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Treatment of ovarian cysts in cattle with lecirelin acetate

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Keywords: anestrous, cows, reproduction.

Ovarian follicular cyst is an important reproductive pathology in cows. This disease has been observed in 11% of the crossbred - Girolando - lactating dairy cattle (Fernandes et al., 2004). Ovarian cysts have a negative effect on reproductive index of the herd, decreasing the efficiency of the milk production in dairy farms. The aim of this study was to evaluate the use of Lecirelin Acetate (AL, 0.1 mg) for treatment of ovarian cysts in dairy cows. A total of 20 animals were introduced to the experiment. The animals aging 3-7 years; were above 90 days postpartum; with 2-5 lactations, mean 22.4 ± 5.2 l per lactation; body condition score (BCS) ranging from 2.5 to 4.0 with an average of 2.9 ± 0.37 (range 1-5). Firstly, the animals underwent the gynecological exam by rectal palpation and ultrasonography (Mindray, DP2200). The cysts were diagnosed as ovarian follicular structures larger than 20 mm in diameter with no observation of the luteal tissue (CL). After the diagnosis, the animals were separated randomly into two experimental groups: G1 (n=12) – one intramuscular (IM) administration of the AL (0.1 mg in 4 ml, Dalmarelin ®) and G2 (n=8, 4 ml of saline). The animals were re-examined 7-8 days later to access the ovarian condition. The disappearance of the cystic structure and the presence of luteal tissue at the second examination were considered as the effectiveness of the treatment. The average diameter of the cyst was compared between groups by Student 't' test and the effectiveness of the treatment was accessed by Fisher's exact test. The mean diameter of the cyst did not differ ($p > 0.05$) between groups (28.8±4.6 and 29.3±4.8 mm for G1 and G2, respectively). There was difference ($p<0.05$) in treatment efficacy in animals of different groups. The CL was observed in 75.0% (9/12) of the animals treated in G1 and this was higher ($p<0.05$) than the observed in the animals receiving a saline injection (12.5%, 1/8). The product used at the indicated concentration was effective for treatment of the ovarian cysts in dairy cattle. However, 25% of the G1 animals remained with the cyst and further studies might help to understand the physiological mechanisms involved in this observation.

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Morphological analysis of embryos anglonubiana breed of goats after freezing

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Keywords: embryo, goats, morphology.

The objective of this study was to describe the morphology and quality of embryos from Anglonubian goats after cryopreservation, evaluating their ultrastructure. As a protocol for superovulation, females received intravaginal implants (CIDR ®) on day 0. At D9 cloprostenol (Ciosin ®) were injected (IM), and started applications of 200mg pFSH (Folltropin ®) divided into six decreasing doses (IM) every 12 hours. The implants were removed at D11 and after 12 hours the heat was observed. Controlled natural mating was performed every 6 hours. The embryo collection was conducted transcervicaly with 21 structures being recovered: 13 morulaes and 8 blastocysts. The embryos were assessed soon after harvest, under a dissecting microscope in accordance with the IETS manual (IETS, 1998). Morula and blastocyst grade 1 and 2 were cryopreserved in a programmable automatic system (TK3000 ®), being subjected to the chilling process starting at room temperature until -120°C trough two negative curves. After thawing, embryos were analyzed for light (13) and transmission electron microscopy (8), at the Laboratory of Embryology FMVZ / USP. There were no differences in embryonic morphology measured under dissecting microscope before and after cryopreservation. The embryos were limited by a perfectly spherical zona pellucida (ZP) with no cracks and, with no cell fragments attached to it. Furthermore, the cell mass presented a spherical contour with symmetrical borders. The blastomeres were nitid and clear, with good compaction and an adequate ratio between the embryo and the perivitelline space. For observation under light microscope 2µm thick sections were stained with 2% toluidine blue and 1% sodium borate solution in distilled water. The trophoblast layer in the morula consisted of slightly elongated cells, bonded to each other, remembering an epithelium lining the inner cell mass (ICM). In the cells arranged internally several cavities of varying dimensions were seen, possibly involved in the formation of the blastocoele. The cells surrounding these wells were noticeably flatter than the others. Ultra structurally the ZP was thinner in the blastocysts with a more homogeneous texture in the inner side and a more porous appearance in the outer face. We also observed cells with degenerative features and cell death in the ICM and the trophoblast. This condition may be due to cryopreservation that damages the cell structure, or was related to the process of blastocoele formation. The most predominant organelle was ribosomes. It was observed that rounded mitochondria were prevalent in relation to other forms. These results are similar to the ones obtained in bovine embryos using the same techniques, however, further studies are still needed.

