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Effect of expression of pluripotency markers at blastocyst stage on bovine embryo development during elongation

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Keywords: cattle, embryos, pluripotency.

The development of assisted reproductive technologies has been an important advance on animal production improvement. The use of techniques such as embryo transfer, in vitro fertilization and somatic cell nucleus transfer has contributed to accelerate the multiplication of high valuable animals. However when embryos are produced or manipulated in vitro their developmental potential decreases significantly, this impinges upon the production of viable offspring. The lower quality of in vitro produced embryos compared with in vivo-derived ones is due to changes in the gene expression pattern as a result of the response to the in vitro conditions. In bovine, blastocysts for transfer are selected based on the morphology, this does not reflect their developmental potential since many developmental crucial genes might be aberrantly expressed in embryos with an otherwise normal morphology. In this sense the use of genetic markers could be of remarkable value to select good quality bovine embryos. We propose that the expression of pluripotency markers (Oct4, Sox2 and Nanog) at blastocyst stage will correlate with their development potential during the peri-implantation (elongation) period. For this, in vitro produced grade I blastocysts were split in halves; one of half was selected for gene expression analysis while the other was transferred to recipient cattle. Transferred embryos were recovered at day 17, classified by the elongation stage and used for gene expression analysis (Oct4, Sox2, Nanog, Cdx2 and TP1). More than 65 % of the split embryos generated two viable hemi-embryos with the same ability of in vitro re-expansion, similar cell number as well as homogenous gene expression. From 15 embryos that were transferred, 9 (60 %) were collected with different grades of elongation (1-15 cm). A correlation analysis showed that the expression level of pluripotency markers (Oct4, Sox2 and Nanog) at blastocyst correlates with the expression level of the same genes at the elongation stage, but neither with the expression level of trophoblastic markers (Cdx2 and TP1) nor with embryo length.

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Follicular dynamics, corpus luteum growth, and regression in buffalo heifers and buffalo cows in the colombian humid tropics

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Keywords: buffaloes, corpus luteum, follicular dynamics.

Buffaloes are a species of growing economic importance worldwide; however, there are few studies on their reproductive physiology, especially under tropical conditions. The aim of the study was to characterize the follicular dynamics and luteal growth and regression pattern of multiparous (MB) and heifer (HB) Murrah buffaloes in Colombian humid tropical conditions (Puerto Salgar, Cundinamarca. Lat. 5° 39.075"N and Long. 74° 34.843"O). The HB had an age of 24.72±1.45 months while the MB had 81.97±31.75 months, 2.7 ±0.8 births and 79.5±16.0 days in milk. The animals grazed on *Brachiaria mutica* pastures, with free access to water and mineral salt. 10 MB and 10 HB were synchronized with progesterone-releasing intravaginal device, application of estradiol benzoate on days 0 and 9 and prostaglandin when the device was removed. However, artificial insemination of animals was not performed. Seventeen animals responded to the protocol and 15 days later, the daily ultrasound monitoring began to determine the number and diameter of the structures present in both ovaries (follicles and corpus luteum). Student's *t* test was used to evaluate differences between means of MB and HB. All data are presented as mean ± standard deviation. The estrous cycle length was 22±4.5 for MB and 22±2.7 days for HB. The follicular growth occurred in one wave (n=1; 5.89%), two waves (n=14; 82.35%) or three waves (n=2; 11.76%). In all animals, the first wave emerged the day after ovulation showing 8.33±2.06 and 10±2.72 follicles in MB and HB, while the second wave started on day 11±2.00 and 10.5±2.82, presenting 8.37±2.26 and 8±1.51 follicles, respectively. The third wave began on 16.21±3.1 showing 6.5±1.7 follicles, happening only in MB. The follicular deviation occurred 3.77±1.89 days after emergence in all waves and in both groups, moment when the largest follicle had a diameter of 9.65±1.62 mm. Likewise, the preovulatory follicle maximum diameter was 17±4.6 and 14±2.9 mm for MB and HB. The maximum diameter of the corpus luteum was 19.58±4.16 mm and 17.74±3.32 mm and, its regression started at 15.22±5.26 and 17.62±1.68 days in MB and HB, respectively. There were no significant differences between groups for all of these variables. These results show that MB and HB have estrous cycles with 1, 2 or 3 follicular waves and that 2 wave cycles are the most common, similar to previously reported by others. Futures studies should provide a better understanding of the follicular development in buffaloes in tropical conditions and for the establishment of reference values of clinical relevance.

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The effect of blocking (pro)renin receptor on progesterone synthesis during luteinization in cattle

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Keywords: aliskiren, ovulation, prorenin.

The renin-angiotensin system is a target for research in physiology of reproduction. In mammals, a possible role of prorenin, independently of renin was suggested in increasing P4 levels in response to the LH surge, because LH release increases prorenin synthesis, although not renin levels, in the blood plasma and follicular fluid (Itskovitz *et al.,* 1988, Ann. N. Y. Acad. Sci., 541, 179-89). Recently the (pro)renin receptor [which binds to prorenin and renin; (P)RR] has been identified in bovine theca and granulosa cells (Ferreira *et al*., 2011 JRAAS, 12, 475-82). However, the role of prorenin/(P)RR binding in the peri-ovulatory period remains unclear. The aim of this study was to evaluate the effect of blocking (P)RR in the plasma concentration of P4 during luteinization. Thus, european cows with body score ≥3 had a new follicular wave induced by hormonal protocol according to Santos *et al.* (2012, JRAAS, 13, 91-8). Cows which reached follicular diameter ≥12mm received GnRH (100 mg of gonadorelin acetate, IM) and received randomly an intrafollicular injection of 10μM aliskiren (direct renin/prorenin inhibitor; Novartis, Intermed, Wiesbaden, Germany) diluted in PBS (n=6) or PBS alone (control group, n=4). Intrafollicular injections were guided by ultrasound using a 7.5MHz convex probe. The amount of aliskiren injected was determined according to the volume of the fluid in each follicle, which was estimated by linear regression equation V=-685.1+120.6D, where V is the estimated volume and D is the measured diameter of the follicle (Ferreira *et al.,* 2007, Reproduction, 134, 713-9). After intrafollicular injection, the follicles were monitored by ultrasound at 24, 48 and 72h and blood samples were collected from the jugular vein, at 6 and 8 days after GnRH analogue injection, for P4 immunoassays by electrochemiluminescence (CV 1,23%; sensitivity 0,030ng/ml). Cows that showed a ≥2mm reduction in follicular diameter within 24h after treatment were excluded from the study. Ovulation was characterized by the disappearance of the large follicle between two consecutive evaluations followed by corpus luteum detection. The absence of ovulation over a 48h period associated to a decrease in follicle diameter was characterized as follicular atresia. The data from P4 assay in different treatments and days were compared by two-way ANOVA. All animals in the control group (PBS, 4/4) and four cows (4/6) in the aliskiren group (66.66%) ovulated. Considering only the cows that ovulated, the P4 concentrations (ng/ml) were reduced in the aliskiren group (3,89±0,73) compared to the control group (6,89±1,56) on day 6 ($P<0,05$), whereas no difference was detected in P4 concentrations between aliskiren group (6,56±0,77) and control group (8,64±1,48; $P>0,05$) at day 8. In conclusion, prorenin/(P)RR participates positively in P4 synthesis during early luteinization in cattle.

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Flemish: a breed in extinction – reproductive profile of Flemish cattle in Southern Brazil

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Keywords: flemish, follicular growth, progesterone levels.

The understanding of physiological phenomena associated with follicular growth and ovulation is essential for the optimization of reproductive biotechniques and, thus, the productive efficiency of the herd (Baruselli et al., Rev Bras Reprod Anim, 31, 205-211). Such biotechniques may determine the reproductive success in threatened species or breeds and, therefore, their maintenance. The introduction of specialized bovine breeds in our herds lead to a gradual disinterest on the Flemish breed, resulting in a drastic decrease in the herd size, with approximately only 50 animals remaining of such breed in the EPAGRI Research Station in Lages, Southern Brazil (Zago et al., Acta Scientiae Veterinariae, 38, 2, 770). The objective of the present study was to describe the behavior of the dominant follicle and variation of plasma progesterone during the estrous cycle of Flemish cows, comparing them to Holstein cows that served as control group. Two groups of female non-lactating pubertal cows, aged from 4 to 6 years-old, of Flemish (FLE, n=5) and Holstein (HOL, n=4) breeds, had the estrus synchronized with two doses of 500 µg sodium Cloprostenol (Sincrocio®, Ouro Fino, Cravinhos, Brasil) IM, at 14 day intervals. As they manifested estrus, the animals had their ovaries evaluated by transrectal ultrasound (M5Vet®, Mindray, Shenzhen, China) every 24 hours, until detection of the second ovulation. The dominant follicle (DF) of each follicular wave was identified, measured and its diameter registered considering the day of the cycle. Blood samples were obtained from each animal, with at intervals of 5 days between collections, until the 20th day of the cycle, and the correspondent plasma was submitted to radioimmunoassay for determination of progesterone plasma levels. For data analysis ANOVA followed by *t* test and Tukey were used. Progesterone plasma concentrations did not differ between groups in none of the evaluated periods (P>0,05), reaching minimum levels (mean±SEM) of 0,155±0,016ng/ml for FLE and 0,300±0,048ng/ml for HOL on estrus and maximum levels of 6,651±1,868ng/ml and 5,957±1,233ng/ml on diestrus, respectively, for groups FLE and HOL. The maximum diameter (mean±SEM) of the dominant anovulatory follicles was 12,96±0,52mm for FLE and 12,63±0,65mm for HOL (P>0,05). The ovulatory follicles showed maximum diameter of 13,20±0,44mm and 14,68±0,85mm, respectively (P>0,05). The dominant follicles of Flemish cows had a day mean growth of 1,237±0,093mm, while for HOL group this measure was 1,172±0,173mm (P>0.05). It is concluded that Flemish cows have follicular growth and progesterone plasma profile similar to Holstein cows.

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Dickkopf 1 (DKK1), a canonical WNT signaling inhibitor, promotes development of the trophectoderm cell lineage in bovine blastocysts

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Keywords: bovine embryo, cell differentiation, DKK1.

Activation of canonical WNT signaling in bovine embryos at day 5-post insemination in vitro impairs blastocyst development and decreases cell number, particularly of trophectoderm (TE) cells. Deleterious effects of canonical WNT activation can be rescued by DKK1 (Denicol et al., 2013 Scientific Reports, 3, 1–7). Moreover, transfer of embryos exposed to DKK1 from day 5 to 7 increased pregnancy success at day 32 of gestation in lactating Holstein cows (Denicol et al., abstract ID 282728 – Society for the Study of Reproduction, 2013 meeting). The objective of this study was to determine the effect of inhibition of canonical WNT signaling by DKK1 during the morula-to-blastocyst transition on development of TE cells. Embryos were produced in vitro from slaughterhouse-derived oocytes. Maturation time was 20–22 hours and fertilization, 8-10 hours. Both processes took place under 38.5°C and 5% CO_2. Putative zygotes were randomly allocated in groups of 25 to 30. Culture conditions were 38.5°C, 5% O_2 and 5% CO_2. On day 5 after insemination, embryos were treated with vehicle (DPBS/BSA 0.1%) or 100 ng/ml human recombinant DKK1. Blastocyst development was evaluated on days 7 and 8. Blastocysts were harvested on both days for staining with immunofluorescence-labeled antibodies. Briefly, embryos were fixed, permeabilized and incubated with a blocking solution. Immunofluorescence was accomplished by sequential incubation with primary and secondary, FITC-conjugated antibody, for detection of CDX2+ nuclei. Hoescht 33342 was used for staining of all cell nuclei. Embryos were observed with a 40x objective using a Zeiss Axioplan 2 epifluorescence microscope (Zeiss, Gottingen, Germany) and Zeiss filter set 02 (DAPI) and Zeiss filter set 03 (FITC). Digital images were acquired using AxioVision software (Zeiss) and a high-resolution black and white Zeiss AxioCam MRm digital camera. Cell count was performed using ImageJ (National Institutes of Health). The GLM procedure of SAS® (version 9.3; Cary, NC) was used for data analysis. Replicate was considered a random effect. Cells were counted in 125 embryos and 3 replicates. DKK1 did not affect the proportion of blastocysts that were expanded or hatched on either day 7 or 8 ($P = 0.22$). DKK1-treated blastocysts had fewer cells on day 7 (107.7±4.9 vs 125.9±5.7) but there was no difference on day 8 (123.7±4.4 vs 122.2±5.7; $P = 0.08$; treatment x day: $P = 0.02$). Exposure of embryos to DKK1 increased the percent of cells that were TE on both days 7 (71.9±1.8 vs 61.6±2.1) and day 8 (73.8±1.6 vs 67.6±2.1) ($P < 0.05$). In conclusion, DKK1 promotes development of cells of the TE lineage without promoting overall cell proliferation.

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Comparison of uterine vascularization by Doppler ultrasound of pregnant and not-pregnant cows submitted to FTAI

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Keywords: color Doppler, resistence index, uterus.

The aim of this work was to study the uterine vascularization by Doppler ultrasound of inseminated cows, verifying if there is any difference between the resistence index values (RI) and the uterine vascularization score (VS) after AI of cows that became pregnant and the ones not-pregnant. Nelore cows (n=182) were used between 50 and 70 days postpartum assessed by Doppler ultrasonography (Mindray, M5Vet), in spectral (RI) and color Doppler (VS, 0 to 4) modes in three different moments: 30 hours before AI, 4 and 24 hours after AI. The spectral mode was used to objective (RI) evaluation of blood flow of uterine arteries. The localization of uterine arteries was done according to Bollwein et al. (2000, Theriogenology, 57, 2053–2061). Color mode was used for subjective evaluation (VS) of the uterine vascularization, being the uterine horns scanned and movies recorded. After that, the images were analyzed by two different examiners. The mean of two examiners was used for statistical analyses. The pregnancy diagnosis was done 30 days after AI, with 99 cows being pregnant (54.39%). The data of pregnant group (n=99) and not-pregnant group (n=83) were analyzed by PROC MIXED of SAS (9.3) and a level of significance of 5%. As there was no interaction between pregnancy diagnosis and time of evaluation (RI: p=0.97; VS: p=0.95), the data was studied according to diagnosis, independent of time and according to time, independent of diagnosis. No differences was observed for RI (p=0.51) and uterine VS (p=0.39) of cows that became pregnant (RI=0.68±0.01; VS=2.36±0.05) and the not-pregnant (RI=0.67±0.01; VS=2.36±0.05). Differences were noted between times of evaluation (RI: p<0.001; VS: p<0.001). Vascularization was higher 4 hours after AI (RI=0.63±0.01; VS=2.61±0.06), the second highest value of vascularization was observed 24 hours after AI (RI=0.67±0.01; VS=2.31±0.05) and the values of 30 hours before AI were lower (RI=0.74±0.01; VS=2.04±0.06). Is not possible to identify changes in the uterine vascularization after AI in cows according to pregnant or not-pregnant status; however, it is possible to note vascular alterations in the uterus during the different periods of the estrous cycle.

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Presence of conceptus modulates expression of interferon-tau stimulated genes in peripheral blood immune cells of pregnant and non-pregnant beef cows

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Keywords:, interferon-tau, monocytes, pregnancy.

