejaculate. During processing, 0.0 (control) or 4.5 mg/ml of semen of chlorogenic acid were added to the insemination doses, which were maintained at room temperature in dark conditions for 120 minutes and then stored at 15 ºC. At 0, 24 and 72 hours of storage, 10 ml of semen were incubated in test tubes in a water bath at 37 °C and added of 0.0 (control) or 8.0 mM of caffeine. The sperm membrane integrity was evaluated by Eosin-Nigrosin at 0 and 120 minutes of incubation. At 60 minutes of incubation, the malondialdehyde (MDA) was quantified by the commercial kit. The data were subjected to analysis of variance and Scott-Knott test at 5%. Until 24 hours of storage, the chlorogenic acid and caffeine did not influence ($P>0.05$) the quality of semen. At 72 hours the chlorogenic acid reduced ($P<0.05$) the MDA concentration and increased ($P<0.05$) the sperm viability. Caffeine ($P>0.05$) did not affect these parameters, regardless of the storage time. According to these results it may be stated that the chlorogenic acid can be added in the cooled boar semen, but the caffeine addition must be better studied.

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Osmotic resistance and acrosome integrity of boar semen frozen with addition of chlorogenic acid in freezing media

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Cryopreservation of spermatozoa has been a great resource for the trade in genetic material and for gene banks. However, cooling and freezing are traumatic events for the sperm, and the heat shock generates significant cell damage, reducing the seminal quality post-thawed. Some reports have shown that the cryodamage induced by freezing and thawing can be minimized by antioxidants. Chlorogenic acid exhibits antioxidant activity decreasing the amount of reactive oxygen species produced during the cryopreservation process and, consequently, reducing the cellular oxidative stress. This study aims to evaluate the effects of adding different doses of chlorogenic acid to the frozen boar semen on their osmotic resistance and acrosome integrity after thawing. Twelve ejaculates from three boars (four ejaculates per animal) belonging to the Swine Experimental Center of the Federal University of Lavras were utilized. Immediately after collection, the ejaculates were diluted 1:1 in BTS® (Beltsville Thawing Solution) and cooled at 17 °C for 24 hours. Subsequently, the semen was frozen according to an adapted Westendorf method, adding different doses of chlorogenic acid (0; 1.5; 3.0; 4.5 and 6.0 mg/ml) to the freezing media. After two weeks in liquid nitrogen, the semen samples were thawed for 20 seconds in a water bath at 37 ^{o}C, resuspended in BTS (1:5) and maintained at 37 ^{o}C for 240 minutes. In the beginning and after 240 minutes of incubation the acrosome integrity was evaluated by staining POPE and the osmotic resistance by the hypo osmotic test. The data were submitted to the non-parametric analysis using Kruskal-Wallis test at 5%. There was no difference ($P>0.05$) between the levels of chlorogenic acid, regardless of the incubation time. The osmotic resistance and acrosome integrity decreased with time, regardless of the levels of chlorogenic acid. According to these data is possible to conclude that the addition of different concentrations of chlorogenic acid to the boar sperm freezing media did not affect the osmotic resistance or the acrosome integrity.

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Imprinted genes during the process of pluripotency acquisition in bovine cells

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The discoveries of pluripotency induction mechanisms revolutionized studies about the cellular events involved in the processes of undifferentiating and nuclear reprogramming, and although the generation of induced pluripotent stem cells (iPS) has been reported in several species, results in cattle are still inconsistent. In this study bovine fibroblasts and mesenchymal cells were subjected to direct reprogramming through integration and expression of known reprogramming factors (*Oct4*, *Sox2*, *Klf4* and *c-Myc murine-OSKM*) and the expression of imprinted genes *IGF2R* and *H19*, relevant for embryonic and fetal development, was analyzed during the reprogramming period. These genes have specific epigenetic regulation centers, known as Differentially Methylated Region (DMR), which promotes a different expression in the reprogrammed cells. The pluripotency induction was performed in three biological replicates in both cell types. Cell samples of D0 (control), D5, D12 and stabilized reprogrammed cells were collected. Colony formation of induced pluripotent stem cells (iPS) were derived only from fibroblasts. The alkaline phosphatase detection test was positive for the mentioned formed colonies. Expression patterns varied during the pluripotency induction process and in the established iPS colonies. The transcript *H19* level was higher ($p<0.05$) in fibroblasts (1 ± 0.58) than in mesenchymal cells (0.022 ± 0.032) without any changes ($p>0.05$) in these mesenchymal cells during the induction. The expression fibroblasts *H19* decreased ($p<0.05$) from D5 (1.67 ± 1.19) to D12 (0.67 ± 0.5), reaching 0.35 ± 0.23 in iPS cells. Regarding *IGF2R* transcripts, levels were initially lower ($p<0.05$) in fibroblasts (1 ± 0.92) compared to mesenchymal cells (3.14 ± 2.4). No difference in variations ($p>0.05$) was observed between D12 samples (1.5 ± 0.97) and stabilized iPS cells (0.08 ± 0.01), whereas this transcript level in mesenchymal cells decreased in D5 (0.75 ± 0.13) compared with the initial period. Therefore, we conclude that the in vitro induction of pluripotency in bovine fibroblasts and mesenchimal cells promotes changes in expression of imprinted genes, possibly by epigenetic modifications in the Imprinting Centers that regulate these genes, and the fact that mesenchymal cells were not efficiently reprogrammed is probably related to the initial epigenetic profile of the cell lines, referring to an importance of the "cellular memory" of the cells to be submitted to the pluripotency induction process.

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Evaluation of motility, vigor and total amount of sperm morphological abnormalities of boar semen stored at 15°C added with chlorogenic acid and caffeine

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One of the limiting aspects of the liquid storage of boar semen is the reduction in sperm quality over time, due to oxidative damage unleashed by the reactive oxygen species (ROS) which are generated during storage. In order to prolong the seminal viability during storage, substances with the potential to modulate cellular response and protection against oxidative damage are added to the dilution extender. Among these substances caffeine and chlorogenic acid can be mentioned. Caffeine is considered a metabolic activator by inhibiting the action of phosphodiesterase, allowing the maintenance of intracellular cAMP, whereas the chlorogenic acid is a polyphenol with antioxidant characteristics, reducing the amount present in the ROS. The aim of this study was to evaluate the motility, vigor and total amount of sperm morphological abnormalities of the boar semen cooled at 15°C and stored for 72 hours after the addition of caffeine and chlorogenic acid. Twelve ejaculates were evaluated and processed. The experiment was a 2x2 factorial design and arranged in a randomized block design, with split plot and 12 repetitions, represented by the ejaculates. During processing, different amounts of chlorogenic acid were added to the insemination doses, yielding final concentrations of 0.0 and 4.5 mg/ml of semen. The insemination doses were maintained at room temperature and protected from light for120 minutes and then stored at 15ºC. At 0, 24 and 72 hours of storage, aliquots of 10 mL were incubated in test tubes in a water bath at 37°C receiving or not 8 mM caffeine. Evaluation of motility and vigor were performed in optical microscopy at the beginning and at 120 minutes of incubation. The total amount of sperm morphological abnormalities were performed in the same times in phase-contrast microscopy after fixation of semen samples in formaldehyde-citrate solution. Motility data were subjected to analysis of variance and Scott-Knott test, and vigor and morphology data were subjected to the Friedman test. No difference ($P > 0.05$) was found between treatments before and after 24 hours of storage. After 72 hours cooling, the motility was influenced by the chlorogenic acid and caffeine ($P < 0.05$). The addition of both substances, isolated or associated, improved sperm motility compared to the control group at 10 minutes of incubation. At 120 minutes of incubation only samples supplemented with chlorogenic acid, associated or not with caffeine, showed better motility than other treatments. The sperm vigor was influenced by caffeine ($p<0.05$), since the samples that received this substance showed higher intensity of movement than the other samples, only at 10 minutes of incubation. The increased motility in the samples that received caffeine may be associated with the increased concentration of intracellular cAMP by blocking the action of the phosphodiesterase. The intracellular cAMP activates protein kinases that promoted phosphorylation of proteins of the flagellum, promoting cell movement. Chlorogenic acid, due to its antioxidant function, protects cell membranes from oxidative damage, thus maintaining its integrity and functionality during periods of storage and incubation. The total amount of sperm morphological abnormalities shown did not change with the use of chlorogenic acid and caffeine ($P > 0.05$), regardless of storage time and treatment. Therefore, the addition of 4.5 mg of chlorogenic acid, associated or not with 8 mM caffeine, provides better motility of boar semen stored for 72 hours. Furthermore, the use of caffeine associated or not with chlorogenic acid promotes greater motion intensity in reheated semen.