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Monitoring of uterine involution and fluid accumulation during buffaloes puerperium

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Keywords: bubaline, postpartum, uterus.

This study aimed to describe the dynamics of uterine involution process and intrauterine fluid accumulation in buffaloes during the postpartum period. For that, 16 pluriparous Mediterranean buffaloes, with body condition score equal or greater than 3 (1 to 5 scale), were submitted to gynecological examinations on days 7, 14, 21, 28 and 35 postpartum (considering parturition as day 0). Only animals that presented normal parturition without retained placenta were included in the study. During gynecological examination, the uterine involution degree (GRUNERT, 1979) was evaluated via rectal palpation, as: 1 (slightly involuted uterus with the total mass into the abdominal cavity, and a large horn asymmetry, examination of all uterine extension was not possible), 2 (uterus with part of its mass into the abdominal cavity, with reasonable assymmetry between horns, palpation and examination of uterine entire length is possible) or 3 (whole uterus into the pelvic cavity, with symmetrical or slight assymmetry horns). Ultrasound examinations were performed to classify intrauterine fluid accumulation (KRUEGER et al., 2009) in a 0 to 3 scale, where: 0 – no fluid, 1 – from 0 to 1.5 cm, 2 – between 1.5 to 2.5 cm, 3 – more than 2.5 cm. For statistical analysis, data were submitted to ANOVA and Tukey test at 5% probability. Mean uterine involution and fluid accumulation were respectively 3.00 and 2.87 at day 7, 2.87 and 1.50 at day 14, 2.56 and 0.44 at day 21, 1.50 and 0.19 at day 28, and 1.06 and 0.62 at day 35. There was significant difference in uterine involution degree between days 7, 21, 28 and 35 postpartum. Accumulation of fluid differed significantly between days 7, 14 and 21 after parturition. These results suggest that buffalo uterine involution is fast, being complete around day 35 postpartum. Intrauterine fluid of postpartum buffaloes decreases dramatically during the first three weeks postpartum, coinciding with the period of lochia expulsion.

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Plasma concentration of anti-mullerian hormone and response to the superstimulatory treatment in nelore cows

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Keywords: AMH, follicular population, super ovulations.