Interferon-tau secretion by the pre-implantation conceptus is crucial for corpus luteum maintenance and establishment of pregnancy. This cytokine stimulates the expression of specific genes (ISGs) in peripheral blood mononuclear cells (PBMCs) in dairy cows and sheep. This evidence may serve as the basis for the development of molecular techniques for early pregnancy diagnosis. Ultimately, such approaches may become helpful tools to reduce the interval between inseminations and to optimize beef cattle production. The aim of this study was: (1) to evaluate ISGs transcripts abundance during early pregnancy in beef cows; and (2) to determine the feasibility of detecting non-pregnant cows based on ISGs expression between days 12 and 20 post-AI. Nelore cows (n=27) were submitted to TAI. All animals were treated with estradiol benzoate (2 mg) and received a P4-releasing device (1 g). After 8 days, the devices were removed and the animals received a PGF injection (0.5 mg). Ovulation was induced by GnRH treatment (10 µg) and animals were inseminated 48 hours after P4 device removal. Blood samples were collected from jugular vein on days 12, 15, 20, 22, 30, 45 and 60 post-AI for PBMC isolation by Ficoll® (GE Healthcare) gradient. Pregnancy was diagnosed by ultrasonography on days 25, 30, 45 and 60 post-AI; 10 pregnant animals were detected on day 25 post-AI and one pregnancy was lost between days 45 and 60. Isolated PBMCs from pregnant and non-pregnant cows (n=6/group) were used for RNA extraction and cDNA synthesis. The abundance of the ISGs 2'-5'-oligoadenylate synthetase 1 (OAS-1) and myxovirus resistance 2 (Mx-2) was measured by qPCR. Cyclophilin was used as housekeeping gene for data normalization. Repeated variables were analyzed by spli-plot ANOVA using the PROC MIXED procedure (Version 9.2; SAS Institute). For OAS-1 and Mx-2 gene expression, an effect of group, day and their interaction were detected ($P<0.05$). Expression of OAS-1 and Mx-2 genes in PBMCs progressively increased from day 15 post-AI, reached a peak at day 20 and decreased sharply until day 22 and progressively until day 45 in pregnant cows. Gene expression did not differ across time in non-pregnant cows. On the peak of ISGs expression (day 20 post-AI), the lowest values for relative expression of OAS-1 and Mx-2 were 0.29 and 0.05, respectively. In non-pregnant cows the relative expression of ISGs was never higher than these values. In conclusion, the expression of OAS-1 and Mx-2 genes in PBMCs is stimulated between days 18 and 30 post-AI, and the greater abundance of ISGs on day 20 post-AI may be used for the development of technologies to diagnose early gestation in beef cattle.

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Oocyte quality in post-partum of 3/4 and 7/8 Holstein x Zebu primiparous crossbred cows

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Keywords: crosses, oocyte, ovum pick-up.

Crossbred Holstein x Zebu cows are the basis of milk production in Brazil, representing about 75% of the milked cows. In post-partum these cows undergo a period of negative energy balance (NEB), as it occurs in Holstein cows, which causes delay in the return to ovarian activity, associated with decreased oocyte quality. The present study aimed to evaluate oocyte quality in first 57 days post-partum in crossbred cows. Holstein x Zebu primiparous cows 3/4 crossbred (n=13) and 7/8 crossbred (n=14) were used, which were fed a corn silage and concentrate based diet. Ovum pick-ups (OPU) were performed, in average, on16, 32, 43 and 57 days post-partum, after follicular wave synchronization by puncture of follicles larger than 6 mm, 72 hours before OPU. Oocytes recovered in PBS medium (supplemented with 0.05% fetal calf serum and 20 IU/mL heparin) were classified as viable (grades I, II and III) or not viable and used for *in vitro* embryo production. The variables oocyte total number, viable oocytes and not viable oocytes were analyzed by ANOVA (PROC GLM), while oocyte rate was evaluated by logistic regression (PROC LOGISTIC). In both cases the effects of cross, post-partum days and their interaction were considered. Analyses were performed using SAS software, v.9.2. Crossbred 3/4 cows produced less ($P<0.05$) total oocytes, viable and not viable oocytes than the crossbred 7/8 cows, that were respectively, 3.52 ± 0.39, 2.44 ± 0.30 and 1.06 ± 0.20 in 3/4 crossbred cows, and 8.43 ± 0.94, 6.48 ± 0.79 and 1.93 ± 0.27 in 7/8 crossbred cows. In contrast, there was no effect ($P> 0.05$) of post-partum day on the rate of viable oocytes, which was 74.89% (516/689). Also there was no effect ($P> 0.05$) of post-partum day on oocyte production. On average, 4.89 ± 0.87, 4.36 ± 0.82, 4.36 ± 0.86 and 4.82 ± 1.28 viable oocytes were produced after 16, 32, 43 and 57 days postpartum, respectively. No interaction between the cross and the post-partum days was detected. The lack of influence of days post-partum on the production of oocytes suggests n similar energy balance pattern between groups, which possibly indicates that differences observed between the cows groups is probably due more to individual variation of donors used than to a difference in the cross itself. The lower milk production of crossbred cows compared to Holstein cows, suggests that the intensity and duration of negative energy balance are reduced in crossbred animals, so that during early post-partum (57 days) variation in production and oocyte viability was not observed. However, further studies using a larger number of animals are necessary to establish the relationship between energy balance and oocyte quality in crossbred cows.

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Characterization of the histone H3R26me2 modification during *in vitro* development of bovine embryos

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Keywords: embryo development, epigenetics, IVP.

The *in vitro* production (IVP) of bovine embryos is a biotechnology of great economic impact for use on genetically superior animals, resulting in greater reproductive efficiency. However, despite several technological advancements, the developmental rates of IVP embryos are much lower than those for *in vivo* embryos. *In vitro* culture is thought to cause adverse effects on embryo development and subsequent gestation. Epigenetics, the regulation of gene expression without changing the DNA sequence, is essential for proper embryo development and may be affected by *in vitro* culture. One of these epigenetic events is the remodeling of histone proteins which are responsible for DNA conformation and required for proper embryonic development. The aim of this study was to evaluate the modification of histone H3R26me2 during pre-implantation development of IVP bovine embryos cultured with and without serum. After *in vitro* maturation and fertilization, bovine embryos were cultured with either 0 or 2.5% serum. Embryos were collected at 2-cell, 4-cell, 8-cell, 16-cell, morula and blastocyst stages from both groups and fixed in 4% paraformaldehyde. Fixed embryos were then used from immunofluorescence utilizing an antibody for H3R26me2. Images of stained embryos were analyzed as a percentage of total DNA. Levels of H3R26me2 changed for both groups over development. In the group cultured in 0% serum, the greatest amount of H3R26me2 staining was at the 4-cell ($P<0.01$), 16-cell ($P<0.05$) and morula ($P<0.05$) stages. In the 2.5% serum group, only 4-cell stage embryos were significantly higher than all other stages ($P<0.01$). These results suggest that the modification of histone H3R26me2 is regulated during development of pre-implantation bovine embryos, and that culture conditions greatly alter this regulation.

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Supplementation with sunflower seed increases the conception rate in recipients of in vitro produced bovine embryos

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Keywords: embryonic mortality, linoleic acid, PGF2α.

Embryo mortality between 15 and 19 days of pregnancy in cattle is apparently due to increase in endometrial PGF2α release, resulting in the corpus luteum regression. The PGF2α synthesis may be inhibited in animals supplemented with linoleic acid-rich compounds, among them a sunflower seed. In previous study (Peres et al., 2008, Acta Scientiae Veterinariae, 36, 639), Nelore cows supplemented with sunflower seed for 22 days starting at the day of timed AI (TAI) had a higher conception rate (66.7% *vs.* 46.3%; p = 0.02). This study aimed to evaluate the effect of supplementation with sunflower seed on conception rates in embryo recipients. Crossbred heifers received an intravaginal device containing progesterone (1g; CRONIPRESS®; Biogenesis Bago) associated with an intramuscular (IM) injection of estradiol benzoate (2mg; BIOESTROGIN®; Biogenesis Bago). The devices were removed eight days after, when heifers were treated with D-cloprostenol (150µg; CRONIBEN®; Bago Biogenesis) and eCG (400IU; Folligon®, Intervet), both IM. Heifers were treated IM with estradiol benzoate (1mg; BIOESTROGIN®; Biogenesis Bago) 24 hours after device removal. Two days after the removal of the device (D0), it was expected to occur the estrus. Heifers were split into two groups to receive 1.7 kg /animal/day of the following treatments: 40% soybean meal 44% crude protein (CP) and 60% sunflower seed (Sunflower Group; n = 106) or 53% of soybean meal with 44% CP and 47% corn (Control Group; n = 111). Both supplements were balanced with 72% TDN and 24% CP. Supplements were given for 22 days from D-2 to D19. In D7, *in vitro* produced embryos, Holstein (13.82%) and Nellore (86.18%) breed, were transferred to the recipient by FTET. Pregnancy diagnosis was performed by ultrasonography on D30. Blood samples were collected at D-2, D7 and D19 for measurement of plasma progesterone (P4), total cholesterol, triglycerides, HDL and LDL. Data were analyzed using the SAS procure GLIMMIX. The conception rate was greater ($p<0.01$) in the Sunflower Group (55.66%; 59/106) than in the Control (36.94%; 41/111). The P4 concentration did not differ ($P>0.05$) on D7 between the Control (4.92 ± 0.24 ng/mL) and Sunflower (4.72 ± 0.23ng/mL) groups. Greater concentrations of total cholesterol were observed in the Control Group compared to Sunflower on D7 (306.02 ± 11.61 *vs.* 277.10 ± 11.88mg/dL, respectively; $p <0.05$) and D19 (260.51 ± 7.98 *vs.* 231.95 ± 7.95mg/dL, respectively; $p <0.01$). Greater concentrations of HDL cholesterol were observed in the Control Group compared to the Sunflower on D7 (166.82 ± 5.88 *vs.* 139.43 ± 5.74 mg/dL respectively; $p <0.01$) and D19 (162.01 ± 4.67 *vs.* 135.57 ± 4.76mg/dL respectively; $p <0.01$). Supplementation with sunflower seed increases the conception rate in recipients of *in vitro* produced embryos.

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Effect of recombinant somatotropin (rbST) on the superovulatory response and early embryo development in bovine (*Bos taurus*)

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Keywords: bovine, embryo, superovulation.

In dairy cows, the fertility rates have shown a decrease in proportion to the increase in milk production in the last 30 to 40 years. This is a continuous trend that occurs at an annual rate of 0.45% in the USA and 1.0% in the UK. Due to this situation, extensive research has been carried out in the last decades in order to enhance fertility in dairy cattle. Recombinant somatotropin treatment at the moment of the artificial insemination has shown a positive effect over pregnancy rates in dairy cows. Nonetheless, the mechanisms by which this effect is held remain to be determined. The objective of this research was to evaluate the effect of rbST on the superovulatory response, ovulation rate, number of recovered oocytes, fertilization rate, stage of embryo development and embryo quality of bovine donors. Female donors of Holstein (16), Brown Swiss (16) and Simmental (16) breeds (n=48), between 2 to 7 years old, in adequate body condition score (2.5 to 3.5) and >100 days in milk, without any diagnosed reproductive disorder were used. The cows were kept in an intensive management system with 50% concentrate and 50% forage. Superovulation was performed according to the following protocol: day 0 DIB insertion (1.0 g progesterone), 1 mg estradiol benzoate (EB), 40 mg coprostenol (PGF2α) and 50 mg injectable progesterone; from day 4, 300 mg FSH (Folltropin V, Bioniche Animal Health, Canada), divided in 8 decreasing doses were administered, day 7 DIB withdrawal (Syntex SA, Argentina) and administration of 40 mg cloprostenol, on day 9 artificial insemination (twice, 12 and 24 h post-estrus) and on day 16 collection and evaluation of embryos. The administration of rbST was at first artificial insemination (day 9, AM, n=24). The procedures of collection, washing, recovery and classification of embryos were carried out under standards of the IETS Manual, 2010. The ovulation rate (n of corpora lutea, day 16) and the number of recovered oocytes were not statistically different ($p>0.05$) between control and rbST groups. However, the quantity of viable embryos (4.2 vs 6.1), proportion of embryos at blastocyst stage – stage 5, 6 and 7 (25 vs 55%), rate of freezable embryos (70% vs 83%) and rate of embryos of excellent quality – grade 1 (42 vs 75%) showed statistical significance ($p <0.05$) between control group vs rbST group. In conclusion, the administration of recombinant somatotropin in superovulated cows at the first insemination improved embryo quality and sped up early embryo development.

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Body morphometry related to gender in *Trachemys scripta elegans* turtles (WIED, 1839)

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Keywords: body morphometry, testudine, turtle.

Trachemys scripta elegans is an exotic underwater turtle, introduced in Brazil with no proper control by authorities. The abandonment in lagoons, rivers and bodies of water threatens the maintenance of the local biological diversity, due to the extinction of native species by competition or genetic extinction by hybridization. Therefore, studies on the biological aspects of this species are necessary to reduce the risks of environmental impact, to help on its population control or as an experimental model. This study aimed at determining the body biometrics of males and females of *T. scripta elegans*, in order to provide information on their biology when away from their natural habitat. Nine males and 33 females had corporal volume (VC, mL), mass (CM, g), length (LCar, cm) and maximum width (WCar, cm) of the carapace; ;length (LPla,cm) and maximum width (WPla,cm) of the plastron; ;body height (Ch) (Malvasioet et al.,1999, *Arq.Brasil.Zootec,* 16, 91-102) and sexual dimorphism index (SDI) (Gibbons & Lovich,1990, *Herpet. Monogr.,* 4, 1-29) determined. Averages were analyzed by using ANOVA followed by Tukey test and simple correlation coefficients (r) were analyzed among the variables (Assistat 7.6 beta). This study was approved by the CEDEP[1]from UNIVASF[2] (Protocol nr 0001/160412). In the population analyzed, the gender ratio was 3.67:1, where 21.0% (N= 9) were male and 79.0% (N = 33) female. The males presented CM (685.0±298.3), VC (691.8±368.5), LCar (16.1±2.6), WCar (12.9±1.7), LPla (14.8±2.2), WPla (9.6±1.1) and Ch (5.6±1.4) lower ($P<0.05$) than the females (1178.0±362.3; 1164.3±368.0; 19.8±3.0; 15.0±2.9; 18.7±2.5; 12.2±1.6 and 7.3±1.6, respectively). SDI in the population analyzed was 1.72 for CM, 1.68 for VC, 1.23 for LCar, 1.16 for WCar, 1.26 for LPla, 1.27 for WPla and 1.30 for Ch. In males, very high positive correlations ($P<0.01$) were observed between CM and VC, WCar, LPla and Ch, between VC and WCar and LPla, LCar and WCar, WCar and LPla and between LPla and WPla, while the positive correlations ($P<0.05$) occurred between CM and LCar, VC and LCar, WPla and Ch, LCar and LPla, WCar and WPla and Ch and between LPla and Ch. On the other hand, in females, very high positive correlation ($P<0.01$) was observed among all parameters analyzed, except for Ch that presented positive correlation ($P<0.05$) with VC and CPla. The results lead to the conclusion that, in the population analyzed: 1-females presented average body mass larger than the males, 2-SDI was, in average, 1.23 times larger for females in all parameters analyzed, 3-the body development of the females occurred uniformly among mass, carapace and plastron, while, in males, it was not observed for WPla, CM and LCar and for Ch, LCar and WPla. These data provide scientific support on the reproductive biology of this exotic species

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Different media for selection of swine oocytes with Brilliant Cresyl Blue – effects on parthenogenetic embryo development

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Keywords: Brilliant Cresyl Blue, parthenogenesis, swine oocyte.