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Effect of chlorogenic acid on sperm viability and total amount of sperm morphological abnormalities after thawing

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Technologies in animal reproduction are studied during many years with significant improvement. Regarding the conservation of male gametes, many techniques are being developed to improve the reproductive efficiency, whereas the cold shock causes considerable cell damage during the freezing and thawing process in boar sperm, reducing seminal fertility rates. Antioxidants, such as chlorogenic acid, have been reported to have protective effects on several cells through the scavenging of the reactive species of oxygen and nitrogen. The aim of this study was to evaluate the addition of chlorogenic acid to boar semen cryopreservation media and its effects on viability and total amount of morphological abnormalities of spermatozoa after thawing. Twelve ejaculates from three boars (four ejaculates per animal) belonging to the Swine Experimental Center of Federal University of Lavras were utilized. Immediately after collection, the ejaculates were diluted 1:1 in BTS® (Beltsville Thawing Solution) and cooled at 17°C for 24 hours. After this, the semen was frozen according to an adapted Westendorf method, adding different doses of chlorogenic acid (0; 1.5; 3.0; 4.5 and 6.0 mg/ml) to the freezing media. After two weeks in liquid nitrogen, the semen samples were thawed for 20 seconds in a water bath at 37 ^{o}C, resuspended in BTS (1:5) and maintained at 37 ^{o}C for 240 minutes. Evaluation of sperm viability was performed in Eosin-Nigrosin staining at the beginning and after 240 minutes of incubation. The total amount of sperm morphological abnormalities were performed in phase-contrast microscopy only after 10 minutes of incubation. The obtained data was analyzed using the non parametric test of Kruskal-Wallis at 5% level. No differences ($p>0.05$) were found between the levels of chlrorogenic acid, regardless of the incubation time, for all parameters. The sperm viability decreased ($p<0.05$) at 240 minutes of incubation, regardless of the chlrorogenic acid levels. It was concluded that the chlorogenic acid added during semen processing does not affect the sperm viability and total amount of sperm abnormalities of thawed boar semen.

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Objective assessment of the effect of different extenders on frozen-thawed epididymal sperm from collared peccaries (*Pecari tajacu*)

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In Latin America, the captive breeding of collared peccaries has been stimulated due to the international demands of its pelt. Thereby, information concerning its reproductive biology and the application of reproductive biotechniques, such as sperm cryopreservation, are fundamental for the success of this commercial activity. In conventional sperm analysis, motility is evaluated subjectively, but the problem found in this kind of evaluation is the considerable variation existent between observers for the same sample. In order to improve sperm assessment, the use of computer-assisted semen analysis (CASA) has increased in the last years, providing an accurate, quick and objective assessment of different sperm parameters of motility. Thus, the aim of this study was to assess motility kinetic parameters of frozen-thawed collared peccary epididymal sperm cryopreserved with two different extenders, using the CASA. Epididymal cauda pairs from 12 sexually mature male collared peccaries were used. These animals belonged to the Centre of Multiplication of Wild Animals from UFERSA and they were submitted to an annual planned cull for population control, respecting all ethical and animal welfare standards. Epididymal sperm were recovered using the retrograde flushing technique. For each animal, one epididymis was flushed with 5 mL of powdered coconut water (ACP-116c) based extender, while the other was flushed using 5 mL of a buffered media based on Tris. After recovery, samples were evaluated for overall quality and diluted in the same extenders plus 20% egg yolk and 6% glycerol. Finally, samples were cryopreserved using a protocol previously defined for the species. After two weeks samples were thawed at 37ºC for 1 min. The evaluation of the motility kinetic parameters was conducted by using the CASA (HTM-IVOS 12, Hamilton Thorne Research, USA). Five fields per sample were selected for the evaluation of the total motility (TM), progressive motility (PM), velocity average pathway (VAP), velocity straight line (VSL), velocity curvilinear (VCL), amplitude lateral head (ALH), beat cross frequency (BCF) and straightness (STR). Comparison between extenders was performed by t-test ($P < 0.05$). Among all the results, we verified that Tris extender promoted a more efficient ($P < 0.05$) preservation of TM (47.6 ± 7.9 vs 12.3 ± 2.9 %), PM (21.8 ± 4.3 vs 4.5 ± 1.0%), and VCL (108.7 ± 7.6 vs 86.9 ± 6.1 µm/sec) parameters than ACP, respectively. Regarding other kinetic parameters, no differences were observed between the extenders. In addition, it was observed that egg yolk was not totally dissolved when ACP was used, which resulted in the formation of a great amount of debris that interfered in the CASA results and, probably, in the availability of the extender components for the sperm during the cryopreservation process, reflecting on a poor post-thaw quality. In conclusion, the extender based on Tris showed a superior kinetic evaluation in comparison to ACP, becoming the most recommended for the cryopreservation of collared peccary epididymal sperm.

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Evaluation of cholesterol incorporation to the goat sperm membrane by the perivitelline membrane of hen's egg yolk binding test and gas chromatography

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The cryopreservation process causes many injuries to the sperm membrane such as destabilization of cell membranes. The sperm membrane sensitivity to changes in temperature is most related to its lipid-protein composition and the presence of cholesterol and phospholipids. Species with a higher concentration of cholesterol have less damage to the membrane during the cryopreservation process. The aim of this study was to add cholesterol carried by cyclodextin (Cyclodextrin-Cholesterol Complex - CCC) to goat semen to see if the inclusion of cholesterol is able to improve the fertilizing capacity of cryopreserved sperm, and to evaluate whether this CCC was effective incorporated in sperm. These assessments were performed by perivitelline membrane of hen's egg yolk (MPEY) binding test and gas chromatography evaluation, predicting the fertility of spermatozoa and to quantitatively evaluate the inclusion of cholesterol in the sperm membrane respectively. Four male goats were used: 2 Saanen and 2 Parda Alpine. The collections were by artificial vagina and after obtaining ejaculates, they were divided into the following treatments: TG – Tris-glycerol diluent; TGCCC – Cyclodextrin-Cholesterol Complex + Tris-glycerol diluent. The experiment was conducted in a completely randomized design. To perform the perivitelline membrane of hen's egg yolk (MPEY) binding test and gas chromatography, the methodology described by Barbato et. al (Barbato et. al, 1998, Biology of Reproduction, 58, 686-699) and by Collins et. al (Collins et al., 2006, Fundamentals of Chromatography, 453p.) was used. For the MPEY biding test they were compared by the Kruskal-Wallis test. The level of significance adopted was $\alpha = 0.05$. Data referring to gas chromatography was submitted to square root transformation ($x' = \sqrt{x}$) to normalize the data, then submitted to analysis of variance (ANOVA) and Duncan Test at 5% probability. The CCC did not improve ($P>0.05$) the binding capacity of the goat sperm to the MPEY (TG – 1147.8±257; TGCCC 1282.2±172.9). This may have been due to the excess of sperm incubated with the perivitelline membrane not having enough challenge to the sperm and also because of the long period of incubation. The insemination dose has a great importance in the test, in order to avoid saturation of spermatozoa with MPEY due to the limited number of available binding sites on the membrane. The lack of differences between the treatments suggest that the cholesterol did not incorporate to the sperm membrane, which is demonstrated in gas chromatography (TG – 26.3±11.5 ppm; TGCCC 27.4±15.8 ppm). This may be related to the concentration of CCC (1 mg), which showed insufficient to increase the concentration of cholesterol in the goat sperm membrane, showing no difference ($P>0.05$) between the treatments. The concentration of sperm membrane cholesterol increased linearly according to the raise in the concentration of cholesterol-cyclodextrin complex incubated with the sperm. Therefore, both tests are effective to determine the presence of cholesterol in the cryopreserved goat sperm.