Embryo transfer (ET) contributes significantly to the genetic improvement of cattle and the superstimulatory treatment has been successfully used in ET programs. Recent studies indicate that the concentration of anti-Mullerian hormone (AMH; a family member of the transforming growth factor β - TGFβ) produced by the granulosa cells of preantral and antral follicles, can be used as an indicator of follicular population in cattle (Ireland et al. Reprod Biol 79:1219-25) and also associated with the superstimulatory response (Monniaux et al. Repro Fert and Dev 22, 1083-91). The aim of this study was to investigate, in Nelore (Bos indicus), the possible correlation between the plasma concentration of AMH in follicular population and embryo production after the superstimulatory treatment. Nelore cows (n=20) were superstimulated with the P-36 protocol: cows received an intravaginal progesterone device (D0, 1.0 g, Primer®, Tecnopec, Sao Paulo, Brazil) and 2 mg of estradiol benzoate (IM, RIC-BE®, Syntex, Buenos Aires, Argentina). The administration of FSH (total dosage of 160 mg Folltropin-V®, Bioniche Animal Health, Ontario, Canada) started at D4 and was performed in decreasing doses for 4 consecutive days. At D7 a PGF2α analog (150 mg d-cloprostenol, IM, Prolise®, RARS SRL, Buenos Aires, Argentina) was administered and the primer was removed after 36 h. In D4, blood samples from all animals were collected (jugular venipuncture, Vacuntainer®) and the ovaries were examined by ultrasound (Aloka 900, 7.5 MHz transducer, Tokyo, Japan). Cows were subjected to the protocol four times and after the treatments, animals were divided into two groups according to their average follicular populations: groups with low (17.9±1.15) or high (29.6±1.3) follicle count. Plasma concentrations of AMH were determined using the human kit AMH/MIS genii - ELISA (Beckman Coulter, Brea, CA, USA). The results were analyzed by ANOVA using Proc GLM in SAS (SAS 9.2). Plasma concentration of AMH was higher ($p<0.02$) in animals of the group with high follicle count (718.5±74.65 pg/mL) when compared to the group with low follicle count (307.8±67.5 pg/mL). The mean of total embryo production was higher ($p<0.02$) in animals in the group with high follicle count and higher concentrations of AMH when compared to animals with low counts and lower follicular AMH concentration (2.9±0.43 vs 1.39±0.4). The same was observed in the mean of viable embryos (1.68±0.21 vs 0.84±0.2; $p<0.02$). In conclusion, the plasma concentration of AMH in Nelore cows can be used as a response indicator to the superstimulatory treatment, assisting in the selection of prospective donors.

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Evaluation of osmolarity in amniotic fluid in thoroughbred mares - preliminary data

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Keywords: gestation, foaling, foetal viability

The performance of amniocentesis and subsequent analysis of amniotic fluid are used to study the amniotic fluid volume and composition as a routine part of evaluation of the biochemical process that involves fetal-placental unit and the viability of the fetus. The aim of this study was to relate the osmolality of the amniotic fluid with ions and enzymes, according to the time of pregnancy in mares at term. For this study, it has been observed 36 pregnant mares, which were classified according to age into two groups, young mares (age <6 years, n=16) and mature mares (age > 7 years, n = 20). There were raised in open field given birth, in an equines ranch property of Thoroughbred horse in the city of Bage during the reproductive season of 2010-2011. The collection of amniotic fluid was performed using a 20mL syringe and a 40x16 needle by the amniocentesis method, once the amniotic sac was exposed in the first stage of labor. The material was immediately transferred to 15 mL Falcon ® tubes and later, frozen and stored at - 20 ° C for later evaluation. The biochemical evaluations performed on amniotic fluid were: Osmolarity, Creatinine, Urea, ions Sodium, Calcium and Potassium and were determined using commercially available kits by colorimetric method. The statistical analysis was performed by "t" test and Pearson's correlation, at the significance level $p<0.05$. The results of mean and standard deviation for Osmolarity, Creatinine, Urea, Sodium ions, Calcium and Potassium were respectively: 274.67+94.37; 4.79+3.16; 33.68+12.56; 53.97+13.15; 3.84+1.27; 1.76+0.68. The mean gestation length was 343+11 days (minimum 322 and maximum 375 days), but it was not correlated with the osmolarity of the amniotic fluid. The osmolarity was positively correlated with calcium ($r=0.53$; $p=0.0007$), Urea ($r=0.53$; $p=0.0008$), Potassium ($r=0.55$; $p=0.0004$), Creatinine ($r=0.56$; $p=0.0003$) and Sodium ($r=0.60$; $p=0.0001$). A difference in osmolarity ($p = 0.03$) was observed between groups of young mares and mares aged more than seven years. The evaluation of osmolality in amniotic fluid, as well as creatinine, is a routine part of the assessment of fetal maturity in human. However, in horses more studies are required to establish these relationships with the fetus. The results obtained in the present study showed that there are positive correlations between the osmolarity of the amniotic fluid, the age of the dam and enzymes and electrolytes in amniotic fluid, however there was no correlation with gestational length of Thoroughbred mares at term.