Parthenogenetic activation is a crucial step for the establishment of other technologies such as nuclear transfer. However, regarding swine, more effective protocols need to be established to provide best oocyte selection and to increase rates of oocyte parthenogenesis. Considering this, the oocyte selection by the viability dye Brilliant Cresyl Blue (BCB) is a useful tool. The stained oocytes are considered the most suitable for IVM. Within this context, the aim of this study was to compare development rates by parthenogenesis of swine oocytes selected with 13 µM of BCB, in a richer medium, called modified Porcine Zygote Medium (PZM-m) and in PBS solution, the most utilized medium. Prior to IVM 1,621 oocytes were incubated for 60 min at 39 ºC in different media, with BCB. The oocytes were classified as positive (stained) or negative (no staining), except for group 1 which was washed in PFF and were not incubated, being considered the general control. The oocytes were distributed in the following groups: Porcine Fluid Follicular (PFF) (n=62); PBS-control (PBSc) (n=336); PZM-m control (PZMc) (n=371); PBS BCB positive (PBS+) (n=336); PBS BCB negative (PBS-) (n=161); PZM-m BCB positive (PZM+) (n=293); PZM-m BCB negative (PZM-) (n=90). IVM was performed in NCSU-23m with eCG, hCG, hypotaurine, β-mercaptoethanol, cysteine, EGF, AMP-c and PFF, in the first 24 hours, followed by NCSU-23 without eCG, hCG and AMP-c, for additional 24 hours. The oocytes were parthenogenetically activated with 20 µM ionomicyn for 5 minutes prepared in TCM Hepes medium and 2 mM 6-DMAP for 3 hours in PZM-3, the same medium for embryo culture. On day 4, 10 % of fetal calf serum was added to embryo culture. The results were analyzed by Chi-Square using the software Statistix 9.0. The cleavage rate in group PFF was 67.7% (n=42); 45.1% in PBSc (n=139); 52% in PZMc (n=193); 58.6% in PBS+ (n=197); 0% in PBS- (n=0); 55.3% in PZM+ (n=162); 23.3% in PZM- (n=21). The embryo rate on D7 (blastocyst and morulae) was 1.6% in group PFF (n=1); 14.9% in PBSc (n=46); 18.9% in PZMc (n=70); 8.6% in PBS+ (n=29); 0% in PBS- (n=0); 14.7% in PZM+ (n=43); 1.1% in PZM- (n=1). These results showed that cleavage rates in PZM+ were superior ($p<0.05$) to PBS+ and PBSc. However, this rate was not superior to PFF. The embryo results showed no difference between the groups PZMc, PZM+ and PBSc, which obtained the best rates. The groups PZM+ and PFF had the best cleavage ratesf. However, parthenogenetic activation did not show influence of the medium. At the same time, the PZM+ oocytes showed better embryo development than PBS+. Nevertheless, more research and experiments are underway to confirm these findings.

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Expression of competence genes in cumulus cells from immature and *in vitro* matured cumulus-oocyte complexes (COCs) morphologically classified into different grades

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Keywords: cumulus cells, gene expression, IVM.

Efficiency of oocyte *in vitro* maturation (IVM) is directly associated to the intrinsic quality of oocyte from the ovarian follicle. Oocytes undergoing IVM are classified according to their morphological characteristics in grades I, II and III, however, little is known about the expression of developmental competence related genes in COCs of different quality grades. The objective of this study was to assess the expression of cumulus cells (CC) competence genes in immature and *in vitro* matured COCs classified into grades I, II and III (Khurana & Niemann, 2000; n=3 replicates/group). Therefore, bovine COCs from 3-8 mm follicles were obtained from slaughterhouse ovaries and separated into three groups according to their morphological classification: grade I (GI, oocytes having an homogeneous, evenly granulated cytoplasm surrounded by a compact CC with more than three layers), grade II (GII, oocytes having an homogeneous evenly granulated cytoplasm with fewer than three CC layers) and grade III (GIII, partially denuded oocytes). The CC from 20 COCs were separated by successive pipetting before (Immature group) and after IVM with bicarbonate TCM199 supplemented with 6% BSA, pyruvate (11 μg/μL), amikacin (16.67 mg/uL), FSH (0.1 mg/mL, Pluset®, Serovet, Rome, Italy), LH (50 mg/mL, Lutropin®, Bioniche, Belleville, Ontario, Canada) and estradiol (1 ug/uL). Total RNA was extracted by RNeasy® kit (Qiagen) and 100ng of RNA was reverse transcribed by SuperScript III® enzyme (Life Technologies). Expression of amphiregulin (AREG), FSH receptor (FSHR) and progesterone receptor (PGR) mRNA was investigated by real-time PCR with StepOnePlus® (Life Technologies) using PowerSybrGreen® (Life Technologies) reagent. Relative mRNA quantification was calculated using ΔΔCt method normalized by cyclophilin (CYC-A) as housekeeping gene. Effects of COC grade and time of maturation were tested by ANOVA and groups were compared by Tukey-Kramer HSD test. Differences were considered significant when $P<0.05$. The results showed effect of maturation for all target genes. Expression of PGR and AREG mRNA was higher in matured CC compared to the immature, regardless of morphological degree. In contrast, FSHR mRNA expression decreased with IVM. No differences were found for AREG and FSHR mRNA among grades I, II and III in immature or matured CC, assessed separately. PGR mRNA expression was higher in GI CC compared to GII CC when *in vitro* matured. We conclude that IVM influences expression of competence genes in CC and the regulation of PGR mRNA in matured CC suggests that immature morphologically fittest COCs have better ability to support *in vitro* development. Furthermore, the decreased gene expression of FSHR suggests downregulation of the receptor, possibly associated with IVM inefficiency.

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Production rates of *in vitro* produced bovine embryos with different developmental kinetics

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Keywords: kinetic, quick embryo, slow embryo.

The quiet (or slow) embryo hypothesis mentions that embryo viability is associated with slower metabolism than with faster metabolism, since slow embryos give off less energy to correct genome, trascriptome and proteome damages (Brison et al., 2004, Hum Reproduction, 19, 2319-2324; Leese et al., 2007, Human Reproduction, 22, 3047-3050). However, since *in vitro* produced bovine embryos are cultured in groups, the efficiency of blastocyst formation from slow and fast embryos is not known. In this sense, the objective of this study was to evaluate the rates of cleavage and of conversion to blastocyst in fast and slow *in vitro* produced bovine embryos. In addition, kinetics and standards of embryonic/secretion and consumption of metabolites, by evaluating the culture media by Raman spectroscopy and gas chromatography coupled with mass spectrometry (GC/MS), will be correlated in the future. To evaluate the cleavage rates, the cumulus-oocyte complexes were aspirated from slaughterhouse ovaries, selected (grade 1 and 2), placed in 90 µl IVM drops (M-199 + HEPES (20 oocytes / drop) and cultured for 22 hours at 38,5°C and 5% CO_2. The matured oocytes were in vitro fertilized in 90µl droplets of IVF medium (Parrish et al., 1988, Biol. Reprod., 38, 1171-1180) in an atmosphere of 5% CO_2 in air at 38,5°C and high humidity for 18 hours. Subsequently, the zygotes were transferred to individual 20 µl culture medium droplets (SOF medium supplemented with essential and nonessential amino acids and 5% fetal bovine serum) in a well of the well system (WOW) (adapted by Feltrin et al., 2006, Proceedings of the 58th Annual Meeting of the SBPC - Florianópolis, SC), and cultured in an incubator with an atmosphere of 5% CO_2 in air and high humidity at 38,5°C for 7 days. Zygotes were classified as fast (4 or more cells) and slow (2-3 cells), according to cleavage rate at 40hpi. Although present, uncleaved embryos after 40hpi were discarded from the analysis. After 168 hours of IVC, embryos were re-evaluated to obtain the blastocyst rates of the fast and slow groups (n=3 replicates). Preliminary data were evaluated by Student's *t* test and there was no difference between the cleavage rates from fast and slow embryos ($p = 0.1208$) with averages of 28.77 ± 3.164% for fast embryos and 34.50 ± 1.191% for slow embryos. However, differences were observed between the blastocyst rates ($p = 0.0178$) with averages of 11.6 ± 1.380% for fast embryos and 6.4 ± 1.168% for slow embryos. Therefore, from this study it can be concluded that there is no difference between the number of fast and slow embryos when evaluated at 40hpi. However, there is a higher number of fast embryos reaching blastocyst stage than slow embryos.

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Different periovulatory endocrine profiles and its relation with spatial distribution of transcripts in the reproductive tract of beef cows

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Keywords: cattle, steroids, uterus.

In cattle, fluctuations of progesterone (P4) and estradiol (E2) concentrations modulate endometrial gene expression, histotroph secretion, conceptus development and pregnancy outcome. Our recent RNAseq studies indicated that changes in the periovulatory endocrine milieu associated with the growth and ovulation of different size follicles regulated endometrial gene expression on day 7 of the estrous cycle. Objectives were to study the effect of the uterine horn relative to the ovary containing the corpus luteum (CL) and the region within the uterine horn ipsilateral to the CL on the expression of selected endometrial genes. An additional objective was to verify whether expression was also modulated on the vagina. The follicular growth of multiparous non-lactating Nelore cows was pharmacologically manipulated in order to obtain groups with large (LF/CL; n=11) or small (SF/CL; n=11) preovulatory follicles and corpora lutea (Mesquita F. Reprod. Fertil. Dev., submitted). Cows were slaughtered seven days after the induction of ovulation with GnRH analogue and fragments from mixed portions of contralateral uterine horn, regions of the ipsilateral uterine horn (anterior, middle, posterior), and mixed fragments of vagina were collected. Gene expression assessment was performed by quantitative PCR in six animals/group. Cyclophilin was used as reference gene. Relative expression of target genes were calculated by the delta-delta CT method with correction for efficiency. The SAS PROC MIXED procedure (Version 9.2; SAS Institute) was used for analysis considering the effect of group (LF/LCL and SF/SCL), uterine horns, regions, and their interactions. A probability of $P \leq 0.05$ indicated significant effects. The LF/LCL group had larger pre-ovulatory follicles and CL and greater E2 concentrations on D0 and P4 concentrations from D3 to D7. The abundance of transcripts coding the progesterone receptor was greater in the SF/CL (50%). The LF/CL group had a greater abundance of transcripts coded by the estrogen receptor (ESR2; 100%), aldo-keto reductase family 1, member C4 (AKR1C4; 71,3%) and serpin peptidase inhibitor, clade A member 14 (Serpin14; 50%). There was an effect of region for the expression of ESR2, AKR1C4 and Serpin14. Specifically, expression of ESR2 was greater in the middle section and the anterior section showed greater expression for AKR1C4 and Serpin14. No significant effects of interactions were detected for any of the target genes. There was no group effect on any of the selected genes expression in the vagina. In conclusion, although regional patterns in the genes expression were detected across the reproductive tract, such patterns were not affected by distinct periovulatory hormonal milieu.

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Gene expression of sirtuin and related genes in different temperatures of *in vitro* maturation of bovine cumulus-oocyte complexes

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Keywords: gene expression, sirtuin, *in vitro* maturation.

The adaptations that animals suffer due to high environmental temperatures lead to reduction of food intake and endogenous production of heat. In parallel there are many changes in energy metabolism. It is believed that the changes that occur in response to heat stress are mediated by sirtuins. These proteins act as an interrupter between energy metabolism and cell signaling cascades of other physiological processes. SIRT1 regulates gene expression programs in response to the metabolic state of the cell, thus coordinating the metabolic adaptation of the whole organism. The precise mechanisms regulating this process at cellular level are poorly understood. The aim of this study was to evaluate the expression of sirtuins (SIRT 1 e SIRT 2) and correlated genes (Hes1, BCL11, p53) on cumulus-oocyte complexes at different temperatures of *in vitro* maturation. Bovine ovaries were collected at a local slaughterhouse and transported to the laboratory. Only grade I cumulus-oocyte complexes (COCs) were selected. After that, COCs were placed in an incubator with high humidity and 5% CO_2 in air at temperatures of 37 ° C, 38.5 ° C and 40 ° C for 24 h. After maturation oocytes and cumulus cells were separated, and each group was stored in RNAlater solution (Qiagen, Hilden, Germany) at - 20 °C. Each sample contained 40 oocytes or the corresponding amount of cumulus cell. Three replicates of each group were submitted to RT-PCR and the data were normalized using the endogenous gene RPLPO. To identify differences in expression and their statistical significance the data were submitted to ANOVA including n the model the effect of cell and temperature. The averages were compared by Tukey Test at 5%. The results show that, independent of maturation temperature, the cumulus cell presented differences in expression between mature and immature COCs. The genes SIRT 2, BCL11 and p53 increased their expression, while SIRT 1 showed a reduction of expression after maturation. The only exception was the Hes1, which did not significantly alter the pattern of expression in any situation. In oocytes, the single gene that significantly changed the expression pattern was SIRT1, which presented similar expression between immature (0,048) and oocytes matured at 40° C (0,068) and different expression in oocytes matured at 37 and 38,5º C (0,075; 0,088). Although there was no significant difference among the temperatures of maturation, the oocytes matured at 40 ° C showed the lowest expression among matured (37º C = 0,075; 38,5º C = 0,088: 40º C = 0,068), which allows us to speculate about the reduced expression of this gene when COCs undergo maturation temperatures above 38.5 ° C. Other experiments are being conducted to explain the correlation between genes and their expression in oocytes and cumulus cells.

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Use of preimplantation genetic diagnosis (PGD) in equine embryo sexing

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Keywords: equine, preimplantation genetic diagnosis, sexing.

The preimplantation genetic diagnosis (PGD) is the removal of a small number of cells from an early development embryo for genetic analysis. In horses, despite PGD presenting enormous potential for determining the sex of the embryos before transfer, the progress for establishment of genetic diagnosis techniques has been slow. In the present study, we investigated new methods based on human reproductive techniques for PGD to improve the efficiency of embryo sexing in horses. Twelve expanded blastocysts were biopsied in Syngro ® Holding medium (Bioniche) using a laser (Hamilton Thorne, USA) and a beveled injection pipette (15 um diameter) coupled to a Narishige micromanipulator. Biopsy pipettes were used to puncture the zona pellucida and the capsule in the region opposite to the inner cell mass followed by aspiration of 10 to 30 trophoblast cells adjacent to the region of the opening of the zona pellucida. The cells were then transferred to microcentrifuge tubes, which were centrifuged at 11.000xg for 10 minutes and stored at -20 ° C for future genetic analysis. The WGA (Whole Genome Amplification) method was performed based on fragmentation of genomic DNA followed by isothermal amplification and PCR cycles. The samples were incubated at 50°C for 1 h and heated to 99°C with Single Cell Lysis & Fragmentation Buffer. Then universal oligonucleotide primers were used to amplify the DNA fragment with 25 cycles of PCR. PCR products were loaded on a 2% agarose gel containing ethidium bromide and electrophoresed for 2 h at 60 V or 1 h at 110 V. Results were visualized and photographed using UV light transillumination. The amplified DNA was initially evaluated for sex by PCR for the *SRY* gene, which generated a fragment of 131bp. Because in this test, absence of the male *SRY* signal could occur due to failure of the PCR, the amplified DNA was also evaluated for sex by a duplex PCR for the gene for RNA-binding motif protein, Y-linked (*RBMY* – fragment of 225bp), which is Y-chromosome specific, and glioma pathogenesis- related protein 1 (*GLIPR1* – fragment of 113bp), an autosomal gene. Specificity of the primers was confirmed during a blinded study of 6 blood samples collected from mares and stallions, and the sex was correctly determined in all samples. The sex of the embryos could be detected in 10/12 embryos (83.3%), of which 7 were females and 3 were males. The time required for the confirmation of sexing was approximately 8 hours. All embryos re-expanded after the procedure. The biotechnology here described is simple and fast providing the equine vet a suitable alternative for clinical use.

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The antioxidant system in preterm and term canine neonates

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Keywords: antioxidante, canine neonates, lung.