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Comparative analysis of three extenders for buffalo semen cryopreservation

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The aim of this study was to test three different extenders containing egg yolk for cryopreservation of buffalo semen. The experiment was conducted at the Center for Biotechnology in Bubalinocultura - UFMG, in Pedro Leopoldo, MG, using six Murrah bulls, aged 29-36 months. Six ejaculates were collect by artificial vagina. After semen collection, analyzes of subjective motility, vigor, force tourbillon, sperm concentration (Neubauer chamber), sperm morphology and hypoosmotic test (phase contrast microscopy) were performed. The ejaculates were separated and diluted according to the treatment until it reached a final concentration of $50x10^6$ sperm/mL. Three extenders were used: Tes-Tris and Tris-citric acid, both containing 20% egg yolk, and commercial extender Botubov®, according to the manufacturer's recommendations. The diluted semen was packaged in 0.25 ml straws, and cooling was performed on a computerized machine (TK 4000), using a cooling rate of -0.25°C/min to 5°C. Semen was kept in balance at 5°C for 4 hours. The straws were frozen in an ice chest, kept at 5cm from the surface of liquid nitrogen for 20 minutes and then immersed in liquid nitrogen. The samples were kept bottled in nitrogen until thawing. After thawing (two straws for each treatment) thermal resistance test (TTR) assisted by CASA (120 minutes, with assessments conducted every 30 minutes), hypo-osmotic test and membrane integrity by fluorescent probes (CFDA / Pi) were done. The average results of these three tests were compared by Anova followed by Tukey test ($P < 0.05$). The post-thaw motility was similar ($P > 0.05$) between groups (41.0 ± 14.2, 37.1 ± 10.1, 32.8 ± 11.8; respectively for commercial extender, Tes-Tris and Tris-citric acid). Thirty minutes after thawing, the commercial and Tes-Tris extenders showed higher ($P < 0.05$) motility compared to Tris-citric acid (31.7 ± 12.6, 12.6 ± 26.1 versus 18.8 ± 8.6, respectively). The values of VCL, VSL, VAP, LIN and BCF were higher ($P < 0.05$) in the commercial extender compared with Tes-Tris and Tris-citric acid. There was no difference ($P > 0.05$) between treatments regarding the integrity of membranes, or when evaluated by the hypoosmotic test (47.3, 49.2 and 50.2 reactive) or when assessed using fluorochromes (39.2, 27.4 and 29.8 of intact). The commercial and Tes-Tris extenders similarly cryopreserved buffalo sperm.

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Oocyte recovery from Santa Ines ewes subjected to laparoscopy follicular aspiration by a modified lateral position approach

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The laparoscopic ovum pick-up (LOPU) in small ruminants is a reproductive biotechnology that has been widely applied over the past years for *in vitro* embryo production research with efficient results. However, one of the challenges of videolaparoscopic positioning is the cardiorespiratory depression caused by the Trendelenburg position (a reverse bench press 45°) that is conventionally used for this procedure. The aim of this study was to evaluate the oocytes' number and quality recovered by LOPU from Santa Ines ewes positioned conventionally in Trendelenburg (GT, n=12) and in the right lateral decubitus position with an inclination of 10 ° (GL, n=12). Both groups did not receive previous hormone treatment and were submitted to a unique follicular aspiration session using an 18G needle. The oocytes were recovered in collecting media consisting of phosphate buffered saline (PBS) supplemented with 10 IU/ml heparin at 36 °C. At laboratory the oocytes were observed and classified using a stereomicroscope. Statistical analysis was performed with the aid of R® software (R Foundation for Statistical Computing, Vienna, Austria). The experimental design corresponded to a completely randomized design. Data were analyzed by analysis of variance and when the difference between the treatments was significant the means were compared by Tukey test ($P < 0.05$). No differences ($P>0.05$) were found in the number (4.75±3.82 and 5.08±3.90) and quality (1.92±1.62 and 2.17±1.75) of oocytes recovered between GT and GL, respectively. The oocyte recovery from ewes subjected to LOPU using a right lateral decubitus position with an inclination of 10° were a viable technique with a great potential to improve the assisted reproductive techniques in this species, avoiding diaphragm compression caused by the Trendelenburg position. However, difficulties in performing the technique, anesthetic complications, postoperative recovery, among others, should be investigated in this new lateral position.

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Gestation rate of Santa Ines breed ewes synchronized with Ovsynch protocol using artificial insemination or natural matting

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The present experiment aimed to compare the pregnancy rates of ewes synchronized using the Ovsynch protocol inseminated with frozen semen and submitted to a controlled natural mating. Thirty seven Santa Ines breed cyclic ewes were used in the study, with mean weight of 45kg, from 2 to 4.5 years old, all being dewormed regularly and clinically healthy, fed in pasture (*Panicum maximum* var. mombaça), with balanced ration, mineral salt and water *ad libitum*. In group 1 (G1; n=11), the animals received a dose of 25µg of GnRH analog (buserelin) on day zero (D0), and seven days later (D7) a dose of 37.5µg of PGF2α was injected and on day 9 (D9) a second dose of GnRH analog was administered. The ewes observed in heat were inseminated 12 hours after the detection. In group 2 (G2; n=7), the same hormonal protocol was used, but the ewes were inseminated twice, at the moment of heat detection and 12 hours later. In group 3 (G3; n=9), the same hormonal protocol was used, and as a positive control, the ewes were put into natural matting 12 hours after heat detection. And in group 4 (G4; n=10), the same hormonal protocol was used, and the ewes were submitted to a natural matting at the moment of the heat detection and 12 hours after heat signs. The heat detection was performed immediately after the second dose of GnRH analog, using a teaser at the feedlot for 30 minutes, every 12 hours during 4 consecutive days. The artificial insemination was performed according to the previously described protocol (Wulster-Rradcliffe, Theriogenology, v.62, p.990–1002, 2004). The pregnancy examination was executed by transrectal ultrasonography examination 30 days after the last insemination or matting. The results of gestation rate and heat synchronization rate were compared among groups using Fisher`s Exact test, combined with the four groups` Freeman-Halton extension test, in 5% of significance. From the total of hormone treated ewes, 81.1% (30/37) had shown signs of heat. The sexual behavior started 12 to 24 hours after the end of hormonal treatment (D9) in 90% (27/30) of the synchronized ewes and only 10% (3/30) showed heat between 24 and 48 hours. The pregnancy rate was different ($P<0.05$) among groups: 27.3^{b}% (3/11) in G1, 33.3^{b}% (1/3) in G2, 44.4^{b}% (4/9) in G3 and 85.7^{a}% (6/7) in G4. All observed pregnancy rates are in agreement with the literature (Oliveira, Arch. Zootec., v.58, p.281-284, 2009). G4, in which the ewes were submitted to natural mating at the moment of heat detection and 12 hours later, had the best pregnancy rate among all groups ($P<0.05$). Based on previously reported data, it can be concluded that the Ovsynch protocol used for ewe`s estrous synchronization had a promising result, especially due the higher pregnancy rate observed in G4.

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Pregnancy rates after using FTAI protocols with different doses of GnRH in Nelore cows

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The use of GnRH in estrus synchronization protocols has been largely studied due its importance for the control of gonadotropins release. The present study aimed to compare the pregnancy rates in Nelore cows using protocols of fixed-time artificial insemination (FTAI) with different doses of GnRH. Nelore cows (n=52) were divided in four experimental groups: G1 (n=13) cows received a vaginal implant (second use) and 2.0 mL of estradiol benzoate (EB) on day 0 (D0, 8 am), and on day 8 (D8, 8 am) the implant was removed and 1.5 mL of ECG, 0.5 mL of ECP and 2.0 mL of cloprostenol were administered. On day 10 (D10), 1.0 mL of saline was injected (7 am) and the cows were inseminated (3 pm). G2 (n=13): the same protocol was used, but 1.0 mL of GnRH was administered on D10; G3 (n=13): the same protocol was used, but 0.8 mL of GnRH was administered on D10; and, G4 (n=13): the same protocol was used, but 0.5 mL of GnRH was administered on D10. All drugs were administered intramuscularly. After 45 days the ultrasonography examination was performed to assess pregnancy rate. The statistical analyses used was Fisher`s exact test, with p correction using Monte Carlo independence test for more than two groups, at the significance level of 5%. The pregnancy rate were different ($p<0.05$) among groups: G1: 53.8^{ab}% (7/13), G2: 76.7^{a}% (10/13), G3: 61.5^{ab}% (8/13) and G4: 38.5^{c}% (5/13). G2 (1.0 mL of GnRH) had the better pregnancy rate among all groups, while G4 had the worse rate (even worse than the saline group).