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Use of L-arginine *in vitro* fertilization of bovine oocytes: effect on nitric oxide production and development of bovine embryos

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Keywords: bovine, L-arginine, sperm capacitation.

L-arginine is an amino acid that has been tested as sperm capacitation agent (O'Flaherty et al. 2004, Biochimica et Biophysica Acta 1674, 215-221; Srivastava et al. 2006, Biology of Reproduction 74, 954-958), though its effect on in vitro embryo production has not yet been evaluated. The objective of this study was to assess the use of L-arginine as a promoter of sperm capacitation, by analyzing the fertilization rates and bovine embryo in vitro development. For this purpose, 1146 oocytes derived from slaughterhouse ovaries underwent IVM (TCM 199 modified) for 18 hours in an incubator with 5% CO_2 at 38.5°C. For IVF, sperm from a single bull (Simmental breed) was selected through Percoll gradient and incubated with oocytes in IVF medium for 24h. Initially, a dose response test was undertaken to determine the optimal concentration (5 mM, 10 mM, 20 mM or 50 mM) of L-arginine to be added on the IVF medium. The embryos were cultured in SOF medium under the same conditions as maturation. The nitric oxide metabolite (nitrite / nitrate) production was assessed five hours after fertilization. The cleavage rates and blastocyst formation were evaluated at day 2 and 7, respectively, after fertilization. The results were submitted to ANOVA and Tukey test using the program BioEstat 5.0®. After four repetitions, the 10 mM L-arginine group showed the highest cleavage rate among the L-arginine treatment groups, similar ($p > 0.05$) to the 10 mg/ml heparin group (83.87% vs. 87.5%, respectively), though, regarding blastocyst formation, treatment with heparin showed better results (58.75%, $p < 0.05$) than treatments with L-arginine (43.75%, 50.56%, 47.5% and 12.5 %). Regarding the dosage of nitrite/nitrate in the fertilization medium, we observed a higher production in 10 mM L-arginine and heparin groups (11.29 mM vs. 12.04 mM, respectively, $p > 0.05$). The use of the inhibitor of nitric oxide synthesis (10 mM L-NAME) in the 10 mM L-arginine group reduced ($p < 0.05$) cleavage rate (50.56%), blastocyst formation (25.28 %) and nitric oxide (6.56 mM) in comparison to 10mM L-arginine group without the inhibitor (58.75%, 47.5% and 11.64 mM, respectively). In conclusion, these results show that the L-arginine can be used as a promoter of sperm capacitation in vitro for production of bovine embryos and its action depends on the synthesis of the intracellular signaling molecule nitric oxide.

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Low level laser irradiation for the biomodulation of in vitro produced bovine embryos system

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Keywords: cell cycle, cumulus-oocyte complex, low level laser.