With the onset of pulmonary respiration, an exponential increase in the production of free radicals occurs. In order to counteract such event, the development of an antioxidant defense capable of neutralizing the oxidative stress cytotoxicity is necessary. The aim of this study was to compare the development of the antioxidant system, vitality and gas exchange in canine term and preterm neonates. According to the gestational age, 15 neonates were allocated into: Term Group (63 days of gestation, n = 5), Premature 57 Group (57 days of gestation, n = 5) and Premature 55 Group (55 days of gestation, n = 5). Gestational age was assessed by identifying the LH surge through maternal progesterone assay. The pregnant females were submitted to cesarean section and, after the hysterectomy and removal of fetuses from the uterine cavity, the amniotic fluid was collected to assess the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX), as well as the marker of oxidative stress (TBARS). The quantification of GPX activity was performed by NADPH consumption, SOD through the reduction of cytochrome c by superoxide anion, and TBARS by means of malondialdehyde (MDA) concentration. From neonates, the Apgar score of vitality and body temperature were assessed at birth and after 2 and 4 hours of birth, as well as blood samples were drawn for hemogasometric and SOD, GPX and TBARS evaluation. The experimental design was approved by the Ethics Committee of FMVZ/ USP. Data were compared by ANOVA and Tukey test ($p \leq 0.5$). Four neonates of the Premature 55 Group died within the first hour of life, due to extreme degree of prematurity. There was no statistical difference between groups or among time points for SOD, GPX and TBARS both in serum and in the amniotic fluid. At birth, the Term Group had the highest Apgar score (3.8±0.4), statistically superior to Premature 55 Group (2.5±0.2), but not different from Premature 57 Group. The Term Group reached the satisfactory Apgar score within 2hs of birth (8.4±0.7), while the Premature 57 Group remained low within the first 4 hours of birth (5.3±0.7). There was a progressive increase in body temperature in Term Group during the 4 hours of birth, inferior to Premature 57 Group, with no statistical difference between the times of evaluation. All puppies had blood acidosis, except at 4h for Term Group. Moreover, the Term Group showed higher pCO_2 at birth (76±7.84) compared to Premature 57 (47.7±3.1) and Premature 55 (56.7±3.2) groups. In conclusion, our results suggest that the neonatal antioxidant status does not change according to prematurity, with the same antioxidant ability between premature and term neonates. Puppies have mixed acidosis at birth, but only term neonates can efficiently reverse such imbalance, compared to premature neonates.

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Body condition and return of ovarian activity in lactating woolless sheep

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Keywords: leptin, postpartum, progesterone.

The return of cyclic ovarian activity postpartum (RCOA) in small ruminants depends on hormonal interactions in the hypothalamic-pituitary-ovary axis during lactation. However, in early lactation, the pituitary hormones are more directed towards the synthesis and secretion of milk than for the restoration of cyclic ovarian activity, resulting in a period of postpartum anestrus (Rodrigues et al., 2011, Arq Bras. Zootec Vet Med., 63, 171-179). The nutritional management and body condition of lactating ewes directly influence the ovarian activity postpartum and plasma concentrations of leptin, a hormone important for promoting follicular growth and ovulation (Zieba et al., 2008, Can. J. Physiol. Pharm., 59, 7-18). The aim of this study was to investigate the effects of supplementation and body condition of ewes on ovarian activity postpartum. We used 24 ewes belonging to the Technical School of Bom Jesus-PI. During the latter half of pregnancy and 75 days of lactation the sheep had access to pasture grass *Andropogon gayanus* and were randomly divided into two groups according the levels of concentrate supplementation (0.5% and 1.5% of body weight) based on 70% corn bran, 25% soybean meal, 5% vitamin and mineral supplement. Weight and body condition score (BCS) were weekly measured (Thompson and Meyer, 1994, Oregon State, 4). Blood samples were collected twice weekly for determination of progesterone and leptin, performed by radioimmunoassay (Laboratory of Endocrinology, UNESP, Araçatuba, SP) using commercial kits (Coat A Count Progesterone Kit, Siemens®, Multi-Species Leptin RIA kit, Millipore®). The RCOA was considered when progesterone concentrations were greater than 1.0 ng/mL for more than ten days (Minton *et al*., 2001, J. Reprod. Fertil., 69, 314-320). The percentage of RCOA until the end of lactation was compared by Chi-square between the levels of supplementation and between the classes of BCS (1.0-2.0 and 2.5-3.5). To test the effect of supplementation level and the class of BCS on the plasma concentrations of leptin ANOVA was performed and means were compared by Tukey test. During lactation, 13 animals showed BCS average between 1.0-2.0 and 11 animals showed BCS average between 2.5-3.5. The level of supplementation had no effect on the RCOA and on plasma concentrations of leptin ($p>0.05$). There were no interaction effects of supplementation and the class of BCS ($p>0.05$).). The RCOA rate was higher in the group of animals with BCS between 2,5-3,5 than in the group of animals with BCS between 1.0-2.0 (91,9 vs. 61,5%, $p<0.05$), with effect of BCS categories ($p<0.05$) in the RCOA ($p<0.05$). The sheep with BCS between 2.5-3.5 also had a higher concentration of leptin than those with BCS between 1.0-2.0 (0.80 vs. 0.60 ng/ml; $p<0.05$). It is concluded that the body condition of lactating sheep influences the return of cyclic ovarian activity postpartum independently of the levels of supplementation studied in this work.

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The ovine perinatal period: an uterine and umbilical blood flow survey

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Keywords: gestation, ovine, vascularization.

The pulsed-wave Doppler is a non-invasive diagnostic tool valuable for accurate prenatal follow-up. The present study aimed to evaluate the blood flow of the uterine (UA) and umbilical (UMA) arteries in pregnant ewes from 60 days onwards, during the pharmacological induction of parturition, as well as the uterine blood flow during the puerperal period. Fifteen Santa Inês ewes were used for Doppler ultrasound analysis. Uterine arteries were scanned through a rectal linear probe (5-10 MHz) after the identification of the iliac vessel ramification. Subsequently, the pulsed-wave Doppler was employed to classify the flow velocity waveforms and to automatically calculate the hemodynamic parameters (RI, PI, S/D). In order to localize the umbilical arteries, the abdomen was scanned. A total of nine stable waves were obtained for each artery to calculate the average for each variable. During gestation, ultrasonographic exam was performed at 60, 90 and 120 days. On the 135th day of pregnancy, ewes were subjected to lambing induction with a double injection of 0.33 mL/kg (IM) of the antiprogestagen aglepristone (Alizin, Virbac, Brasil), at a 24h interval. During parturition induction, UAs and UMA were evaluated at 3 time-points: 12hs previous to the first injection (M1) and 12hs after each aglepristone injections (M2 and M3). The onset of lambing occurred 49.8±4.9 hours after the first injection of aglepristone. The UAs were also examined at 1, 3, 5, 7, 15 and 30 days post-partum. During gestation and parturition induction, the overall uterine vascularization remained constant, with a low resistance pattern (RI: 60d-0.58±0.01; 90d-0.59±0.02; 120d-0.6±0.01; M1-0.61±0.02), characterized as continuous and high blood flow in the fetal unit. The UMA hemodynamic indexes progressively decreased along gestation (RI: 60d-0.99±0.02; 90d-0.77±0.01; 120d-0.62±0.02). There was no negative influence of the parturition induction on uterine vascular flow, since the UAs hemodynamic indexes remained unchanged after treatment (RI: M2-0.6±0.02; M3-0.6±0.02), simultaneously with the increase of UMA blood flow. Conversely, there was a decrease in UMA resistance after the induction of lambing (RI: M1-0.61±0.02; M2-0.58±0.01; M3-0.55±0.02), suggesting vasodilation to insure fetal oxygenation during parturition. On the other hand, the vascular indexes immediately post-partum were enhanced (RI: M3-0.6±0.02; 1d-0.77±0.02), compared to the results obtained at lambing. Based on these results, we can infer that the mechanism that triggers ovine parturition along with the steroidogenic change have no direct influence on uterine vascular dilation capable of impairing placental perfusion. Therefore, there was a reduction in uterine vascular flow soon after the removal of the vasodilation stimuli. Hence, the uterine blood flow maintains regardless of the increase in umbilical hemodynamic as a manner to guarantee the adequate vascular flow for fetal survival.

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Correlation between maternal body condition and follicular population in the development of bovine fetal gonads from Nelore breed

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Keywords: carcass yield, fetal ovaries, fetal testis.

The cattle body condition reflects energy intake variation, influencing reproductive function and during gestation, promoting direct and indirect effects on fetal growth, including ovary and testicle characteristics. The aim of this study was to correlate maternal carcass weight (CW), carcass yield grade (CYG, scale 1 to 5), age and antral follicle counts (AFC) with weight and volume from fetal gonads in different gestational ages (3 to 8 months). The study used 534 pregnant cows from slaughterhouse located at Araçatuba region and was conducted from June 2012 until March 2013. All cows were distributed to groups according to their AFC as follows: low ≤ 15 (≥2mm diameter), intermediate 16 to 30 and high ≥31 antral follicles. Fetal age was estimated by the formula: DG= 8.4 +0.087C +5.46√L, where DG= Day of gestation and L= fetal length. The measurements from fetal gonads were: height, width, thickness (cm) and weight (g). Data were analyzed by Pearson correlation test (r) and values with $p<0.05$ were submitted to non linear regression test. Negative correlation was observed between maternal AFC and fetal testicular weight, and volume ($r= 0.3$, $p<0.0001$ and $r= 0.23$, $p= 0.0014$). A positive correlation was observed between maternal CW and fetal weight ovary on the 5th and 7th months of gestation ($r= 0.47$, $p= 0.0192$ and $r= 0.43$, $p= 0.0223$). Cattle CYG was positively correlated to fetal ovary weight on the 5th month ($r=0.55$ and $p= 0.0057$). On the other hand, it was negatively correlated with fetal testicular volume on the 8th month ($r= -0.69$ and $p= 0.0126$). The correlation between maternal age and fetal testicular volume was negative on the 5th and 8th months ($r= -0.35$, $p= 0.0353$ and $r= -0.62$, $p= 0.0334$). There was not influence of maternal CYG on fetal length during the gestation. It is known that nutrition affects LH plasma concentrations, which could be a possible explanation for the increase on fetal ovarian weight found on the 5th and 7th months of gestation. On this period, fetal ovaries begin to exhibit sensitivity of antral follicles to gonadotropin concentrations. To demonstrate this effect, maternal and fetal LH concentrations will be quantified in the next phase of this study. Testicular volume from male fetuses may decrease as consequence from high maternal testosterone concentrations (T), since this can cause a negative feedback on LH secretions by fetal pituitary decreasing testicular growth due to low fetal T concentrations. Collaborating with this assumption, in obese cows high androgen concentrations in the blood and reduction on Leydig cells population in the testes of their male fetuses were observed. Quantification of T and LH concentrations will be held at the next stage of this work, as well as Leydig cells counting, both to prove these assumptions.

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Corpus luteum development and function after supplementation of long-acting progesterone during the early luteal phase in beef cattle

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Keywords: Doppler, estrous cycle, luteolysis.

Progesterone (P4) is essential for establishment and maintenance of pregnancy in mammals. Supplementation of P4 within the first week post artificial insemination has embryotrophic effects in cattle (Carter, Reprod Fertil, 20, 368-75). Paradoxically, there is a reduction in corpus luteum (CL) function when exogenous P4 is given on the days next to ovulation indicating a possible interference with development of the CL (Garret, Prostaglandins,36, 85-96). The objective of this study was to evaluate the effects of long-acting P4 supplementation on days 2 or 3 postovulation on CL development and regression in beef cattle. Nelore cows were synchronized with an estradiol/P4-based protocol and treated intramuscularly with a single dose of 150 or 300 mg long-acting P4 (Sincrogest®, Ouro Fino Saúde Animal, Brazil) on Day 2 or 3 postovulation (n=6-7 cows/group). Color-Doppler ultrasound scanning and blood sample collection were conducted from Days 2 to 21.5 postovulation. Based on plasma P4 concentrations, the beginning and the end of functional luteolysis were estimated for each cow. Timing of structural luteolysis was estimated based on the area of CL measured in each scanning. Continuous variables were analyzed by spli-plot ANOVA using the PROC MIXED procedure (Version 9.2; SAS Institute). Discrete variables related to characteristics of luteolysis were analyzed by one-way ANOVA. Mean comparisons were performed using Duncan's test or Dunnett's test. Fisher's exact test was used for comparisons of frequency data. Plasma P4 concentrations were greater ($P<0.05$) from Day 2.5 to Day 5.5 in the Day2-treated groups and from Day 3.5 to Day 5.5 in the Day3-treated cows than in the control group. CL area and blood flow from Day 2 to 8.5 did not differ ($P>0.1$) among groups, suggesting that there was no effect of P4 treatment on luteal development. The frequency of cows that began luteolysis before Day 15 was greater ($P<0.04$) in cows treated with 300 mg (4 out of 7 cows in each group) than in the controls (0 out of 7). The interval from pre-treatment ovulation to beginning of functional and structural luteolysis was shorter ($P<0.01$) in the combined P4-treated groups than in the control cows (14.3 ± 0.6 d vs. 17.9 ± 1.1 d, and 15.6 ± 0.6 d vs. 18.6 ± 0.7 d, respectively). Collectively, data indicate that an earlier exposure of the uterus to elevated P4 concentrations leads to anticipated onset of mechanisms involved in the luteolytic process. In conclusion, we propose that early P4 supplementation is not associated with a reduced CL size, vascularization or P4 secretion during luteal development, but anticipates on about 3 d the beginning of functional and structural luteolysis in beef cattle.

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Effect of PDE5 inhibition on nuclear maturation of bovine oocytes in vitro cultured

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Keywords: maturation, phosphodiesterase, sildenafil.

Nuclear maturation comprises the reversal of the first meiosis block of the oocyte from germinal vesicle stage up to the second meiosis block in metaphase II (MII). It is well established that the decrease in cAMP levels is required for resumption of meiosis. Similarly, cGMP also mediates the resumption of meiosis in mammalian oocytes (Tornell *et al.*, 1990, Acta Physiol. Scand., 139, 511-517). The levels of cGMP are balanced between synthesis by guanylate cyclase and degradation by cGMP-specific phosphodiesterases (PDEs; PDE5, PDE6 and PDE9; Kass *et al.*, 2007, Cardiovasc. Res., 75, 303-314). The aim of this study was to evaluate the effect of inhibition of one of the cGMP-specific PDEs on the nuclear maturation of bovine oocytes *in vitro* to assess its participation on the process. PDE5 was chosen because a specific inhibitor is available and because we have detected its expression in bovine oocytes and *cumulus* cells (Schwarz K.L., 2011. PhD thesis, FZEA-USP, 106p). Therefore, a dose-response assay was performed with different concentrations of sildenafil (SIL; PDE5 inhibitor) added to the *in vitro* maturation (IVM) medium from the start of culture (0-22h) or from 11h of IVM culture (11-22h). *Cumulus*-oocyte complexes were aspirated from commercial abattoir ovaries and *in vitro* matured (groups of 20) in droplets (100 µL) of TCM199 under mineral oil with different concentrations of SIL (0, 10^{-7}, 10^{-5}, 10^{-3}M; Santa Cruz Biotecnology, Santa Cruz, USA). The control (C) consisted of oocytes matured without SIL. Culture was carried out for 22h at 38.5°C and 5% CO_2 in air. After IVM, the oocytes were denuded, stained (10 µg/mL Hoescht 33342 for 15 min) and assessed under an epifluorescence microscope to determine the rate of nuclear maturation (MII%). Four replicates were performed and the results analyzed by linear regression (SAS v. 9.2), considering a significance level of 5%. In culture with SIL 0-22h, the MII% were 66.3, 74.1, 85.3 and 53.9% for 0 (n=80), 10^{-7} (n=62), 10^{-5} (n=51) and 10^{-3}M (n=76) SIL, respectively. The 10^{-7}M group was similar to C ($P>0.05$) and 10^{-3}M reduced maturation ($P<0.05$). The 10^{-5}M group, however, increased the maturation rate ($P<0.05$) compared to C. When SIL was added between 11-22h of IVM, MII% were 62.0, 45.4 and 60.7%, respectively, for 10^{-7} (n=49), 10^{-5} (n=63), 10^{-3}M (n=79) SIL. The 10^{-7} and 10^{-3}M groups were similar to C ($P>0.05$), whereas 10^{-5}M reduced MII% ($P>0.05$). In conclusion, a higher concentration of SIL inhibits maturation when added 0-22h, suggesting a greater effect on the initial period of maturation. On the other hand, the 10^{-5}M SIL group showed stimulatory effect in 0-22h and inhibitory in 11-22h periods of culture, suggesting that SIL exerts different effects depending on the concentration and maturation phase evaluated. More studies are needed to clarify the role of cGMP and PDE5 in the control of meiosis in bovine oocytes.