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Different transport periods and temperatures of slaughterhouse ovaries on qualitative-quantitative parameters evaluated by morphological criteria and brilliant cresyl blue test in bovine oocytes

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The oocyte quantity and quality can be altered by several factors during all steps of *in vitro* embryo production. After ovary removal, the oscillations of temperature and transport period of slaughterhouse ovaries until follicular aspiration are initially essential conditions for maintaining the oocyte quality. Therefore, the aim of this work was to evaluate the qualitative-quantitative parameters [number of oocyte *per* ovary, recovery rate, GI to GIV oocyte percentages] in bovine oocytes derived from slaughterhouse ovaries transported at different temperatures and periods using morphological criteria and brilliant cresyl blue (BCB) test. Thus, the experiment was conducted in two stages. In the first step, ovaries were transported to the laboratory in saline solution at two different temperatures (25°C *vs.* 35°C) during 2 h. In the second stage, ovaries were transported at a temperature established in the first stage in two transport periods (2 h *vs.* 10 h). For both experiments, follicles (2-8 mm) were aspirated using the system of needle 21 G and syringe 10 mL. After the aspiration, the follicular content was assessed under stereomicroscope and oocytes classified by morphological criteria as: grade I (≥ 3 layers of compact *cumulus* cells and homogeneous cytoplasm), grade II (1-2 layers of compact *cumulus* cells and homogeneous cytoplasm), grade III (< 1 layer of *cumulus* cells and heterogeneous cytoplasm) and grade IV (degenerated oocyte). Oocytes categorized as GI and GII were considered viable while GIII and GIV were non-viable structures. Subsequently, all oocytes were incubated in BCB (26 µM, Sigma, USA) for 60 min at 38.5°C. After exposure, the structures were washed and categorized as viable when they had blue coloration, and non-viable when colorless. All data were analyzed by the Fisher exact test ($P<0.05$). In the first step, after four repetitions, a total of 102 ovaries, distributed in 50 and 52 ovaries, resulted in 105 and 240 structures obtaining an average of 2.1 and 4.6 oocytes/ovary and a recovery rate of 21.7% (105/484) and 42.9% (240/559) for 25°C and 35°C, respectively. Differences ($P<0.05$) were observed for number of oocyte *per* ovary and recovery rate between the temperatures tested. However, no difference was observed for oocyte quality assessed by both morphological criteria for viable [GI and GII to 25°C: 73 (69.5%) *vs.* 35°C: 155 (64.6%), $P>0.05$] and non-viable oocytes [GIII and GIV to 25°C: 32 (30.5%). *vs.* 35°C: 85 (35.4%), $P>0.05$], and for the BCB test for viable [25°C: 75 (71.4%) *vs.* 35°C: 147 (61.3%), $P>0.05$] and non-viable oocytes [25°C: 30 (28.6%) *vs.* 35°C: 93 (38.7%), $P>0.05$]. Such differences are related to the operator and both temperatures could be used. However, observing the maintenance of structures at temperatures near the animal physiological temperature, 35°C was used for further experiments. In the second stage, after five repetitions, a total of 66 ovaries, distributed in 34 and 32 ovaries, resulted in 125 and 127 structures obtaining an average of 3.7 and 4.0 oocytes/ovary and a recovery rate of 41.9% (125/298) and 44.6% (127/285) for 2 h and 10 h of ovary transport, respectively. No difference ($P>0.05$) was observed between the transport periods for the number of oocyte *per* ovary and recovery rate between the transport periods tested. Moreover, the assessment of oocyte quality using morphological criteria did not differ between the times tested for viable [GI and GII for 2 h: 65 (52.0%) *vs.* 10 h: 74 (58.3%), $P>0.05$] and non-viable oocytes [GIII and GIV for 2 h: 60 (48.0%). *vs.* 10 h: 53 (41.7%), $P>0.05$]. However, differences were observed using the BCB test for viable [2 h: 87 (69.6%) *vs.* 65 (51.2%)] and non-viable oocytes [2 h: 38 (30.4%) *vs.* 10 h: 62 (48.8%), $P<0.05$]. Thus, the transport period affected the oocyte quality evaluated by BCB test. In conclusion, the transport of the ovaries using 25°C or 35°C and the period of 2 h and 10 h did not affect the GI and GII oocyte recovery number; nevertheless, the oocyte quality evaluated by BCB staining was reduced when the transport period of slaughterhouse ovaries to the laboratory takes 10 h.

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Evaluation of bovine immature oocyte quality using brilliant cresyl blue and trypan blue

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In vitro embryo production is a tool of high scientific and commercial value that maximizes genetic gain in cattle production. The success of this technique depends on several factors such as the quality of immature oocytes. In general, the oocyte quality before *in vitro* maturation can be determined by morphological criteria. However, other techniques have been developed with the use of dyes, such as brilliant cresyl blue (BCB) and trypan blue (TB). The BCB test is based on the capability of the glucose-6-phosphate dehydrogenase (G6PDH) to convert the BCB stain from blue to colorless. This enzyme is active in the growing oocyte, but has decreased activity in grown oocytes. The TB test is based on assessing the *cumulus* cells viability by exclusion analysis. Therefore, the aim of this work was to evaluate the effectiveness of two methods of selection, BCB *vs.* TB dyes, of bovine viable oocytes. Thus, ovaries derived from a local slaughterhouse were transported to the laboratory in saline solution (NaCl, 0.9%) at 35-37°C. All follicles (2-8 mm) were aspirated using a system consisting of 5 mL syringes and 21 G needles. After the aspiration, oocytes recovered were classified under stereomicroscope by morphological criteria as: grade I≥(3 layers of compact *cumulus* cells and homogeneous cytoplasm), grade II (1-2 layers of compact *cumulus* cells and homogeneous cytoplasm), grade III (< 1 layer of *cumulus* cells and heterogeneous cytoplasm) and grade IV (degenerated oocyte). Only oocytes categorized as GI and GII were considered viable structures and used for staining tests. For the BCB test, structures were incubated with BCB (26 µM, Sigma, USA) for 60 min at 38.5°C. After exposure, oocytes were washed and classified as viable if they had blue stained cytoplasm and non-viable when colorless. For the TB test, *cumulus* cells were removed by repeated pipetting (10-15 oocytes/100 µL of medium). The cell suspension was diluted in TB (0.2%, Sigma, USA) at a 1:40 ratio and viable cells (live/colorless) and non-viable (dead/blue) were counted in a Neubauer chamber. All data were expressed as percentage and analyzed by the Fisher exact test ($P<0.05$). In the BCB test, the percentage of viable oocytes was calculated by dividing oocytes with blue staining cytoplasm by the number of viable oocytes assessed by morphological criteria x 100. In the TB test, the percentage of viable oocytes was calculated as the proportion of oocytes with viable cells by viable oocytes assessed by morphological criteria x 100. Thus, the ratio of viable oocytes by morphological criteria was 100% viable *cumulus* cells. After four repetitions, a total of 64 ovaries resulted in 171 structures, obtaining an average of 2.7 oocytes/ovary. Of these, 50.9% (87/171) and 49.1% (84/171) were classified as viable and non-viable oocytes, respectively, according to morphological criteria. The 87 viable oocytes were distributed in 44 and 43 structures for BCB and TB tests, respectively. The percentage of viable oocytes according to the BCB test was 79.5% (35/44). The percentage of viable oocytes according to the TB test was 69.8% (30/43), calculated on the viable *cumulus* cells (146/212, 68.9% of viable cells of 30 oocytes). No difference was observed between both methods ($P>0.05$). In conclusion, both tests, BCB *vs.* TB, can support the evaluation by morphological criteria for the selection of bovine viable oocytes. Although the TB test excludes the use of these oocytes for the next steps, this test could be an important tool to estimate the oocyte quality by *cumulus* cell viability, which has a fundamental role in the development of *in vitro* competence.

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Morphological distinct sperm cell subpopulations determined by harmonic Fourier descriptors and cluster analysis are found in ejaculates of *Bos taurus taurus* and *Bos taurus indicus* bulls

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Harmonic Fourier descriptors (HFD) are commonly used for description and analysis of shapes and have been used for characterization of sperm morphology. Studies in several species identified different subpopulations of spermatozoa, mainly analyzing kinetic parameters with the CASA system. Some morphological characteristics of sperm cells can also determine different cell clusters, thus the aim of this study was to evaluate and compare the existence of these subpopulations in *Bos taurus taurus* and *Bos taurus indicus* bulls by HFD and cluster analysis. Eighteen Simmental and nine Nelore breed bulls were used. For each animal 100 images of sperm heads considered normal were acquired for HFD and 200 for cluster analysis, using light microscopy (Olympus BX41) with a green filter (Olympus IF550), and 400 X magnification, coupled to a CCD camera (Charge Coupled Detector - SDC-415 , Samsung) connected to a computer. All images were processed using the software Image J. Simmental bull spermatozoa were analyzed by HFD principal component analysis, while cells of Nelore bulls were classified by cluster analysis using the R statistical program, considering the characteristics area, centroid, perimeter and center of mass for each sperm head. Preliminary results showed the existence of three or four distinct cell clusters in the two different subspecies. Ejaculates of two Simmental bulls, analyzed until now, considering the first two HFD components, showed one cluster of cells with high similarity in quadrant pc1 and pc2 0e +00 -0.00004. Considering the same HFD components, two more cell clusters were also observed, characterized by a more pronounced dispersion and lower similarity within its cells and with the cells of the major cluster, in quadrants pc2 0e+00 to 5e-05 and pc1 -0.0007 to 0.00000, that suggests three morphological distinct sperm subpopulations. Fifty five percent of the Nelore bulls evaluated by cluster analysis had four sub-populations, while 45% had three. Distinct morphological features of spermatozoa characterize different cell clusters, present in *Bos taurus taurus* and *Bos taurus indicus* bulls, and can be determined by HFD and cluster analysis.