During maturation, the oocytes prepare their cytoplasm for the fertilization and the early events of embryonic development. Due to its importance, the artificial induction of maturation (IVM) is a critical event and its modification is a possible approach to increase the efficiency of IVP in bovine embryos. In this way, the low-intensity laser (LBI) is an alternative to the biomodulation of these cells. In our previous work, we found that, in cumulus cells (CC), the use of LLLT in IVM was able to induce an increase in mitochondrial activity and a higher number of cells committed to the cycle, with no change in viability. The objective of this study was to evaluate the effect of LLLT (633nm HeNe λ - 1J/cm2 fluency) in the expression of genes related to the cell cycle in oocytes and CC during IVM and its impact on blastocyst rates and viability. For that, COCs grades I and II were selected, irradiated with LLLT and subjected to IVM. Samples were collected after 30 min, 8h, 16h and 24h of the onset of IVM (T30, T8, T16 and T24). Non-irradiated COCs were submitted to the same conditions (control groups - C30, C8, C16 and C24). The COCs were mechanically dissociated and kept at -80 ° C until RNA extraction, cDNA synthesis and RT-PCR for the studied genes (CDK 1, 2 and 4, Cyclins A, B and E and β-actin-reference gene). At the end of IVM, the remaining COCs were in vitro fertilized and it was assessed the rates of cleavage (D2), blastocyst (D7) and cell viability (double staining). RT data were first normalized by the estimated amplification efficiency and the reference gene. RT data, embryo rates and viability were analyzed by ANOVA and Tukey as the post test (Prism 5 (GraphPad Inc.). There were differences in gene expression patterns throughout the IVM in both oocytes and CC. In oocytes, synthesis of CDK1 (C8 <T8, C24> T24), Cdk2 (C8 <T8, C24> T24), CDK4 (C30 <T30, C8 <T8, C16 <S16), cyclin B (C8 <T8, C24> T24) and cyclin E (C30> D30, C8> T8) were different. In CC the changes occurred CDK1 (C8 <T8, C16 <T16), CDK4 (C16 <T16, C24 <T24), cyclin B (C16 <T16, C24 <T24) and cyclin E (C24 <T24) expression. Cyclin A remained unchanged in both cell types. There was no difference between the rate of cleavage, blastocyst and number of non-viable cells per embryo between the groups (C = 66.18 ± 8:00 T = 61.71 ± 8.17, C = 35.62 ± 12.22, T = 29.15 ± 3:43 and C = 1.05 ± 1.07; T = 1.68 ± 2.12, respectively). However, there was an increase in the total number of cells on the treated group (C = 60.47 ± 28.89, T = 77.93 ± 21.97). Based on these results, we conclude that the irradiation with LLLT changed the expression pattern of genes related to cell cycle regulation in oocytes and CC. This change was not reflected in an increase in the embryos rates, but it was reflected in the generation of blastocysts with more cells.

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Progesterone profile in non-cyclic pregnant recipient mares treated with altrenogest during the first 130 days of gestation

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Keywords: gestation, non-cyclic mares, progesterone.

There are few reports about the progesterone (P4) profile synthesized by supplementary corpora lutea (SCL) in non-cyclic pregnant mares, without primary corpus luteum (PCL). Therefore, the aim of the present study is to describe progesterone profile in non cyclic pregnant mares treated with altrenogest during the first 130 days of gestation. Twenty two recipient mares pregnant by embryo transfer (ET) were used and received embryos during the anoestrous or transitional phase (Non-cyclic group, n=5) and during breeding season (Control group, n=7). Prior to embryo transfer, non-cyclic mares were given a single dose of estradiol benzoate (2.5 mg, IM; EstroginTM, Farmavet, Brasil) on day 1 or 2 after donor ovulation and following observation of uterine edema mares were supplemented with 33mg of daily P.O. altrenogest (ProgestalTM, Pro-Ser, Argentina). After completion of ET and gestation diagnosis by ultrassonography, altrenogest administration was continued until 120 days of gestation. Blood samples were collected by jugular vein puncture in heparinized tubes. The collections were performed previously to recipient ovulation in Control group (moment 0) and immediately prior to the first administration of altrenogest in Non-cyclic group (moment 0), with an interval of five days, continuing to 130 days of gestation. Progesterone plasmatic concentration was measured by radioimmunoassay (RIA) by using a solid phase commercial kit (Coat-A-Count Progesterone®, Los Angeles, USA). The antiserum used at RIA has no cross reaction with altrenogest. The progesterone profile of non-cyclic mares remained below 1 ng/mL between moment 0 and 45 days of gestation, due to the absence of PCL. From day 45, with the beginning of SCL formation, a gradual increase in P4 concentration was observed, reaching values of 3.2±0.9 ng/mL (mean±S.E.D.) at 70 days of gestation and 7.5±1.1 ng/mL at 130 days. It should be noted that 20% (3/15) of non-cyclic mares did not show SCL formation and that these animals were in anestrous phase at the beginning of treatment. For the control group (cyclic mares), mean P4 reached 9.3±0.7 ng/mL five days after ovulation, which was attributed to CLP formation. A sharp increase was observed between 35 and 60 days of gestation (maximum 15.1±4.3 ng/mL), due to complementary P4 synthesis by the SCL, which was followed by stable mean concentrations until 110 days (maximum 16.5±4.3 ng/mL at day 75 and minimum 12.6±2.9 ng/mL at day 105 of gestation). Thereafter, a gradual decrease in the hormone concentration was observed until 130 days of gestation (13.9±5.5 ng/mL). Progesterone concentration of non-cyclic mares was lower than that of cyclic ones at all moments evaluated ($p < 0.016$ for all moments). We conclude that the increase in progesterone concentration synthesized by SCL in non-cyclic mares starts in a more advanced gestational period and the hormone profile during the first 130 days of gestation is lower than the profile of cyclic recipient mares.