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Global gene expression in bovine oocytes submitted to heat shock during *in vitro* maturation

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Keywords: bovine, heat stress, oocyte.

Exposure of bovine oocytes to elevated temperature reduces oocyte maturation and developmental competence. The aim of this study was to evaluate global gene expression in *Bos indicus* (Nelore) and *Bos taurus* (Holstein) oocytes exposed to elevated temperature during *in vitro* maturation (IVM). Nelore (NEL n = 13) and Holstein cows (HBW n = 14) were kept in a common installation under the same management conditions. Environmental parameters such as air temperature [28.05 ± 0.28°C (maximum) and 16.88 ± 0.29°C (minimum)] and relative humidity [77.36 ± 1.30% (maximum) and 44.71 ± 1.29% (minimum)] and physiological parameters such as rectal temperature (38.37 ± 0.04°C - NEL and 38.23 ± 0.05°C - HBW) and respiratory rate (28.05 ± 0.44 bpm - NEL and 29.99 ± 0.49 bpm - HBW) were monitored. Cows were submitted to follicular aspiration sessions and cumulus-oocyte complexes (COCs) recovered were submitted to control (38.5°C for 22 hours) and heat shock (41°C for 12 hours followed by 38.5°C for 10 hours) treatments during IVM (Roth and Hansen, 2005, Reproduction, 129, 235-244). Then, COCs were denuded and stored at -80°C until genomic DNA microarray evaluation. Three pools of 25 oocytes were used per experimental group. Total RNA was extracted by RNeasy Mini Kit (Qiagen), samples were submitted to RNA amplification (MessageAmp II aRNA Amplification Kit, Ambion) to obtain 100 ng of mRNA and submitted microrarray assay (Affymetrix GeneChip Bovine Genome Array). Data were analyzed with the software *FlexArray* 1.6.1.1. and the *Affy* package developed in the language R. Genes with fold-change of at least 1.5 and $P \leq 0.05$ were considered differentially expressed. Results indicated 68, 6 and 5 differentially expressed genes between the variables breed, temperature and interaction breed x temperature, respectively. According to the Ingenuity Pathways Analysis functional classification, genes differentially expressed regarding breed x temperature interaction were related to cell signaling (*OSMR*), lipid metabolism (*ACOX1*), protein processing (*CCT4*), gene expression control (*DICER1*) and protein catabolism (*DENND3*). Genes *CCT4*, *DICER1* and *ACOX1* were up-regulated in heat shocked HBW oocytes group (*CCT4*: HBW-41°C vs HBW-38.5°C; *DICER1*: HBW-41°C vs NEL-41°C and *ACOX1*: HBW-41°C vs HBW-38.5°C and NEL-41°C). *OSMR* gene was up-regulated in NEL-41°C oocytes as compared to HBW-41°C and the *DENND3* gene was up-regulated in NEL-38,5°C as compared to HBW-38,5°C. In conclusion, factors such as genotype and temperature modulate expression of genes that play key biological roles in cell growth, differentiation, protein and RNA processing. Functional studies will be needed to better characterize the thermoprotective role of these molecules.

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Natriuretic peptides during ovulation in cattle

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Keywords: EGFR, LH, NPPC.

The Natriuretic Peptides (NPs) have been observed in the local regulation of reproductive functions of mammals, besides their systemic activity. Recently, the NPC precursor (NPPC) mRNA expression downregulation by hCG in granulosa cells has been demonstrated. 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 *in vitro*. Cyclic beef cows were synchronized using a progesterone-based protocol. After intravaginal device removal (day 9), ovaries were examined by transrectal ultrasonography and cows that had GnRH-responsive preovulatory follicles (≥ 12 mm) were challenged with 100 μg gonadorelin acetate IM 12 h after removal of intravaginal device. Treated cows were then ovariectomized at 0, 3, 6, 12 and 24 h post-GnRH via 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 EGFR signaling blockade on LH modulation of NPPC mRNA expression was evaluated using granulosa cell culture. Data were tested for normal distribution using Shapiro-Wilk test and analyzed by ANOVA. 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 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 NPA and NPC 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 LH effect completely abolished after addition of EGFR blocker (AG1478) to 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 EGFR. These results suggest that NPs are involved in the ovulatory process in bovine and that the regulation and function of NPs during ovulation may differ between monovular and polyovular species.

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Occurrence of estrus in cycling bovine heifers after PGF2α application

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Keywords: estrus, heifers, prostaglandin.

Prostaglandin F2α (PG) and its synthetic analogues have been widely studied since its discovery in 1970, as potent luteolytic agents (Fierro et al., 2013, Theriogenology, 79, 399-408). The corpus luteum (CL) maturity at the time of PGF2α application has a marked influence on the luteolytic response, however in the first six days of the estrous cycles there is no luteolytic effect of PGF2α injection (Bó *et al*., 2002, Theriogenology, 57, 53-72). In animals that respond to treatment with PG estrus is distributed in a period of six days (Macmillan K. L; Henderson H. V., 1984, Anim Reprod Sci., 6, 245-254). Thus, the aim of this study was to evaluate the occurrence and time of estrus after PG application in cycling Nelore and crossbred heifers. We used 199 Nelore and 79 crossbred heifers. Heifers received i.m luteolytic dose of 0.526 mg sodium cloprostenol (Sincrocio®, Ourofino Saúde Animal, São Paulo, Brazil) using 3ml disposable syringe with 40x12 needle. The estrus detection started 24 hours after PG application in an observation schedule throughout one hour AM and one hour PM. The occurrence of estrus was 48.93% (n=136/ 278). Nevertheless, in Nelore heifers the occurrence of estrus was 45.23% (n=90/199) whereas in the crossbred heifers was 58.23% (n=46/79). Moreover the distribution of estrus after PG application occurred in 29 animals (27.55%) within 48 hours, 60 animals (43.13%) within 72 hours, 31 animals (22.45%) within 96 hours, 10 animals (7.32%) within 120 hours and in one animal (0.50%) within 144 hours. The estrus distribution among Nelore heifers occurred in 12 animals (6.03%) within 48 hours, 43 animals (21.1%) within 72 hours, 22 animals (11.06%) within 96 hours, 7 animals (3.52%) within 120 hours and one animal (0.50%) at 144 hours. Among crossbred heifers distribution of estrus occurred in 17 animals (21.52%) within 48 hours, 17 animals (21.52%) within 72 hours, 9 animals (11.39%) within 96 hours, 3 animals (3.80%) within 120 hours and none at 144 hours. The results show that cycling heifers respond to PG application and occurrence of estrus is distributed up to 144 hours, with the distribution especially from 48 to 96 after PG application.

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Quantification of total lipids of *in vitro* produced bovine embryos with different developmental kinetics

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Keywords: bovine, embryo, lipids.

The embryo morphology and cleavage and blastocyst rates have been used to assess embryo viability. However, with the advent of new biotechnologies, it has become clear that embryo viability can be severely compromised without noticeable morphological changes. One of these changes is the higher lipid accumulation in IVP embryos. This has been related with a lower cryopreservation efficiency and embryo viability, and could be an indicative of inadequate culture conditions when compared with the *in vivo* system. Based on these data, the hypothesis of this work was that embryos of different developmental kinetics have different characteristics of lipid accumulation, which is reflected in embryo viability. Our objective was to determine the amount of total lipids in embryos of fast (4 cells 40hpi and blastocysts from this group) and slow (2 cells 40hpi and blastocysts from this group) development by lipids staining at different stages of early development. For this, IVP fast and slow bovine embryos (individually cultured) and control (conventional group cultured) were used for total lipids staining by SUDAN BLACK B (Sigma) at cleavage (40hpi) and blastocyst stages (186hpi) (triplicate, n = 8 embryos per group minimum). The cleaved embryos and blastocysts were photographed and the images processed by threshold tool so to only lipids to become evident. After this transformation, we calculated the number of pixels obtained from each image and converted into arbitrary units by a script created in the development environment Matlab using the Image Processing toolbox. The results were submitted to ANOVA with Tukey post test (Prism 5 GraphPad Inc.). There was no difference between groups in the amounts of lipids among the cleavage groups (Fast: 93 884 ± 4331; Slow: 68911 ± 7180; Fast Control: 74622 ± 21180; Slow Control: 70763 ± 20046). However, the amount of lipids was lower for slow blastocyst when compared with the control and fast groups (Slow: 38617 ± 3379; Fast: 122626 ± 30378, Control: 95658 ± 15138). There was no difference between the fast and control groups. These results show that the developmental kinectic and culture conditions have direct influence on lipid accumulation in IVP bovine embryos. Furthermore, these data can contribute to the improvement of the IVP system, especially for the production of embryos for cryopreservation.

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Ovarian evaluation of *Trachemys scripta elegans* (WIED, 1839) turtles raised in Brazil

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Keywords: ovary, testudines, turtle.

Trachemys scripta elegans (*T. scripta elegans*) is an underwater turtle native to North America, however, in Brazil, it is invasive and exotic. The lack of knowledge on the reproductive biology of exotic species may interfere with population control measures or comparative studies. Therefore, this study aimed at describing the ovaries of *T. scripta elegans* turtles to contribute with information on their reproductive biology under Brazilian conditions. The macroscopic and topographic anatomy and the morphometry (mass (MO), volume (VO) and length (LO)) of the right (r) and left (l) ovaries of 40 turtles from the Parque Ecológico do Tietê[1] (IBAMA[2] Record Nr. 2491988) were studied. Mass (g) was estimated in analytical scale, volume (ml) according to the Scherle method (1970) and length using a millimetric precision caliper (Mitotoyo®). Ovarian follicles were determined macroscopically and classified in: Type 1 (T1 = 0.5 – 1.0 cm), Type 2 (T2 = > 1.0 to 2.5 cm) and Type 3 (T3 = > 2.5 cm). The Kruskal-Wallis test, followed by testing, was employed for comparison of means± sd and simple correlation coefficient (R) was determined among variables (Assistat 7.6 beta). This study was approved by the CEDEP[3] from UNIVASF[4] (Protocol nr 0001/160412). The ovaries were irregular structures filled with developing follicles (primary, secondary and tertiary) on a richly vascularized stroma. They were located in the central region of the celomatic cavity, caudally to the intestine, stomach, liver and heart, medially to the uterine tubes, cranially to the urinary vesicle and cranioventrally to the swim bladder. MOd (11.95 ± 8.87 g), VOd (11.28 ± 8.72 ml) and LOd (12.52 ± 5.12 cm) were similar (P>0.05) to MOe (15.48 ±9.89g), and to VOe (15.20 ±9.37 ml) and to LOe (14.40 ±4.61 cm). The average numbers of T1, T2 and T3 follicles were, respectively, 4.15±3.83, 3.80±3.61and 0.60±1.26 on the right antimere and 5.35±4.14, 4.25±4.29 and 0.70±1.76 on the left antimere, and there was difference (P<0.01) among T1, T2 and T3 within the same ovary and among ovaries of different antimeres. High correlation (P<0.01) was observed among MO, VO, LO and T2 follicles within each ovary and among the T1 and T3 follicles among ovaries of different antimeres, while there was moderate correlation (P<0.05) between MO and VO and among T2 follicles in ovaries of different antimeres. It is concluded that in specimens studied, ovaries developed symmetrically, ovarian morphometry and the quantity of T2 follicles correlate highly and positively, ovaries had a higher amount of T1 follicles, followed by T2 and T3and the left ovary showed higher quantity of each follicular type. This data provides scientific support on the reproductive biology of this exotic species, helping on its population control and comparative studies.

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ITS supplementation in maturation medium and low oxygen tension during *in vitro* culture improve blastocyst formation in domestic cat ICSI embryos but enhances DNA fragmentation

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Keywords: DNA fragmentation, feline, ICSI.

The ICSI procedure is potentially of great value for felids, and it has not been extensively studied in these species. The objective of this work was to evaluate embryo development and DNA fragmentation of cat ICSI embryos treated with antioxidant conditions during oocyte maturation and embryo culture. Ovaries were recovered from cats subjected to ovariectomy. Cumulus-oocyte-complexes were *in vitro* matured in maturation medium (MM): TCM199 containing 1 IU/ml HCG, 10 ng/ml ECG, 2.2 mM calcium lactate, 0.3 mM pyruvate, 0.3% BSA and 3% antibiotic-antimycotic; or MM supplemented with 1ul/ml of the insulin, transferring and selenium (ITS, a free radical reducer). After ICSI, presumptive zygotes were cultured in SOF at 39°C and atmospheric oxygen tension (21%O_2) or low oxygen tension (5% O_2). The experimental groups were: MM-21%O_2 (n=138), MM-5%O_2 (n=142), ITS-21%O_2 (154) and ITS-5%O_2 (n=206). Control SHAM groups were included for each treatment. We evaluated cleavage on day 2 and blastocyst formation on day 7. The blastocysts from all the ICSI groups were evaluated by TUNEL assay to determine total cell number and the presence of fragmented nuclei. In vitro embryo development was compared by non-parametric Fisher's exact test, differences in total cell number by one-way ANOVA and the proportion of fragmented nuclei over total cell number by the "difference of proportions test", ($p<0.05$). The cleavage rates were lower ($p<0.05$) in the ITS-21%O_2 group (35.7%) than the other three ICSI groups (52.2%, 55.6% and 56.8% for MM-21%O_2, MM-5%O_2 and ITS-5%O_2, respectively). Regarding blastocyst formation, the highest blastocyst rate was observed in the group ITS-5%O_2 (20.9%) *vs.* 8.7%, 7% and 6.5% for MM-21%O_2, MM-5%O_2 and ITS-21%O_2, respectively. No blastocyst development was observed in any of the SHAM groups. The mean of blastocyst cell number did not differ among the groups (177.8±28.7, 105.9±16.7, 128.6±18.3 and 129.4±17.9 for MM-21%O_2, MM-5%O_2, ITS-21%O_2 and ITS-5%O_2, respectively). However, the proportion of TUNEL+ cells was statistically higher in the group ITS-5%O_2 (67.6%), in respect to the other three groups (43.5%, 36.5% and 34% for MM-21%O_2, MM-5%O_2 and ITS-21%O_2, respectively). Our results showed that the antioxidant conditions used in this study (ITS and low oxygen tension) improved embryo development in vitro but increased DNA fragmentation. It is possible that embryo development improved because of higher sperm decondensation in oocytes matured with ITS, as has been previously reported (Yeon et al. 2006, Animal Reproduction Science, 106:13-24), and more physiological conditions using low oxygen tension during culture. We also suggest that these conditions allowed the blastocyst formation of embryos of lower quality and higher DNA fragmentation that would not have developed in the other conditions. More studies are needed to determine the best conditions to generate viable cats after the transfer of ICSI embryos to recipient females.

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Pharmacological blockade of H3K27 trimethylation increases cell apoptosis of in vitro-produced porcine embryos

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Keywords: differentiation, epigenetics, polycomb.