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Comparison of pregnancy rate between primiparous and multiparous Nelore heifers submitted to fixed time artificial insemination

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The aim of this study was to evaluate the reproductive performance of Nelore cows in a fixed time artificial insemination (TAI) program on pregnancy rates by comparing the categories of primiparous and multiparous heifers. For the experiment we used the Nellore females belonging to the same herd on property located in the coastal lowlands of the state of Rio de Janeiro, kept on pasture divided into three groups and submitted to the same synchronization protocol for TAI (D0- 2,0 mg of estradiol benzoate + bovine intravaginal device with 1.0g progesterone, D8- implant removal + 250µg of cloprostenol + 300 IU eCG, D9- 1.0 mg.of Bz. estradiol, D10- IATF). The groups were divided according to rank, in group I, n=150 heifers, in group II n=150 primiparous heifers and in group III n=181 multiparous heifers. The overall pregnancy rate was 86% for group I (BCS = 3.2 ± 0.4), 45.3% for group II (BCS = 2.1 ± 0.2) and 76.8% for group III (BCS = 2.4 ± 0.4). The results indicated significant difference in pregnancy rates between groups I and II and between groups II and III, with no difference between groups I and III However, when compared with BCS the pregnancy rate between the groups showed a significant difference between all groups evaluated. The results indicate that primiparous Nelore cows showed low reproductive performance in the TAI program used when you compare to heifers and multiparous of the same race, with BCS influencing the results found for this category, which affected the reproductive efficiency of studied herd.

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Horse spermatogonial stem cell cryoreservation and transplantation

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Suitable conditions for germ cell cryopreservation and storage are essential for the successful spread of the germ cell transplantation and the preservation of the genetic stock of valuable animals. In this context, the aims of the present study were to evaluate the effects of different cryopreservation protocols on the viability and survival rates of horse spermatogonial stem cells (SSCs) and investigate the success of germ cell transplantation from horse (donors) to mule (natural infertile recipient). Testes from 10 horses were enzymatically treated and the presence of SSCs in the obtained cell suspension was evaluated through GFRA1 receptor immunolabeling and western blot assay. In order to cryopreserve these cells, three different cryoprotectant media were utilized [DMSO+DMEM+10%BFS (1); ethylene glycol (2); DMSO+sucrose (3)], associated with different methods (vitrification, slow and fast-freezing). These cell suspensions were also prepared to perform the germ cell transplantation into the testes of two mules. Based on pre and post-thawing rates of viable SSCs, as well as the number of recovered cells after cryopreservation, the best results were obtained using DMSO-based cryoprotectants and fast (Medium 3) and slow freezing methods (Media 1 and 3). In addition, after culture (24 days), the MTT test data have indicated that the cryopreserved cells were as metabolically active as the fresh cells and they were also expressing GFRA1 protein. Regarding the germ cell transplantation, six months after the surgical procedure we could observe the presence of spermatozoa in the mule ejaculates. Genetic analyses are currently being conducted in order to verify if these cells are derived from transplanted horse spermatogonia. In summary, the results so far obtained have indicated that equine SSCs could be cryopreserved without impairment of their metabolic activity and stemness capacity. In addition, preliminary SSC transplantation findings suggest that mule seminiferous tubules are able to sustain complete development of horse spermatogenesis.

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Efficient gene delivery to Nile tilapia's male germline stem cells by using functionalized carbon nanotubes

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Male germline stem cells (MGSCs) are important cells to study because they possess a unique ability to transmit genetic information to offspring, through sperm generation. In the present study, we aimed to investigate the efficiency of gene delivery to Nile tilapia's MGSCs using different plasmidial DNA delivery strategies. We also investigated the induction of cytotoxicity that these delivery strategies might trigger on these cells. Nile tilapia's MGSCs were obtained in DMEM/F12 supplemented medium after testicular enzymatic dissociation, density gradient centrifugation and differential plating. The plasmidial DNA used was the pAmCyan1-N1 plasmid (Clontech) also containing the inserted sequence of the promoter region from the Nile tilapia's endogenous constitutively active gene: β-actin. The delivery to these cells was performed by using Lipofectamine® 2000 (Life Technologies), electroporation (225V and 50 μF) and carboxylated/hydroxylated multiwall carbon nanotubes (fMWCNTs). The fMWCNTs were produced by the chemical vapor deposition method (CVD) and functionalized through oxidation in nitric/sulfuric acid using microwaves. After transfection, MGSC's viability was determined using the Annexin-V-Fluos Staining kit (Roche). The cyan fluorescent protein's (CFP) expression was accessed through fluorescent microscopy: 24 hours after the delivery and after cells' incubation at 28ºC and 5% CO_2. Through RT-PCR and q-PCR the transgene and β-actin's mRNA production were compared as an indicative of the MGSCs' capacity of expressing the CFP gene. By performing these experiments it was possible to observe that, 24 hours after transfection, the expression of the fluorescent protein was achieved in cultured MGSCs transfected through all the delivery strategies tested. When it comes to the cytotoxicity induced by these different plasmidial DNA delivery strategies, the electroporation induced decrease in SSCs viability (23.5%) at levels higher than those induced by carbon nanotubes (1.4%) and Lipofectamine® 2000 (4.8%). The transgene's transcription rates, accessed by RT-PCR and q-PCR, revealed that the highest value of CFP's mRNA production was obtained on the plasmidial DNA delivery performed by fMWCNTs. These findings provide the first step in establishing a methodology that will allow genetic manipulation of fish MGSC: a key cell for the production of transgenic fishes. So, the results obtained represent an important achievement to contribute to the progress of biotechnologies in aquaculture.

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Cryopreservation of testicular tissue: a potential tool for the conservation of male genetic material from collared peccaries (*Pecari tajacu*)

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Attempts to develop reproductive biotechnologies can contribute to collared peccaries' (*Pecari tajacu*) conservation and multiplication. The preservation of testicular tissue is a relatively new practical and applicable tool for the conservation of threatened or genetic valuable individuals, providing an important material for other biotechniques such as xenotransplantation or in vitro fertilization. Therefore, we aim to establish an appropriate protocol for the male gonadal tissue storage in collared peccaries. From four mature males, both testicles were collected, totalizing 08 testes. Each testes was divided in 07 fragments (~3mm), from which one was immediately submitted to morphology analyses (fresh group). The other fragments were submitted to a protocol for solid surface vitrification using ethylene glycol (EG), dimethylformamide (DMF) or dimethylsulfoxide (DMSO), at a 3 or 6 M concentration, as cryoprotectants. Samples were stored in cryotubes placed on a liquid nitrogen container. After proximately one month, samples were thawed at 37°C, following the washing in growing concentrations of sucrose. Then, the morphological analysis was conducted and 15 micrographs were taken from each sample by using a Leica DM2700® microscope. Seminiferous tubules were evaluated according to the preservation of nucleus (0–5) and epithelia (0–5), following scores in which the lower the score, the better the integrity of the tissue (Milazzo JP et al. 2008. Hum Reprod, 23: 17–28). The effect of different cryoprotectants and its concentration on testicular tissue integrity was verified by Mann-Whitney test ($P < 0.05$). As the main results, a nuclear score of 1.01 and an epithelia score of 0.99 was verified in fresh samples. After thawing, the best nuclear scores ($P < 0.05$) of preservation were achieved in the use of EG 3M (2.53) and EG 6M (2.71), in which the observation of an intact nucleolus was possible in more than 50% of the germ and Sertoli cells. Regarding epithelia scores, the more efficient ($P < 0.05$) preservation was provided by EG 3M (2.32), EG 6M (2.51), DMSO 3M (2.24), and DMSO 6M (2.19), in which only few cell membrane were detached from the epithelia of the seminiferous tubules and a low number of cells presenting vacuoles was verified. In general, results indicate that vitrification promotes several alterations on testicular tissue structure, and the choosing of the ideal cryoprotectant is of paramount importance for the efficiency of the technique. In conclusion, we suggest that testicular tissue derived from collared peccaries could be submitted for solid surface vitrification using 3 or 6 M ethylene glycol as a cryoprotectant, once this substance is the one that more efficiently promotes the preservation of both nuclear and epithelia scores. Furthermore, the technique provides enough intact male germ cells to be destined to other biotechniques. (CEEUA/UFERSA/Process#23091.000254/11-88).