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Effects of FGF8 on cumulus expansion and nuclear maturation in bovine cumulus-oocyte complexes

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Keywords: expansion, FGF8, maturation.

Oocyte secreted factors regulate the maturation of the cumulus-oocyte complex (COC; Zhang et al., 2010 Reproduction, 140, 815-826; Sugiura et al., 2010, Mol Endocrinology, 12, 2303-14). Fibroblast growth factor 8 (FGF8) is expressed by the bovine oocyte and activate receptors expressed by cumulus cells (Buratini et al. 2005, Reproduction, 130, 343-50; Zhang et al. 2010, Science 330, 366-369). In mice, competence to undergo expansion was partially maintained by growth and differentiation factor 9 (GDF9) alone or in combination with FGF8, and by bone morphogenic protein 15 (BMP15) in combination with FGF8 in oocytectomized cumulus cells (Sugiura et al., 2010, Mol Endocrinology, 12, 2303-14), and appears to contribute to maintain meiotic arrest (Zhang et al., 2010, Science 330, 366-369). The aim of this study was to assess the effects of FGF8 on cumulus expansion and progression of meiosis during IVM in cattle. Cumulus-oocyte complexes were aspirated from abattoir ovaries, selected (only grades 1 and 2 were used), transferred in groups of 20 to 200µL drops of TCM 199 supplemented with different doses of FGF8 (0, 1, 10 and 100 ng/ml), and cultured for 22 hours at 38.5°C and 5% CO2. After culture, COCs were visually classified according to the degree of expansion (grades 1, 2 and 3), oocytes were mechanically separated from the cumulus cells and stained with Hoescht 33342 to assess meiosis progression. The data were transformed to arcsine, treatment effects were tested by ANOVA and means were compared with the Tukey-Kramer HSD test. FGF8 did not affect cumulus expansion but decreased the number of oocytes reaching metaphase II at 10ng/mL (52.8%), and 100ng/mL (36%) in relation to control (70%) while increasing the number of oocytes in metaphase I at 10ng/mL (47.2%) and 100 ng/mL (64%) also in relation to control (30%). In conclusion, present data suggest the involvement of FGF8 in the mechanisms that regulate bovine nuclear maturation.

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Histone deacetylase (HDAC) inhibition decreases xist expression in ivp female bovine blastocysts

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Keywords: epigenetics, histone deacetylase, X chromosome inactivation.