Trimethylation of lysine 27 of histone H3 (H3K27me3) is an epigenetic mark that mediates transcriptional repression and controls pluripotency in embryonic cells. This effect occurs through the temporary repression of genes that control embryo development and differentiation of pluripotent cells. Trimethylation of H3K27 is catalyzed by proteins of the Polycomb Repressive Complex 2 (PRC2), however, this process is not entirely known. 3-Deazaneplanocin A (DZNep) can inhibit the action of PRC2, thus preventing H3K27 trimethylation. The aim of this study was to investigate the effect of adding DZNep to the in vitro culture media of porcine embryos. Oocytes derived from ovaries collected in an abattoir were fertilized in vitro and cultured with 0 (control group; n = 101) or 5 μM DZNep, starting on D2 (n = 108) or D4 (n = 120) of embryo culture. Embryos were fixed on D8 and submitted to an immunofluorescence protocol, with rabbit anti-cleaved caspase-3 monoclonal primary antibody and Alexa Flour 488 goat anti-rabbit secondary antibody. The cellular DNA was stained with DAPI. The total cell number and the number of apoptotic cells were determined and the rates of apoptosis between the different treatments were compared using the Chi-square test, with 5% as the significance level. The rate of apoptosis in the control group was 21.4%, lower than the ones of DZNep D2 (45.5%) and DZNep D4 (31.4%) groups. Apoptosis in DZNep D2 group was higher than in DZNep D4 ($P < 0.05$). These data show that, when the inhibitor of PRC2 is added at the beginning of the embryo culture, the induction of apoptosis is more significant and embryonic development is affected more severely. Based on these results, it can be concluded that the Polycomb group proteins play an important role in the regulation of early development of porcine embryos, since their inhibition results in increased apoptosis rate of the embryonic cells. It can also be inferred that, the earlier in embryonic development is the blocking of this complex, the greater is the rate of cellular apoptosis induced.

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Effect of melatonin *on in vitro* maturation of bovine oocytes

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Keywords: *in vitro* maturation, melatonin, recombinant FSH.

In vitro maturation (IVM) is a technique that allows the rescue of large amounts of immature oocytes from the ovary and the culture to produce *in vitro* developed embryos. However, not all oocytes removed from the ovarian follicles acquire full nuclear and cytoplasmic competence. Melatonin is a hormone that exhibits antioxidant and antiapoptotic properties, acting in cell protection (Hardeland R. et al., 1993, Neurosci Biobehavioral Rev.,17,347–357), besides regulating several signaling pathways (Tamura et al, 2009, Fert Steril, 92, 328-343). Thus, the addition of melatonin during IVM could be used to improve the competence of bovine oocytes. The objective of this study was to investigate the influence of melatonin on the progression of nuclear maturation in vitro. *Cumulus*-oocyte complexes (COCs) were aspirated from slaughterhouse ovaries, selected and transferred in groups of 25 to 100 µL droplets of IVM medium [TCM-199 with 0.1% polyvinyl alcohol (PVA), sodium pyruvate (25 mM) and gentamicin (25µg/ml)] under mineral oil. For the experiments, the maturation medium was supplemented with recombinant FSH (0.5 µg/ml, positive control) or melatonin (10^{-6} and 10^{-9}M) and cultured for 6, 12, 18 and 24 h at 38.5°C and 5% CO_2 in air. As an additional control, a group was matured without addition of FSH or melatonin. For the analysis of germinal vesicle breakdown rate (GVBD), the oocytes cultured for 6 and 12 h were labeled with anti-lamin A/C-DAPI (Prentice-Biensch et al., 2012, Theriogenology, 78, 1633–1638) and to analyze the rate of maturation (metaphase II, MII) oocytes cultured for 18 h and 24 h were stained with Hoechst 33342 (10µg/ml, 15 min). Data from three replicates were analyzed by Chi-square at a significance level of 5%. As expected, all oocytes in groups matured for 6 h remained immature in germinal vesicle (GV). For groups cultured for 12 h, the control showed a lower proportion of oocytes in GVBD (n=50; 46%, $P < 0.05$) than the other groups (FSH or melatonin), which were similar among themselves (n=57 to 68; 66.1 to 78.9%, $p > 0.05$). At 18 h of IVM, FSH group had MII rates (n=57; 73.6, $P < 0.05$) superior to 10^{-9}M (n=42; 52.3%), while the others were similar to both (n=41 and 47; 71 and 57.4%, respectively for the control and 10^{-6}M, $P > 0.05$). At 24 h, 10^{-9}M (n=45; 46.6%, $P < 0.05$) was inferior to the others (n=44 to 58; 70.4 to 86.2%, $P > 0.05$). In conclusion, meiosis resumption was stimulated by melatonin in a similar manner as the addition of FSH. However, meiosis progression was stimulated only by the highest concentration of melatonin. Therefore, the results suggest a possible role of melatonin in the control of oocyte maturation in cattle. Further studies are in development to evaluate the role of melatonin in cytoplasmic maturation and its antiapoptotic and antioxidant functions in bovine oocytes.

Acknowledgments: Fapesp, CNPq and Capes.

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Sperm pretreatment prior to ICSI is not necessary for an adequate *in vitro* bovine embryo development

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Keywords: bovine, embryo, ICSI.

Efficiency of ICSI in bovine is lower than in other species due in part to a lack of optimal conditions for its implementation, which has prevented to achieve high rates of embryonic development and the birth of live offspring. The aim of this study was to evaluate the effects of pretreatments of bovine sperm on the in vitro developmental potential of embryos generated by ICSI. Cumulus-oocyte complexes were aspirated from follicles 2-8 mm in diameter and in vitro matured. Motile sperm were selected by Percoll gradient technique. Sperm were treated with 1 mM NaOH for 1h or 5 mM DTT 20 min and sp-TALP for 120 min at 38.5ºC, respectively. ICSI was performed in microdrops under mineral oil using a Nikon TS100 inverted microscope. Each sperm was selected and subjected to tail scoring before being aspirated into the injection pipette (9 mm internal diameter). The injected oocytes were activated by exposure to 10 µM ionomycin for 5 min and 5 µg/ml cycloheximide for 5 h and cultured in drops of KSOM medium (culture medium regularly used in our laboratory) under mineral oil at 38.5°C and 5% CO_2. Cleavage was recorded at 72 h, blastocysts rate on day 9 and pronuclear formation was evaluated at 18 h post activation (Hoescht staining; Bevacqua et al., 2010, Theriogenology, 74, 922-31). Quality of embryos was assessed by staining with Hoechst and propidium iodide (Fouladi-Nashta et al., 2005, Reproductive BioMedicine Online, 10, 497-502). The proportional data were transformed to arcsine, treatment effects were analyzed by ANOVA and means were compared using Scheffe test. Pronuclear formation was analyzed by a Chi-square test with Bonferroni´s correction. Results of 8 replicates with a total of 455 oocytes injected with spermatozoa pretreated with DTT (ICSI-DTT), NaOH (ICSI-NaOH) and sp-TALP (ICSI-ST) showed no differences in the cleavage rate in any of the groups (69, 71 and 69%, for ICSI-ST, ICSI-DTT and ICSI-NaOH, respectively). Similar results were observed in the blastocysts rate (29, 22 and 20% de blastocistos for ICSI-ST, ICSI-DTT and ICSI-NaOH, respectively). The fertilization rate observed, as assessed by the presence of male (or decondensed sperm head) and female pronucleus was 77, 77 and 67% for ICS-ST, ICSI-DTT and ICSI-NaOH, respectively. Quality of embryos was not different between treatments. In conclusion, we describe here for the first time the effects of NaOH treatment on *in vitro* embryonic development after ICSI and demonstrated that classical sperm pretreatment with DTT is not essential for an appropriate in vitro embryo development in the bovine species.

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Natriuretic peptides stimulate the cumulus oophorus expansion in bovine

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Keywords: cumulus expansion, forskolin, natriuretic peptides.

The cumulus-oocyte complex (COC) is influenced by the preovulatory peak of LH, which in addition to initiating meiosis resumption in the oocyte, induces expansion of the compact layer of *cumulus* cells that surrounding it. However, the absence of this gonadotropin receptors in COCs (Peng et al., 1991, Endocrinology, 129, 3200-3207) suggests that LH does not act directly on the female gamete, but by stimulating intrafolicular mediators that act on a paracrine way (Park *et al.,* 2004, Science, 303, 682-684). The Natriuretic Peptides (NP) were discovered after infusion, in rats, of atrial tissue extract, causing rapid decrease in blood pressure, vasodilation and increased urinary sodium excretion (De Bold et al., 1981, Life Science, 28, 89-94). In mice, it is known that the C-type NP (NPC) has the capacity to block meiosis resumption (Zhang et al. 2010, Science, 330, 366-369), but there is no effect proven on *cumulus* expansion. Similarly, the is little knowledge of forskolin and NP on this function. The objective of this research is to propose an *in vitro* model for the study of bovine *cumulus* expansion and evaluate the effect of NP on this process. The COCs were aspirated from abattoir ovaries, using grade 1 and 2 oocytes. Each COC had images captured and their total area (μm^2/CCO, LeicaApplication) measured immediately before (0h) and at the end of the culture period (10 COCs per well). Data were evaluated using randomized blocks, with different treatments performed simultaneously and each replication considered as a block, and the results were processed by PROC RANK for the purpose of applying parametric tests. Experiment 1: groups of COCs were allocated to 12 or 24h of culture with TCM alone; TCM+0.5µg/ml FSH; TCM+100µM forskolin; TCM+100µM forskolin+0.5µg/ml FSH. Experiment 2: we evaluated the dose-response effect (10, 100 or 1000nM) of each NP, for 12h of maturation in stimulating *cumulus* expansion inhibited by 100µM forskolin. In all experiments the oocytes started culture with similar sizes. In experiment 1, after 12h of *in vitro* maturation the group TCM+0.5µg/ml FSH showed the highest growth ($P < 0.05$). At that time, the TCM, TCM+100µM forskolin+0.5µg/ml FSH and TCM+100µM forskolin were not different ($P > 0.05$). After 24h of culture the effect observed in group TCM+100µM forskolin was not maintained. In experiment 2, after 12h of *in vitro* maturation the positive control group (TCM+0,5µg/ml de FSH), 1000nm ANP, 100nM BNP and10nM CNP showed total area similar and higher than the negative control (TCM+100µM de Forskolin+0,5µg/ml de FSH). These results validate a model for *in vitro* study of *cumulus* cells expansion in bovine using forskolin, and furthermore, demonstrated the involvement of ANP, BNP and CNP in *cumulus* expansion.

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Ooplasmic tranfer on the development of zona-free IVF bovine embryos

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Keywords: bovine, IVF, ooplasmic transfer.

Ooplasmic transfer (OT) has been mainly used to improve compromised oocytes and SCNT embryos development. The objective of this work was to evaluate the effect of the OT on development and quality of zona-free IVF embryos (ZF-IVF) and their individual blastomeres (IB) after disaggregation on day 1 post-insemination. COCs were aspirated from slaughterhouses ovaries and selected for standard IVM. A group of oocytes was subjected to IVF and presumptive zygotes were denuded and ZP removed. During gametes coincubation, a second group of matured oocytes was subjected to denudation and to ZP removal prior to enucleation. One (1+ group) or two (2+ group) ooplasms were fused to one presumptive zygote (2 pulses of 60V during 30 usec length and 100 msec interval). A ZF-IVF group (without ooplasm fusion) and a standard IVF (ZP-IVF) group were used as controls. For evaluation of IB development, cleaved embryos at day 1 post-insemination from ZF-IVF, 1+ and 2+ were subjected to hard pipetting for blastomeres disaggregation. ZF-zygotes and IB were cultured using the WOW system. Cleavage and cell numbers of ZF embryos were evaluated on Day 2. Blastocysts rates and total cell numbers were evaluated on Day 7 in all groups. Data were analyzed by Fisher´s test ($p<0.05$). The groups 1+ and 2+ showed a higher number of cells (>9 cells) on Day 2 (62/144; 43% and 49/138; 35.5%) than the ZF-IVF embryos (81/318; 25.5%, $p<0.05$). On the contrary, a higher proportion of cleaved ZF-IVF embryos showed 5 to 8 cells on Day 2 (149/318; 47%). The overall cleavage and blastocyst rates were significantly higher in the ZF-IVF (88% and 24%) and the 1+ (82% and 25%) groups than in the 2+ group (61% and 14%). The ZP-IVF group showed the highest blastocysts rates (131/343; 38%). Surprisingly, 1+ and 2+ groups showed blastocyst cell numbers (60.8±16.38 and 56.50±26.00, respectively) similar to the ZP-IVF group (58.26±6.65) and higher ($p<0.05$) than observed in the ZF-IVF control blastocysts (43.94±11.69). Interestingly, the highest percentage of blastocysts was obtained in groups showing an increased proportion of cells on Day 2: 62% of the blastocysts in 1+ group were obtained from embryos that were in 9 to 16-cell-stage, while 50% of the blastocysts of 2+ group had more than 16 cells-stage on Day 2. A total of 20 disaggregated embryos (49 IB) in the ZF-IVF group resulted in 24 blastocysts (120%); significantly higher than the other experimental groups (27% and 0% for the 1+ and 2+ group, respectively). Additional experiments are being carried out to identify the effects of OT in zygotes in terms of transcriptional pattern, pregnancy establishment and post-vitrification survival. In addition, we confirmed in all groups a positive correlation between more advanced stages of development at Day 2 and higher blastocysts rates. However, 1+ and 2+ reconstructed embryos did not improve blastomeres development. In conclusion, OT improved embryo development when 1 ooplasm (1+) was added, but 2 ooplasms transfer (2+) showed to be excessive and harmful to the embryo.

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The natriuretic peptides system is present in the cumulus-oocyte complex and stimulates the meiosis resumption in bovine

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Keywords: forskolin, natriuretic peptides, oocyte maturation.

The process of meiosis resumption in oocytes, blocked since fetal life, is triggered by the preovulatory peak of LH. However, the absence of this gonadotropin receptors in cumulus-oocyte complexes (COCs; Peng et al., 1991, Endocrinology, 129, 3200-3207) suggests that LH does not act directly on the female gamete, but stimulating intrafollicular mediators that act of paracrine way (Park *et al.,* 2004, Science, 303, 682-684). Among the components of natriuretic peptides (NP) system, only the NP C-type (NPC) has demonstrated its capability to block meiosis resumption in mice (Zhang *et al.,* 2010, Science, 330, 366-369). However, there is no knowledge about the role of this system in controlling meiosis in monovular species. The aim of this study was to characterize the system natriuretic peptides in COCs and demonstrate its role in meiosis resumption in bovine oocyte. The COCs were aspirated from abattoir ovaries, selected (grade 1 and 2) and immediately used for the characterization of the NP system by PCR (experiment 1) or cultured for 12h in TCM199. In experiment 2, the maturation medium was supplemented with different doses (10, 100 and 1000nm) of NP A-type (ANP), B-type (BNP) and C-type (CNP) to evaluate the effect on blocking the meiosis resumption. In the third experiment, the oocytes remained arrested in germinal vesicle (GV) by action of forskolin (100μM; for all maturation), and it was evaluated the dose-response effect (10, 100 and 1000nm) and possible associations of the three NP in stimulating the meiosis resumption. After the maturation period (12h) the oocyte meiotic progression was visualized using Hoechst 33342. The differences between treatments were calculated using a statistical model for categorical data (PROC CATMOD). Initially, we demonstrated the presence of mRNA for ANP, CNP, and the natriuretic peptide receptor 1 (NPR-1), NPR-2 and NPR-3 in cumulus cells, and only NPR-2 mRNA in oocyte. In the second experiment, any dose or association of NP were able to maintain meiotic arrest. In experiment 3, ANP (1000nm), BNP (10 nM) and CNP (1000nm), induced meiotic resumption (73.4, 66.6 and 58.8%, respectively) after 12h of maturation compared to the negative control (24,0%), and moreover, we observed that association ANP + BNP (62.9%), ANP + CNP (69.6%), BNP, CNP + (62.5%) and ANP + BNP + CNP (64 9%) were effective in stimulating the meiosis resumption, but with similar rates seen when used each NP separately. Therefore, we demonstrate for the first time the NP system in COCs, and functional studies suggest that in monovular species such as bovine, the NPs are involved in stimulating the meiosis resumption.

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Effect of preovulatory follicle size on the pathway of eicosanoids biosynthesis in the endometrium of Nelore cows during early diestrus

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Keywords: cattle, estradiol, progesterone.