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Final maturation and ovulation in *Leporinus macrocephalus* submitted to different hormonal treatments

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The aim of this study was to evaluate the effect of different hormonal treatments on *Leporinus macrocephalus* final maturation and ovulation processes. Mature females were submitted to the following treatments routinely used in fish farms: mammalian gonadotropin-releasing hormone in liquid form (mGnRHa – L, single dose) (7 μg kg^{-1}); mammalian gonadotropin-releasing hormone in solid form (mGnRHa – S) (20 μg kg^{-1}, single dose); crude extract of carp pituitary extract (CPE) (0.5 and 5.0 mg kg^{-1}, 12h interval) and human chorionic gonadotropin (hCG) (5 and 10 IU kg^{-1}, 12h interval). CPE and mGnRHa - S, but not GnRH - L and hCG, have provided viable embryos. Ovulation rates in mGnRHa - L, mGnRHa - S (100%) and CPE (71.4%) were similar. Fertilization success of the eggs of mGnRHa - S (93.7 ± 3.0%) and CPE treatments (63.6 ± 16.5%) were superior to mGnRHa - L treatment (6.3 ± 2.5%) ($p<0.05$). Hatching success in CPE (59.1 ± 15.3%) and GnRHa - S (89.2 ± 1.7%) treatments were higher in comparison to m GnRHa - L treatment (0%). By means of histological evaluation we observed, at the time of ovulation, that the volume density of post ovulatory follicles (POFs) was higher in mGnRHa - S comparing to hCG and mGnRHa - L treatments. The average diameter of the eggs was higher in CPE and mGnRHa - S when compared to mGnRHa - L in all periods post fertilization evaluated ($p<0.05$). In carps, the germinal vesicle break down (GVBD) process with a consequently successful ovulation has been reported with a concomitant shift in the steroidogenic profile at the time of ovulation with an increase in circulating 17α,20β-dihydroxy-4-pregnen-3-one (17,20-P) and a transient decrease of estradiol (E_2) levels. However, in *L. macrocephalus* a successful ovulation was not necessarily related to a 17α - OHP (the main precursor of 17.20-P) peak, which was found in CPE and mGNRH - L, but not in GnRH - S treatment. Moreover, in all the treatments E_2 plasma levels kept increasing up to the time of ovulation and a transient decrease was not observed. The best reproductive performance, found in mGnRH – S treatment, was associated with the highest levels of POFs in ovaries evaluated at the time of ovulation, and with a regular process of egg hydration. The failure in obtaining viable eggs in mGnRHa – L treatment seemed to be associated with a less intense process of egg hydration in this treatment. Concluding, differently from what has been observed in carps, we did not find an association between a steroidogenic shift and successful ovulation in *L. macrocephalus*. GnRH – S and CPE treatments are effective for obtaining viable embryos in this species and the failure in obtaining viable embryos with mGnRH – S seems to be related to a less intense hydration.

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Innovative use of *Aloe vera* as a cryoprotectant for the collared peccaries (*Pecari tajacu*) semen freezing

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The collared peccary (*Pecari tajacu*) is one of the most hunted species in Latin America due to the appreciation of its meat by local populations and the value of its leather in the international market. Once these animals have demonstrated a great potential for captive breeding, the enhancement of reproductive biotechnologies would help the species conservation and the improvement of reproductive management in commercial explorations. In order to improve semen freezing protocols, the search for alternative cryoprotectants has been proposed and the use of *Aloe vera* (AV) is highlighted since it presents a similar action as the conventional cryoprotectants, but also presents the advantage of avoiding the possibility for disease dissemination when using animal substances such as egg yolk. The aim of the study was to evaluate the effect of *Aloe vera* in substitution or association to egg yolk on the cryopreservation of collared peccary semen. Ejaculates from eight sexually mature male collared peccaries were used. These animals belonged to the Centre of Multiplication of Wild Animals from UFERSA. Initially, they were physically restrained using a hand net and then anesthetized using intravenous administration of propofol (Propovan®, Cristalia, Fortaleza, Brazil), given as a bolus (5 mg/kg). Semen collection was performed using an electroejaculator (Autojac®, Neovet, Campinas, SP, Brazil) connected to a 12 V source. After collection, semen was evaluated and extended, at room temperature (27 °C), in Tris-fructose and then divided into three treatments: isolated Aloe Vera at 20%, isolated egg yolk at 20%, or associated *Aloe vera* plus egg yolk both at 10%. All the groups were added of 3% glycerol, packed in plastic 0.5 mL straws, and frozen in liquid nitrogen. After two weeks, thawing was performed at 37ºC/1 min in a water bath. The total sperm motility was assessed by a Computer Assisted Semen Analysis – CASA (IVOS 12.0, Hamilton-Thorne, Beverly, MA, USA). The sperm membrane integrity was evaluated using diacetate 6-carboxy-fluorescein (C-FDA) and propidium iodide (PI) association analyzed under fluorescence microscopy. Comparisons among different treatments on the semen parameters were evaluated by ANOVA, followed by the Student's t-test ($P < 0.05$). As the main results, the association of *Aloe vera* plus egg yolk provided the most efficient preservation of sperm motility (54% ± 6.2 %) and membrane integrity (44 ± 6.0%) after thawing ($P < 0.05$). As follows, the isolated use of *Aloe Vera* 20% (30.6 ± 4.2% and 27.8% ± 70%, respectively) was more efficient ($P < 0.05$) than the isolated use of egg yolk 20% (8.2 ± 2.3% and 28.6 ± 4.5%, respectively). Interestingly, the best results were achieved in the use of *Aloe vera* that is known for containing a lot of substances such as glucose and xylose, tannin, steroids, organic acids, antibiotics, various enzymes, a protein with 18 amino acids, vitamins, minerals, and others. Its exact mechanism on cell cryoprotection, however, is not fully elucidated. Beyond nutritive action, some of those substances present antioxidant properties that can directly contribute to sperm viability. In addition, on the contrary to egg yolk, we emphasize that *Aloe vera* does not contribute to debris formation on the semen samples, which facilitates the analysis on CASA. In conclusion, we suggest the use of the *Aloe Vera* and egg yolk association as an effective cryoprotectant solution for collared peccary semen cryopreservation.

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Histological evaluation of ear tissue of collared peccary (*Pecari tajacu*) after different vitrification techniques

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Tissue sample cryopreservation of wildlife species, especially the collared peccary, is an interesting step in biodiversity conservation. The use of cells derived from skin tissue has been increasing due to the possibility of its application in somatic cell nuclear transfer (cloning). This reproductive biotechnique is interesting when associated with cryopreservation, allowing both the conservation and production of the specie. The collared peccary have a great scientific, ecological and commercial importance, being necessary for the advances of cloning and tissue vitrification that are interesting systems for reproductive knowledge of this specie. Therefore, the aim of this work was to compare two vitrification techniques for ear tissue using histological analysis. Thus, adult auricular tissues were recovered from four males derived from the wildlife animal multiplication center (UFERSA, Mossoró, Brazil). Fragments (9 mm^3) were cryopreserved by conventional vitrification directly in cryovials (CVC) or solid-surface vitrification (SSV). For both methods, vitrification solution (VS) consisted of Dulbecco Modified Eagles Medium (DMEM) plus 3 M dimethylsulfoxide, 3 M ethyleneglycol, 0.25 M sucrose and 10% fetal calf serum (FCS). For the CVC technique, fragments were inserted into cryovials containing 1.0 mL of VS, which were immersed in liquid nitrogen (LN_2) after 15 sec. For SSV procedure, fragments were exposed to 1.8 mL VS for 5 min, and then tissues were removed from the solutions and dried. These samples were then individually placed on the surface of a metal cube partially immersed in LN_2, and the vitrified fragments were transferred into cryovials for storage in LN_2. After cryostorage for 2 weeks, the vitrified material was removed from the LN_2, kept at 25°C for 1 min, and then immersed in a water bath at 37°C until the VS was completely melted. The cryoprotectants were removed from the ear tissue by washes in DMEM supplemented with 10% FCS and decreasing concentrations of sucrose (0.5 M, 0.25 M and 0.0 M). Fresh (control group) and vitrified tissue samples were fixed in Carnoy for 12 h, dehydrated in a graded series of ethanol, clarified with xylene, embedded in paraffin wax, and serially sectioned (7 µm). Sections were then placed on albumin-coated glass slides, stained using haematoxylin-eosin and analyzed by light microscope (x400 magnification). The data were expressed as percentage of each evaluated parameter by the total of animals. Thus, a total of 12 samples were analyzed for four animals (3 samples/animal), distributed equally for CVC, SSV and control groups, and evaluated for morphological features of epidermis and dermis layers. The cornified and granular layers of epidermis did not show any alteration between CVC and SSV procedures (for both layers, CVC: 75%, 3/4; SSV 75%, 3/4 and control 100%, 4/4). In the spinous layer, an intercellular space without alterations was observed only in 75% (3/4) of the SSV samples, while 100% (4/4) for CVC tissues and control. However, the percentage of the normal cell cytoplasm of the spinous layer was similar between CVC (25%, 1/4) and SSV (25%, 1/4) samples and differed for the control group (100%, 4/4). The nucleus of these cells in the spinous layer was normal in 50% (2/2) of SSV samples (CVC: 100% (4/4) and control: 100%, 4/4). The normal demis-epidermis junction was verified in only 25% (1/4) of the SSV samples (CVC: 50%, 2/4 and control: 100%, 4/4). Similar results were observed between CVC (50%, 2/2) and SSV (50%, 2/2) tissues for the basal layer. Additionally, collagen of dermis shows no alteration in 25%, 1/4 of the CVC and 25%, 1/4, SSV samples (control: 100%, 4/4). Histological changes were observed between the vitrified and fresh samples. However, both vitrification procedures showed similar results in most evaluated parameters. Other studies on *in vitro* culture of fragments are needed to establish the optimal protocol for ear tissue cryopreservation of collared peccary, aiming for the application of these cells in cloning.

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Aloe Vera use on short-term preservation of Collared Pecari (*Pecari tajacu*) semen

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Due to ecological and socio-economic importance, the captive breeding of collared peccaries (*Pecari tajacu*) has been stimulated in order to produce meat and pelts for the international market. Therefore, the improvement of assisted reproductive techniques, such as semen cryopreservation, is needed to help this species' conservation and multiplication. In spite of semen collection, evaluation and freezing being recently demonstrated for this species, studies on short-term liquid preservation remain scarce. Since egg-yolk and *Aloe vera* have been shown as effective cryoprotectants for semen preservation in domestic species, we aimed to evaluate the short-term preservation at 5 ºC of collared peccaries' semen using these two substances added to Tris extender media. The ejaculates from 5 collared peccaries, bred under captivity at the Centre of Multiplication of Wild Animals (CEMAS – UFERSA, Mossoró, RN, Brazil), were collected by electroejaculation. Semen was evaluated for sperm motility (%) and vigor (0 – 5). Samples were divided in two aliquots that were diluted in Tris plus *Aloe vera* (20%) or Tris plus egg-yolk (20%), reaching a final concentration of 100 x 10^6 sperm/mL. Samples were balanced for 40 minutes on Styrofoam boxes containing biological ice in order to reach 5 ºC, and then transferred to a biological oxygen demand incubator also at 5° C. Samples were re-evaluated immediately after initial dilution (0h) and every 12 h up to 36 h. Results were expressed as means ± SD. The efficiency of the cryoprotectants on the preservation of sperm motility and vigor was compared by Student's t test and non-parametric Mann Whitney's test, respectively ($P < 0.05$). After collection, samples diluted in both treatments presented 92 ± 1.2% motile sperm with vigor 4.6 ± 0.2. A significant decrease on sperm motility was noted after 12 h of incubation both for *Aloe vera* and egg yolk, in which values of 53 ± 9.4% and 67 ± 7.7% were found, respectively ($P < 0.05$). After 24 h incubation, no significant decline was verified for sperm motility, and values of 35 ± 13.4% and 37 ±12.2% were found for *Aloe vera* and egg yolk, respectively. At 36 h incubation, a significant reduction on sperm motility was found for *Aloe vera* that presented values of 22.5 ± 8.5% ($P < 0.05$). Otherwise, egg yolk efficiently preserves the sperm motility, providing values of 42.5 ± 6.3% at 36 h ($P < 0.05$). Regarding vigor, there was no statistical difference ($P < 0.05$) in the egg-yolk treatment throughout the experiment, and a value of 2 ± 0 was reached at 36 h. On the other hand, a significant decline was found for the *Aloe vera* group at 12 h ($P < 0.05$), but vigor remained constant after that, reaching values of 1 ± 0.4 at 36h. By comparing both treatments, we verify that there were no differences regarding sperm motility or vigor preservation throughout the incubation time ($P < 0.05$). Although these preliminary results do not show statistical difference between groups, it is noted that egg yolk provides a more efficient preservation of sperm quality in collared peccaries up to 36 h. The use of *Aloe vera*, however, can be indicated for up to 24 h, since this cryoprotectant provided acceptable values of sperm motility higher than 30% at that time. In conclusion, we verify that *Aloe vera* can be used as an alternative cryoprotectant for short-term liquid preservation of collared peccary semen for up to 24 h. However, further studies should be conducted in order to better evaluate its cryoactivity.

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Duration of spermatogenesis in the "common vampire bat", *Desmodus rotundus* (Geoffroy, 1810) (Chiroptera: Phyllostomidae: Desmodontinae)

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The *Desmodontinae* subfamily comprises three species of haematophagous bats, monotypic and sympatric, in which *Desmodus rotundus* is the most abundant. Largely distributed in the new world, *D. rotundus* is found from North to South America (northern Mexico, Central America, and northern Argentina. Despite of the different reproductive strategies of bats, little emphasis has been given to the morphology and kinetics of their spermatogenic process. Therefore, our aims in the present study were to investigate the testis function and the duration of spermatogenesis in the "common vampire bat", *D. rotundus*, which is a species considered of epidemiological importance. Testes from six adult males were routinely processed for histological 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 for the *D. rotundus* were, respectively, 33.4 ± 0.04g and 0.15 ± 0.01g, providing a gonadosomatic index of 0.46 ± 0.01%. Based on the development of the acrosomic system in spermatids, twelve stages of the seminiferous epithelium cycle were characterized in this species. Stages VIII and XI presented the highest frequencies (~16% in both stages), whereas stages VI and XII showed the lowest frequencies (respectively ~5 and 4%). The most advanced germ cell types labeled at 1 hour and 14 days after BrdU injections were, respectively, preleptotene spermatocytes at stage VII and round spermatids at stage IX. Based on the stages frequencies and the most advanced labeled germ cells, each spermatogenic cycle and the entire spermatogenic process lasted respectively 6.5 ± 0.2 and 29.3 ± 0.7 days. These findings indicate that, compared to the mammalian species already investigated, this Chiroptera species presents one of the shortest duration of spermatogenesis. In order to better characterize testis structure and spermatogenic efficiency in this species, we are currently evaluating several other important parameters.

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Viability evaluation of Curimba (*Prochilodus lineatus*) spawned oocytes after *in vitro* storage

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For many fish species, survival time of oocytes is usually short after spawning. Knowledge of the longevity of gametes, and time post-spawning that preserves the viability of oocytes is essential to develop some techniques such as cryopreservation and vitrification. The aim of these processes was the conservation of the genetic information of these animals for a longer time. The purpose of this study was to evaluate the effect of *in vitro* storage of curimba spawned oocytes on viability. Six females of curimba (1.19 ± 0.3 kg) kept in captivity in the Fish Culture Unit hydroelectric power plant Itutinga-MG were used, maintained at 28 ° C. The experiment was conducted between January and February, 2014. Spawning was induced with crude carp pituitary extract (CCPE) in two doses, 0.5 mg / kg and 5mg/kg respectively, with an interval of 12 hours between doses, by intramuscular injection. The extrusion was performed through a ventral manual massage and oocytes placed in plastic beakers. Ovulated oocytes were subjected to analysis of viability by staining with Trypan Blue (TB) 0.2% at 0 hour, at the time of spawning (CP-Positive Control) and 5g samples of oocytes (oocytes ± 5700) were separated and placed in a falcon tube with nutrient medium and addition of different supplements. The culture media used suffered previous adjustments on pH and osmolarity according to plasma average for these variables on the animals. The 5 g samples previously separated were submitted to one of the following treatments, T1 (treatment 1) in 20 ml of Leibowitz 15 medium (L-15) and adding 10% fetal bovine serum (FBS), T2 (second treatment) in 20 ml of L-15 and supplemented with 10% bovine serum albumin (BSA) and Negative Control (CN) with the sample of oocytes without any treatment or supplementation with nutrient medium. T1, T2 and CN treatments were maintained at room temperature (approximately 26 ° C) for 4 hours. After this period in order to evaluate the integrity of the oocyte membrane, 200 oocytes were counted per treatment, by staining technique on TB, identifying unstained (viable) and stained (nonviable) oocytes. Data were subjected to analysis of variance and subsequently averages compared by Tukey test with a significance level of 5%. There were no significant differences ($P< 0.05$) between the CP and the T1 and T2 regarding membrane integrity. The average percentage of viable oocytes observed was 61%, 64% and 59% for CP, T1 and T2 respectively. It can be inferred that the storage of oocytes in culture media proposed in this experiment (T1 and T2) allow the maintenance of the oocyte viability during the first 4 hours of storage. No significant difference was observed in oocyte viability between the two supplementations of maintenance medium (FBS and BSA). The CN presented an abrupt decrease in the oocyte viability, nearing the end of 4 hours with an average of 10% in these conditions. It is possible to conclude that conditions of *in vitro* storage of oocytes tested in this experiment maintained the viability like in the CP for a period of 4 hours after spawning.