In cows, different estradiol and progesterone levels during the periovulatory period act distinctly on uterine tissues and modulate its function through the control of several metabolic pathways. For example, there is evidence that these steroids modulate the synthesis and signaling of eicosanoids. Such compounds are derived from the arachidonic acid and are essential for a range of reproductive processes. It is believed that distinct eicosanoids profiles during the pre-implantation period modulate uterine receptivity and consequent pregnancy success. The objectives were to (1) measure the gene expression of proteins involved in the synthesis, transport and signaling of eicosanoids and (2) quantify a series of eicosanoids in the uterus under different periovulatory hormonal profiles during early diestrus in beef cows. The follicular growth of multiparous non-lactating Nelore cows was pharmacologically manipulated in order to obtain groups with large (LF/CL; n=11) or small (SF/CL; n=11) preovulatory follicles and corpora luteum (Mesquita F. Reprod. Fertil. Dev., submitted). Uterine wash and tissues were obtained seven days after the induction of ovulation with GnRH analogue. Abundance of transcripts from 28 genes involved in eicosanoids synthesis was measured by qPCR and the concentration of metabolites in the endometrial tissue (in pmol/g of tissue) and uterine wash (in pmol/mL of wash) by mass spectrometry. In the LF/CL group, there was a greater abundance of transcripts coded by the genes ALOX12 (1.56±0.09 vs 1.07±0.10; $p<0.05$), PTGIS (1.22±0.09 vs 1.04±0.08; $p<0.05$), PTGES (1.15±0.10 vs 0.90±0.08; $p<0.05$), PTGES2 (1.18±0.08 vs 1.03±0.05; $p=0.07$), AKR1C4 (2.07±0.30 vs 1.12±0.15; $p<0.05$), and CBR1(1.14±0.13 vs 0.83± 0.12; $p=0.07$), responsible for the synthesis of eicosanoids. Identification and quantification of 65 arachidonic acid metabolites was performed in the endometrium and 87 in the uterine wash. However, the quantification of the respective eicosanoids in the wash did not differ ($p>0.05$) between the LF/CL and SF/CL groups in the compounds 12 HETE (0.6±0.1 vs 0.4±0.1), 6-keto-PGF1α (142.9±2.7 vs 203.7±62.7), 8-iso-PGE2 (3.9±0.9 vs 3.3±1.3), PGE2 (27±6.4 vs 27±4.0) and PGF2α (41.5±4 vs 49.7±6.8) in the endometrium and neither the compounds 12 HETE (1.3±0.6 vs 1.7±1.0), 6-keto-PGF1α (1.4±0.4 vs 1.4±0.5) 8-iso-PGE2 (0.02±0.0 vs 0.01±0.0), PGE2 (0,3±0,1 vs 0.2±0.1) and PGF2α (1.9±0.6 vs 1.8±0.8). Despite the LF/CL group expressed greater amounts of transcripts for the synthases enzymes than the SF/CL group, such differences did not reflect in the corresponding eicosanoids composition in the uterus. Therefore, the exposure of uterus to different fluctuations of ovarian steroids modulates the gene expression of enzymes linked to the eicosanoid synthesis without influencing the concentration of such compounds in the uterine wash and tissues in the beginning of estrous cycle in beef cows.

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In vitro culture of bovine embryos in medium without FBS and supplemented with fatty acids and antioxidants: implications on the development, accumulation of lipids, total cell number and percentage of apoptotic cells

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Keywords: antioxidant, fatty acids, IVC.

Different systems of culture and especially the medium composition for *in vitro* culture (IVC) in embryos are the main causes of great variation in the production rates and embryo quality (Thompson & Peterson, 2000). The increased lipid content in embryos caused by the use of FBS causes morphological changes and alterations in gene expression (Lonergan et al., 2003). It is proven that the use of conjugated linoleic acid (CLA) causes a reduction in lipid content (Pereira et al., 2008). Catalase (CAT) is an antioxidant and is responsible for extracellular removal of hydrogen peroxide from the culture medium (Fridovich, 1998). This study was conducted to evaluate the effect of supplementation with conjugated linoleic acid (CLA) and/or catalase in the culture medium in the absence of FBS. COCs (n=1.094) were matured in B199 (TCM-199 with bicarbonate and hormones) supplemented with 10% FBS. After IVF, zygotes were IVC in SOFaa with 8mg/ml BSA-FAF, 100 µM CLA and/or 100 UI catalase in atmosphere of 5% CO_2 in air. Cleavage was evaluated at 48hpi and blastocysts rates at 168hpi when they were stained with Nile Red (SIGMA) for determination of the lipid content and TUNEL *"In situ terminal deoxynecleotidyl transferase mediated dUTP Nick and labeling assay"*, Roche Applied, IN, USA for apoptosis determination. Embryos were evaluated under an epifluorescent microscope and images of embryos stained with Nile Red were analyzed by Q-Pro Image Capture software for determination of the fluorescence intensity. Data were analyzed by ANOVA ($P<0.05$) and the averages compared by Tukey-Kramer HSD. The results are presented as mean ± standard error of the mean. The cleavage rates were 82.5±2.7%^{a} (BSA), 84.2±0.5%^{a} (BSA+CLA), 86.2±1.8%^{a} (BSA+CAT) and 83.9±2.5%^{a} (BSA+CLA+CAT). Blastocysts rates were 18.7±4.7%^{a}, 14.9±2.6%^{a}, 8.3±1.6%^{a}, 6.4±0.6%^{a}, respectively. The fluorescence intensities with Nile Red were similar ($P>0.05$) among groups (0.99±0.08 to 1.04±0.15), as well as the percentage of apoptotic cells (0.36±0.26 to 1.72±0.49). The average number of total cells differed, being 60,0^{b} (BSA), 110,4^{a} (BSA+CLA), 66,0^{ab} (BSA+CAT), 95,7^{a} (BSA+CLA+CAT). Although the rate of embryo production did not differ between groups, the average results were lower than expected. This confirms that the presence of FBS is still essential to promote embryonic development, even if it is added in small amounts. The addition of CLA and CAT during IVC neither altered the amount of lipids of embryos, nor affected the percentage of apoptotic cells. In conclusion, supplementation of CAT and/or CLA in IVC medium without addition of FBS did not improve the rate of blastocyst development or embyo quality. However, supplementation with CLA or CLA+CAT reflected in an increase in the number of blastomeres.

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Effect of dietary supplementation with polyunsaturated fatty acids on the recovery and oocyte quality and serum concentration of progesterone, insulin and leptin

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Keywords: bovine, hormones, polyunsatured fatty acids.

The nutritional status can influence reproduction, especially fertility in bovine females. In dairy and beef cattle, dietary supplementation with various fat sources has been performed to increase the energy density of the diet and, consequently, improve reproductive performance, especially oocyte quality and by promoting the secretion of progesterone and other hormones. The aim of this study was to evaluate the effects of dietary supplementation of Nelore heifers with a source of rumen protected fat enriched with Omega-6 (n-6) and 3 (n-3) PUFAs (Megalac-E ®) on the follicular population, oocyte quality, and plasma concentrations of progesterone (P4), insulin and leptin. Sixteen heifers were randomly divided in two groups in a "cross-over" experiment, in which isoenergetic and isoproteic diets were used as follows: CONTR (n = 8, maintenance diet) and FAT (n = 8, diet for maintenance + 100 g / animal / day Megalac-E ® in its composition). After 60 days of feeding, ultrasound-guided ovum pick-up (OPU) sessions were carried out. A total of six sessions of OPU was performed during the experiment. The follicular population was assessed (counting and measurement of follicular diameter) with the aid of an ultrasound machine Pie Medical ® model Falcon 100. All follicles larger than 4 mm were aspirated to recover cumulus-oocyte complexes that were classified according to the manual of IETS in grades I, II, III, denuded and degenerated. Blood samples were taken at the beginning and after 60 days of dietary supplementation, as well as during OPU sessions. The hormone quantification was determined by radioimmunoassay using previously validated commercial kits. The mean (least squares ± SEM) were analyzed by the GLIMMIX procedure of SAS. There was no difference ($P > 0.05$) in the mean concentration of P_4 (2.6 ± 0.6 vs 3.5 ± 0.6 ng/mL), insulin (13.8 ± 1.6 vs 14.9 ± 1.6 ng/ml), and leptin (2.5 ± 0.3 vs 2.3 ± 0.3 ng/mL), respectively, for CONTR and FAT groups. Likewise, there was no difference in the population of follicles aspirated (17.9 ± 1.0 vs 15.8 ± 1.0), number of oocytes recovered (14.4 ± 1.4 vs 14.5 ± 1.3), and number of viable oocytes (12.1 ± 1.2 vs 12.5 ± 1.1), at the OPU sessions for CONTR and FAT groups, respectively. In conclusion, supplementation with a source of rumen protected fat rich in PUFAs in Nelore heifers had no effect on the recovery and quality of oocytes, as well as on the plasma concentrations of progesterone, insulin and leptin evaluated in this study.

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Methylation pattern of the XIST gene in oocytes from Nelore cows (*Bos taurus indicus*) during oogenesis

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Keywords: DNA methylation, oogenesis, XIST.

DNA methylation is one of the most studied epigenetic events (Zaid et al., 2010, Molecular and Cellular Biology, 30, 4758-66; Guseva et al., 2012, Developmental Biology, 361, 403-11) being responsible for the epigenetic reprogramming that occurs during gametogenesis (Faulk et al., 2011, Epigenetics, 6, 791-7). Understanding how this reprogramming occurs in oogenesis is important to comprehend physiological and genetic aspects involved in female gametogenesis in order to create parameters for oocyte competence. This is important to improve the *in vitro* embryo production, maximizing the use of gametes. The aim of this study was to evaluate the DNA methylation pattern in Differentially Methylated Regions (DMR) involved in the control of XIST gene expression in oocytes from preantral and antral follicles of Nelore cows. The extracted DNA from oocytes was treated with sodium bisulphite and amplified by PCR for the XIST gene, which was cloned into DH5α cells, and then purified and sequenced. The sequences were compared with that sequence in the *GenBank*, and only sequences with a minimum of 90% of homology and 90% of sodium bisulphite conversion were used. The statistical analysis was done using *Kruskal-Wallis* test, followed by the *Mann-Whitney* test. The methylation patterns found for oocytes of primordial, secondary, incompetent antral and competent antral follicles were 91.59 ± 6.4%, 85.70 ± 19.6%, 91.25 ± 7.2% and 92.58 ± 11.7%, respectively, for XIST gene. The hypermethylated pattern of XIST gene suggests that this event may be responsible for epigenetic reactivation of the X chromosome during oogenesis, which is observed in mouse MII oocytes (Kim *et al*, 2009, Nucleic Acids Research, 37, 5656-64) and human MII oocytes (Nesterova et al., 2002 Differentiation, research in biological diversity, 69, 216-25). This suggests that the analyzed region is not undergoing an epigenetic reprogramming process during oogenenesis.

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Intracellular signaling of angiotensin in the control of bovine oocyte nuclear maturation

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Keywords: angiotensin, maturation, oocyte.

Mammalian target of rapamycin (mTOR) is an important intracellular pathway in the control of cell growth and proliferation by growth factors and nutrients stimuli (Murakami et al., 2004, Molecular and Cellular Biology, 24, 6710-6718; Laplante et al., 2012, Cell, 149, 274-293). The inhibition of this intracellular signaling by rapamycin negatively affects oocyte nuclear maturation in mice (Lee et al., 2012, Molecular Reproduction and Development, 79, 356-366). In bovine, the same factors and intracellular pathways have been studied during oocyte nuclear maturation process. During culture of cumulus oocyte complex (COCs) with follicular hemisections, angiotensin II (AngII) reverts the inhibitory effect of follicular cells on oocyte nuclear maturation (Giometti et al., 2005, Theriogenology, 63, 1014-25). The aim of this study was to assess the effects of mTOR intracellular pathway on AngII signaling during bovine oocyte nuclear maturation. Cumulus oocyte-complexes were aspirated from local abattoir ovaries. To obtain follicle hemisections, follicles with 2 to 5mm in diameter were isolated from the ovaries and dissected free of stromal tissue as previously described (Richard e Sirard, 1996, Biology of Reproduction, 54, 22-28). In the first experiment, COCs were cultured during 15 hours in 200µl TCM199 supplemented with rapamycin in different concentrations (2, 10 e 50µM) and further analysis of meiosis progression. In the second experiment, COCs were cultured as the following treatments: without follicular hemisections in the culture (positive control), or in the presence of follicular hemisections (negative control), plus AngII (10^{-9}) and plus AngII (10^{-9}M) + saralasin (10^{-5}M). In the other groups rapamycin (2, 10 and 50µM) plus AngII (10^{-9}M) were used in the culture medium supplemented with follicular hemisections. After culture, the cumulus cells were removed by vortexing and oocytes were stained with Hoescht 33342 to assess meiosis progression. The percentage of meiosis progression were tested by ANOVA (PROC GLM). In the first experiment the number of oocytes that reached MI were significantly lower ($P<0.001$) in the rapamycin (50µM) treatment group (28.57%) when compared to the control (90,19%). With follicular hemisections in the culture, the rapamycin at 10 and 50µM inhibited the AngII effect to promote meiosis progression (36.53 and 31.57%), respectively, likewise the group AngII (10^{-9}M) + saralasin(10^{-5}M) (36.36%), but with significant difference when compared to hemisections plus AngII (10^{-9}) group (56.6%; $P<0.05$). These data contribute to obtain knowledge on AngII intracellular signaling on the control of bovine oocyte nuclear maturation.

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Ovarian response of rats and gilts submitted to different commercial equine chorionic gonadotropins (eCG)

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Keywords: bioassay, biological activity, eCG.

Different commercial preparations of equine chorionic gonadotropin (eCG) have been extensively used to induce superovulation or to stimulate follicular growth in cattle submitted to embryo transfer and artificial insemination programs. However, sometimes eCG treatments result in low ovarian response, suggesting differences in the potency of commercial eCG preparations. This study evaluated the biological activity of different commercial eCG products available in Brazil. In the first experiment, four products (A, B, C, and D) from different laboratories were tested in rats using the classical method of Cole and Erway based on gain of ovarian weight. Immature 21–25 day old Wistar female rats received a single sc injection of 10 IU eCG. Saline and eCG from Sigma were used as negative and positive control, respectively. Autopsy was performed 48 h after eCG or saline injection and the ovaries were collected and weighed. Data were analyzed by the Student t test. Ovarian weight (g) from Sigma eCG treated females (0.076 ± 0.013) was similar to females treated with product A (0.071 ± 0.004), C (0.076 ± 0.005) and D (0.095 ± 0.010), however, there was higher ($P < 0.01$) than saline (0.033 ± 0.002) and product B (0.038 ± 0.002). The second experiment was designed to compare the ovarian response and ovulation rate of gilts treated with two commercial eCG (A and B). Eighteen immature gilts (6 per group) received im injections of 0 (Control) or 750 IU eCG (products A and B). Seventy-two hours later, all females received im 500 IU of hCG to induce the ovulations. Animals were slaughtered on day 5 after hCG and evaluated the number of corpora lutea. Data were analyzed by Chi square test. Female treated with the product A had higher (5/6, 83.3 %) ovulation rate than that those treated with the product B (1/6, 16.7%) or Control (0/6, 0.0%) ($P < 0.01$). In conclusion, there are differences in the bioactivity of commercial eCG products and these differences may contribute to the variability of ovarian response. Experiments using animals is a way of assessing the quality of these products. However, considering the ethical and political pressures on the use of laboratory animals, there is a need to develop alternative methods of analysis, such as in vitro and physical chemical assays.

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Effects of supplementation of medroxyprogesterone acetate in the pregnancy rate of sheep embryos recipients

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Keywords: embryo, progesterone, sheep.