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Curimba (*Prochilodus lineatus*) sperm DNA fragmentation under different freezing curves

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Semen cryopreservation is a biotechnology technique that can increase flexibility and operationalization of the reproductive period and assisted reproduction programs in Brazil native fishes. However, this technique can cause damage to sperm DNA, a factor that must be considered in the evaluation of freezing protocols. Futhermore, this study aimed to evaluate the effect of different freezing rates on the sperm DNA fragmentation of curimba (*Prochilodus lineatus*). Semen samples were cryopreserved with 10% dimethyl sulfoxide and diluted in 5% BTS (Beltsville Thawing Solution®) at a ratio of 1:4 (semen: freezing medium), filled in 0.5 mL straws and processed in biofreezer (Icecube®) using 3 curves: T1: 15°C/min, T2: 30°C/min and T3: freezing directly in liquid nitrogen, and a control treatment (CT) using semen *in natura* which was not frozen. After 30 days the straws were thawed, submerged in a water bath at 60°C for 8 seconds. The thawed semen was diluted in glucose 277 mM with an approximate concentration of $10x10^{-6}$ spermatozoon/mL. The DNA fragmentation was evaluated by Comet Assay with analysis of 100 nucleoids in epifluorescence microscope (400X) after staining with propidium iodide (40µL in a 1.5 µg/L solution). The nucleoids were evaluated and characterized in scores of 0 to 4, observing the tail size and diameter of comet head: Score 0 - no damage, intact nucleoids, without tail; Score 1 - little damage, the nucleoids presented a smaller size in the tail than the diameter of the head; Score 2 - intermediate damage, the nucleoids tail size was the same as the diameter of the head; Score 3 - high damage, the nucleoids tail size was 2 times of the diameter of the head; Score 4 - maximum damage, the nucleoids presented tails with size greater than 2 times the diameter of the head. The scores were multiplied by the nucleoids frequency that presenting one score for each treatment, obtaining a value of Arbitrary Unit (AU) or Damage Index (DI), which ranges fluctuate from 0 to 400 for each treatment by the formula: DI: (0 x A) + (1 x B) + (2 x C) + (3 x D) + (4 x E); where A, B, C, D and E are the numbers of nucleoids found in each category analyzed. A sample of semen *in natura* was also subjected to the comet assay. Data were subjected to analysis of variance and means evaluated by Dunett and Scott Knott test at a significance level of 5% by R computer program. The results showed that DI was significantly higher in all treatments compared to CT ($P<0.05$). When the frequency of damage in each score was evaluated it could be observed that in score 0, CT presented more frequency than all treatments and these were similar to each other ($P>0.05$). In the score 1 the damage frequency was lower for T2 ($P<0.05$) and similar to the control. For score 2, T1 and T2 were similar but smaller than samples freezing directly in liquid nitrogen (T3) ($P<0.05$). In score 3, T1, T2 and T3 had similar frequencies, except TC and in score 4 the damage frequency was lower for T2 when compared to T1 and T3 ($P<0.05$). In conclusion, the freezing curves tested caused fragmentation in sperm DNA of curimba, however, the curve of 30°C/min showed less harmful effect to DNA, with the lowest rate of injury in the highest score class for comet assay.

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Colonization and proliferation of GFP rat germ cells transplanted to tilapia (*Oreochromis niloticus*) testes

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Germ cell transplantation consists in the removal of spermatogonial stem cells (SSCs) from the testis of a donor species and the transferring of them into the testis of a recipient animal with depleted endogenous spermatogenesis, where these cells will develop to form mature functional spermatozoa presenting donor genetic characteristics. Using the Nile tilapia (*Oreochromis niloticus*) as an experimental model, we demonstrated in our laboratory the production of donor fertile spermatozoa in the recipient testis and these spermatozoa were able to generate healthy progeny. In order to provide a technique to investigate the plasticity of the tilapia testis environment, our aim in the present study was to investigate the development of rat germ cell in the tilapia testes. To deplete endogenous spermatogenesis, young sexually mature male recipient tilapias, maintained at a temperature of 35° C, were treated with two intracelomic injections (18 and 15mg/kg/BW) of the chemotherapeutic drug busulfan (Sigma, MO, USA), with an interval of two weeks between each injection. The donor germ cells were harvested from the testes of an eGFP rat (8-10 days of age) through enzymatic digestion according to Zhang (2003) with some modifications. The recipient fish received the donor germ cells through the common spermatic duct using a glass micropipette under a stereomicroscope (Olympus SZX-ILLB2-100). Fifteen tilapias that received eGFP rat germ cells were sacrificed at 15, 30, 45, 60 and 90 days post-transplantation. Fifteen days post-transplantation donor spermatogonia were seen in contact with the recipient tilapia Sertoli cells. Rat germ cells were more frequently observed in testes evaluated 30, 45 and 60 days post-transplantation, suggesting that GFP rat cells were able to colonize and proliferate in the recipient testes. Since only spermatogonia were present in the 8-10 days old donor rat testes, the rat germ cells observed in the tilapia testes were probably originated from the transplanted rat spermatogonia. The fluorescence analyses showed that rat eGFP germ cells were also present in the recipient tilapia testes evaluated ninety days post-transplantation. Therefore, the proliferation of rat germ cell within the tilapia testes suggests the plasticity of the seminiferous tubules environment of this species, providing a new and important tool to investigate SSCs biology and spermatogenesis in vertebrates. In order to verify if fertile rat sperm is eventually formed in the tilapia testes we are currently developing new experiments.

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Jundiá catfish (*Rhamdia quelen*) germ cell transplantation using the Nile-tilapia (*Oreochromis niloticus*) as a sexually mature recipient fish

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Spermatogonial stem cell (SSCs) transplantation has proven to be the most valuable technique for investigating spermatogenesis and stem cell biology. Besides that, this technique presents several important potential applications such as the production of transgenic animals, assisted reproductive technologies in aquaculture, and the preservation of endangered species. Because all the necessary approaches important for syngeneic (intra-species) SSCs transplantation in adult tilapia were successfully developed in our laboratory, resulting in donor spermatogenesis and fertile sperm, the establishment of xenogeneic (inter-species) germ cell transplantation using spermatogonia from commercially valuable fish would be of great interest. In the present study we investigated the viability of adult Nile tilapia as a recipient for xenogeneic spermatogonial transplantation, using donor cells from Jundiá (*Rhamdia quelen*) that belongs to a different taxonomic order. Twenty young sexually mature male tilapia (*Oreochromis niloticus*) had their endogenous spermatogenesis depleted with the chemotherapeutic drug busulfan (Sigma, MO, USA) (18 and 15mg/kg/BW) associated with the temperature of 35°C. These fish received through the common spermatic duct the donor catfish germ cells labeled with PKH26. The presence and development of catfish germ cells were investigated in the Nile tilapia seminiferous tubules in several different periods (weeks) post-transplantation. From two to four weeks post-transplantation, the fluorescence microscopic analyses showed the presence of several PKH26 labeled spermatogenic cysts, of different sizes, in the recipient seminiferous tubules. Spermatocytes at different stages of development were observed at seventy days after transplantation in the recipient seminiferous epithelium. Labeled spermatids and sperm were observed, respectively, at ninety days and one hundred and twenty days after germ cell transplantation. These results confirm that the Nile tilapia testis microenvironment can support the development of catfish spermatogenesis. In order to evaluate if the catfish germ cells transplantation could generate healthy offspring, further analyses are being performed.

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