The embryonic death in early pregnancy may be a limiting factor for commercial production of embryos in sheep production. These animals are corpus luteum dependent in the first third of gestation, with the placenta participating effectively in the production of progesterone from 50-60 days of this phase. Whereas the low concentration of progesterone is related to the high rates of embryo death, it is possible that, by ensuring high levels of this steroid from exogenous applications, there higher rates of pregnancy can be achieved in this species. The objective of this study was to determine the effect of medroxyprogesterone acetate (MAP), administered through intravaginal sponges, on the pregnancy rate in sheep receiving embryos in commercial embryo transfer programs. Sixteen Dorper sheep were used as donors of embryos, and 101 crossbred Santa Inés sheep were used as recipients. Estrus synchronization was performed by applying intravaginal sponges impregnated with 60 MAP (Progespon®) for a period of 11 days, plus a 300 IU equine chorionic gonadotropin (Novormon®) at the time of the removal of the sponges. Superovulation of donors was stimulated with a total of 256mg FSH (Foltropin _ 1659 ® _ 1669), administered in smaller doses. Five days after conception, the embryos were collected and transferred. The recipients were randomly distributed into two groups, the Experimental group (GE) and the control group (GC). The pregnancy diagnoses were carried out by ultrasound from 21 and 55 days after the embryo transfer. GE females (n = 53) initially received a sponge with 60 MAP at the time of transfer of the embryos, which was replaced every 11 days, in a total of 5 substitutions. The recipients of the GC (n = 48) did not receive sponges. The results between the groups were compared statistically by the Chi-square test, which differed significantly from each other ($P < 0.01$) (GE: 85.7% and 55.5% GC of pregnancy rate). This study demonstrated the effectiveness of MAP supplementation on the pregnancy rate in sheep receiving an embryo. It was observed that the administration of the progestin promoted a gain exceeding 30% in pregnancy rate when compared with the control group. This difference is probably due to the fact that the high level of progestin from the intravaginal sponges ensured a uterine environment favorable for embryonic development in the early period of pregnancy, in which period the sheep is corpus luteum dependent for maintaining the pregnancy.

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Influence of nitric oxide in the levels of cyclic nucleotides and the resumption of meiosis during in vitro maturation of bovine oocytes

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Keywords: cAMP, cGMP, nitric oxide.

The oocyte maturation in vitro is a limiting factor in the production of embryos. Nitric oxide (NO) acts via the guanylate cyclase (GC) by increasing intracellular levels of cGMP in cumulus-oocyte complexes (COCs). The cGMP in turn, can control the levels of cAMP, which acts in the control of meiosis resumption (Richard, 2007. J Anim Sci, 85, 4-6). The objective of this work was to verify the influence of SNAP (S-nitroso-N-acetyl-DL-penicillamine, Sigma, Germany, NO donor) on the levels of cAMP, cGMP and NO and on germinal vesicle breakdown (GVBD) during IVM in cattle. The COCs were aspirated from ovaries obtained from abattoirs, matured (groups of 20 COCs) in droplets (100 µl under mineral oil) of IVM medium (TCM199 + 0.25 mM sodium pyruvate, 0.1% PVA and 25 µg/ml gentamicin) in an incubator at 38.5° C and 5% CO_2 in air. The COCs were divided into three groups: CONTROL – IVM medium; SNAP - IVM medium with 10^{-7}M SNAP and SNAP+ODQ (1H-[1,2,4] oxadiazole [4,3-a] quinoxalin-1- one, Sigma, Germany, inhibitor GC) - IVM medium with 10^{-7}M SNAP and 10^{-4}M of ODQ. After 9 hours IVM, NO quantification in the medium and the rate of oocyte GVBD (5 replicates) were determined. cAMP and cGMP levels were measured in COCs matured for 1, 2 and 3 hours (3 replicates). GVBD was observed using anti-lamin A / C-DAPI (Prentice-Biensch et al., 2012, Theriogenology, 78, 1633-1638). NO quantification was determined using an indirect method by quantifying nitrate (Griess method) using the Griess Reagent System kit (Promega Corporation, Madison, USA) and to measure cAMP and cGMP levels, an enzyme immunoassay was used (EIA cGMP e EIA cAMP kits, Enzo life Sciences, Farmingdale, USA), following the manufacturers´ instructions. Data were analyzed by ANOVA followed by Tukey test for GVBD and levels of NO, cAMP and cGMP were analyzed using ANOVA with two criteria followed by the Bonferroni test. The significance level was 5%. GVBD in SNAP (53.4%) decreased ($P < 0,05$) when compared to the other groups (78.4 to 73.4%, $P > 0.05$). Nitrate concentration in SNAP (48.3 µM nitrate) was superior ($P < 0.05$) to the others (37.8 and 38.0 µM $P > 0.05$). cGMP levels in SNAP at 1 h IVM (4.0 pmol/COC) were similar to immature 0h control (4.4 pmol/COC, $P > 0.05$) and both were superior ($P < 0.05$) to all other groups and time points (1.2 to 3.0 pmol/COC, $P > 0.05$). However, cAMP levels did not differ between the groups at all intervals (0.1 to 0.4 pmol/COC, $P > 0.05$), and all were lower than 0 h IVM (1.0 pmol / COC, $P < 0.05$). In conclusion, the use of NO donor (SNAP) reduces the rate GVBD by increasing the levels of NO and cGMP. The elevation of cGMP by NO, even if only on the first hour of culture was sufficient to delay meiosis resumption, but this effect was not mediated by cAMP pathway. Additional studies are underway to determine the involvement of other factors on the effects observed.

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Effect of chemical activation treatments on the development and quality of bovine embryos generated by intracytoplasmic sperm injection (ICSI)

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Keywords: activation, DMAP, ICSI.

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technique that has been used with considerable success in humans to overcome certain male infertility problems (Palermo et al., 1992, Lancet, 340, 17–18). In bovine, however, the efficiency of this technique is far from optimal. Oocyte activation has been recognized as one of the key steps during the ICSI procedure in this species. The objective of the present study was to evaluate the effect of three chemical activation treatments 6-dimethylaminopurine (DMAP), cycloheximide (CHX), and ethanol (ETOH) on the development and quality of bovine embryos generated by ICSI. Cummulus-oocyte complexes were aspirated from abattoir ovaries, selected and matured in 400 ul drops of standard TCM-199 maturation medium for 22 h at 38.5°C and 5% CO_2. ICSI was performed using an inverted microscope with Hoffman optics (Eclipse TS100F, Nikon Instruments Inc., NY, USA) using hydraulic micromanipulators (Narishige International USA). Motile sperm were selected and subjected to tail scoring before being aspirated into the injection pipette. Following breakage of the oolemma, the spermatozoon and the aspirated ooplasm were expelled back into the oocyte. Injected oocytes were randomly assigned to the different activation treatments and cultured in 50 mL drops of KSOM medium (culture medium regularly used in our laboratory) under mineral oil at 38.5°C and 5% CO_2, 5% O_2 and 90% N_2. Cleavage was recorded at 72 h and blastocysts rate at 192 h. Quality of embryos was assessed by staining with Hoechst and propidium iodide (Fouladi-Nashta et al., 2005, Reproductive BioMedicine Online, 10, 497-502). The data were transformed to arcsine, treatment effects were analyzed by ANOVA and means were compared using Tukey´s test with Statgraphics Plus 2 Software. Partial results with a total of 246 injected oocytes (95, 104 and 47 for DMAP, CHX e ETOH, respectively) showed differences in cleavage ($p<0.01$) in DMAP and CHX groups (83 and 72%, respectively), relative to ETOH (20%). Similarly, the rate of blastocysts at 192 h was higher with DMAP and CHX (34 and 21%, respectively), relative to ETOH (5%). Quality of embryos was not different among CHX and DMAP treatments (ETOH was not included in this analysis due to the low number of blastocysts obtained with this treatment). In conclusion, the results show that activation of bovine oocytes after ICSI is more efficient with DMAP and CHX, compared to EtOH, although the quality of the embryos was not different. Studies are underway to establish the effect of these treatments on the ploidy of the embryos.

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Apoptosis in bovine embryos produced in vitro with different kinetics of development

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Keywords: bovine, development kinetics, embryo.

The kinetics of embryonic development may be related to the viability of IVP embryos, regarding the cellular stress. Although it still remains the hypothesis that embryos presenting higher metabolism could have a higher viability, embryos that develop faster in the beginning also present higher stress response. Based on these data, our goal was to characterize in bovine embryos of different developmental kinetics, the pattern of cell death induced by apoptosis. For that, the cumulus-oocyte complexes were aspirated from slaughterhouse ovaries, selected (grades 1 and 2), placed in drops with 90µl of IVM medium (M-199 with bicarbonate + hormones) (20 oocytes / drop) and cultured for 22 hours at 38 5 ° C and 5% CO_2. Matured oocytes were fertilized in vitro in 90µl drops of IVF medium (Parrish et al. 1,988 Biol. Reprod. 38th, 1171-1180) at 38.5°C, an atmosphere with 5% CO_2 in air and high humidity for 18 hours. Subsequently, the zygotes were transferred to individual 20µl droplets of culture medium (SOF supplemented with essential and nonessential amino acids, and 5% fetal bovine serum) in a well well system (WOW) (adapted from Feltrin et al., 2006, Proceedings of the 58th Annual Meeting of the SBPC - Florianópolis, SC), and left for 7 days in an incubator with 5% CO_2 in air and high humidity at 38.5 ° C. Embryos were classified as fast (4 cells at 40hpi) and slow (2 cells at 40hpi) and were evaluated for DNA fragmentation (TUNEL, Invitrogen) and the presence of caspase-3 and 7 (Invitrogen) at cleavage (40hpi) and blastocyst (186hpi) stages. TUNEL data were assessed as the relation of TUNEL positive cells / total cells expressed in percentage. Regarding the caspases, embryos were classified according to the intensity of the staining in “high”, “medium, and “no caspase”. The results were analyzed by Student's *t* test from a total of 3 manipulations with at least five embryos per group (Prism 5 GraphPad Inc.). There was no difference between the cleaved embryos regarding the presence of caspase 3 and 7. The group of fast blastocysts showed a tendency for higher caspase compared to slow blastocysts. There was no difference between cleaved embryos (fast: 14.1 ± 10.1; Slow: 3.1 ± 3.1) and blastocyst (fast: 7.2 ± 3; slow: 11.9 ± 2.6) for the number of TUNEL positive cells relative to total cells. These results demonstrate that the different developmental kinetics does not affect embryonic viability in relation to the induction of cell death by apoptosis.

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Influence of vaccination against IBR, BVD and leptospirosis in the reproductive health in cows it the Amazon region

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Keywords: conception rate, farrowing rate, pregnancy loss.

In previousstudiestherewas a high percentageofcowswith positive serology for BovineHerpesvirus 1 (BoHV-1), Bovine Viral DiarrheaVirus (BVDV) andLeptospirahardjo (Flores et al., 2005, Pesquisa Veterinária Brasileira, 25, 125-134; Junqueira et al., 2006, Ciências Agrárias, 27, 289-298). This study aimed to evaluate the pregnancy loss from 60 days after FTAI until the moment of the parturition in beef cows from Nelore (n = 4534), vaccinated (n = 2266) or not Nelore breed (n = 2268) for IBR (Infectious Rhinotracheitis Bovine), BVD (Bovine Viral Diarrhea) and Leptospirosis. The work was conducted in the Northern region of Brazil, the city of Boca do Acre - AM, at NossaSenhoraAparecida farm, where the animals used in the experiment were randomly assigned into two groups. The animals in the group that was vaccinated were vaccinated for IBR, BVD and Leptospirosis (Cattle Master® + 4 L5, Pfizer, São Paulo-SP) 5 mL intramuscularly, the first dose was given at the beginning of the FTAI protocol (day 0) and the second dose at diagnosis of pregnancy (day 30). To avoid statistical bias the same estrous cycle handling protocol was used between the groups, with a duration of 11 days, where on Day 0 (D0) the intravaginal device releasing progesterone (P4) was introduced, which is unused (CIDR®, Pfizer, São Paulo-SP) and the application of 2 mL of estradiol benzoate, IM (2,0 mg estradiol benzoate, Estrogin®, Farmavet, São Paulo-SP), after seven days was applied PGF2α, IM (12.5 mg de Dinoprost, Lutalyse®, Pfizer, São Paulo-SP). On day 9, the CIDR® was withdrawn, and 0.5 mg estradiol cypionateinjected IM (E.C.P.®, Pfizer, São Paulo-SP) and the calves were removed for 48 hours (Shang). All animals were inseminated 46 to 52 hours after CIDR®. The inseminations were performed by the same inseminator. Miscarriages were evaluated from 60 days after FTAI until parturition. Miscarriages were calculated by subtracting the total of pregnant females at 60 days from the numbers of calves born. Data were analyzed by Chi-square. Conception rates did not differ ($p = 0.19$) between vaccinated and unvaccinated cows [53.57% (1215/2268) vs. 56.48% (1280/2266), respectively]. The birth rate did not differ ($p = 0.23$) between vaccinated and unvaccinated cows, [92.18% (1120/1215) vs. 95% (1216/1280), respectively]. The rate of pregnancy loss differed ($p = 0.02$) between vaccinated and unvaccinated cows, [7.81% (95/1215) vs. 5% (64/1280), respectively]. We concluded that females vaccinated for IBR, BVD and Lepetospirose have lower pregnancy losses between 60 days after FTAI and parturition.

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Functional ultrasound characteristics of corpus luteum during the first weeks post-insemination in pregnant and non-pregnant beef cows

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Keywords: corpus luteum, pregnancy diagnostic, progesterone.

Pregnancy diagnostic methods that detect non-pregnant cows before the following ovulation (≈ day 21-post-ovulation) are needed to reduce the interval of inseminations (AI) in beef cattle. This study aimed to: (1) identify functional characteristics of corpus luteum in pregnant and non-pregnant cows during early gestation in beef cattle; and (2) determine the accuracy of detection of non-pregnant animals through the evaluation of luteal function between 18 to 22 days post-AI. Nelore cows (n=27) were submitted to timed-AI after synchronization of ovulation. Doppler and B-mode ultrasonography and plasma progesterone (P4) concentrations were measured on days 8, 12, 15, 18, 20, 22 and 30 post-AI for ovulated cows (n=22). In each ultrasonography exam the dimensions and peripheral and total blood flow of the CL, and presence of the embryonic vesicle were evaluated. Loss of pregnancy was detected based on the occurrence of structural luteolysis, that was defined by the following two criteria: A) reduction in ≥25% of luteal area on days 18, 20 or 22 compared to day 8 post-AI; or B) CL with area <2cm^2 and blood flow ≤25% of total CL area. Repeated variables were analyzed by split-plot ANOVA using the PROC MIXED procedure (Version 9.2; SAS Institute), considering the effects of group (pregnant and non-pregnant), day and interaction. Pregnancy was diagnosed in 45.5% (10/22) of cows on days 25 and 30 post-AI. An effect of group, day and their interaction was detected in all variables (P<0.05). There was a reduction in the mean P4 concentration and CL area in the non-pregnant group starting on day 20 post-AI (P<0.05). CL diameter and volume were reduced in non-pregnant cows on day 18 post-AI, and were greater on day 12 post-AI in pregnant cows (P<0.05). Luteal blood flow (peripheral and total) was greater on days 8, 12, 20, 22 and 25 post-AI in the pregnant group than in the non-pregnant group (P<0.05). Although P4 concentrations did not differ (P>0.05) on day 12 post-AI, the greater vascularization and volume of CL on pregnant cows indicated a greater luteal development, which may favor maintenance of pregnancy. Based on the two criteria used to diagnose non-pregnant cows, 25% (3/12), 66.7% (9/12) and 91.7% (11/12) of non-pregnant cows were detected by criteria A, and 25% (3/12), 91.7% (11/12) and 91.7% (11/12) by criteria B, on days 18, 20 and 22, respectively; no false-negative diagnostic was observed. The results suggested that CL of pregnant beef cows are larger and more vascularized on day 12-post AI. In conclusion, the detection of structural luteolysis by ultrasonography on days 20 and 22 post-AI is a reliable tool for the early diagnosis of non-pregnant Nelore cows.

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