Major X chromosome inactivation (XCI) in bovine occurs between hatching and implantation, and in vitro culture conditions may disrupt the silencing process, increasing or decreasing X–linked gene expression. Epigenetic mechanisms including histone deacetylation are involved in XCI. In the present study, we aimed to address the roles of histone deacetylase inhibition by trichostatin A (TSA) on female preimplantation development. The hypothesis tested was that by enhancing histone acetylation, TSA would reduce embryonic arrest at 8-cell stage, and delay XCI in IVF embryos. For that, culture medium was supplemented (SOF 2.5% FCS and 5mg/mL BSA) with 5nM TSA from the 8 cell stage (70 h.p.i.) onwards, in embryos fertilized with sexed sperm. Developmental progression was evaluated on day 5 (48h after TSA supplementation), by nuclei counting after HOECHST staining. Histone acetylation levels were measured by H3k9ac immunofluorescence at day 5 (individual blastomeres from 9-16 cell embryos) and at day 7 (ICM and TE of blastocysts), and evaluated using Adobe Photoshop software. XIST expression was detected by qPCR, using SYBR green system. Endogenous control was GAPDH, and results were compared using a standard curve. We noticed that after TSA treatment, the rate of embryos that reached the 4th and 5th cell cycle 118 h after IVF was similar to Control (C: 36.7, T: 37.1; Chi-Square test, p=0.05). Acetylation levels in individual blastomeres of 8-16 cell embryos were increased by 2-fold on treated embryos (C:98.4, T:204.2*; T Test, p=0.05) , and the same was detected for blastocysts (C: ICM 71.5^{b} and TE 64.4^{b}; T: ICM 139.8^{a} and TE 120.9^{a}; ANOVA and Tukey Test, p=0.05). Changes among blastomere levels within the same embryo were diminished on TSA group, and embryos were homogeneous regarding H3k9ac distribution. Gene expression analysis revealed that XIST expression was 8-fold decreased (C:23.3^{a}, T:2.8^{b}; Mann Whitney Test, p=0.05), and we also detected a major decrease in the percentage of blastocysts expressing detectable XIST level after TSA treatment (C:90^{a}, T:44.4^{b}; Chi-Square test, p=0.05). Based on these results, we conclude that HDAC is involved on XCI process in bovine embryos, and its inhibition might delay X chromosome silencing. Further studies are required to elucidate if XIST down regulation is beneficial for post implantation development.

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Effect of maternal age on placental characteristics and foal weight, height and thorax perimeter at birth

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Keywords: equine, neonate, placenta.

In equine, maternal age may influence placental and fetal development, and consequently neonate performance. Thus, we monitored deliveries of 25 Quarter Horse mares in 2011 (Brotas, Sao Paulo - Brazil) in order to investigate the effect of mare's age on maternal, placental and neonatal parameters. The following data was collected: number of births, mare's weight before and after birth, length of gestation, placental delivery time, placental weight, umbilical cord length, microcotiledonary density, and neonatal weight, height and thorax perimeter. Additionally, microcotiledonary density was evaluated by alantocorion stereologic analysis in pregnant and non-pregnant uterine horns. Mares were divided into three age groups: G1 (1-7 years, n = 3), G2 (8-12 years, n = 16) and G3 (≥ 13 years, n = 6). Performed to compare means of these parameters according to each group by the T test of SAS System for Windows ($p < 0.05$). The mean ± standard error for the maternal variables were: number of birth (G1: 2.33 ± 1.20, G2: 2.81 ± 0.27 3.83 ± 0.79 and G3), mare's prepartum weight (kg) (G1: 573.33 ± 35.22; G2 : 607.68 ± 17:59 and G3: 25.23 ± 571.00); mare's postpartum weight (kg) (G1: 502.33 ± 51.35, G2, G3 and 535.25 ± 15.90, 504.66 ± 25.98), length of gestation (days) (G1: 340.33 ± 5.33, G2: 336.56 ± 1.58; and G3: 336.83 ± 3.80), and placental delivery time (min) (G1: 76.66 ± 32.82; G2: 68.12 ± 3.14 and G3: 50.00 ± 9.21). For the placental variables, placental weight (kg) and length of umbilical cord (cm) were, respectively, (G1: 5.30 ± 0.62 and 58.33 ± 9:35; G2: 5.38 ± 0.38 and 64.68 ± 4.14; G3: 5.60 ± 0.45 and 69.33 ± 4.51); microcotiledonary density (g/cm3) of the pregnant and non-pregnant uterine horn (G1: 2.66 ± 0:37 and 2.10 ± 0.41; G2: 3.41 ± 0.22 and G2: 2.39 ± 0.16; G3: 2.85 ± 0.64 and 2.65 ± 0.37), respectively. Considering the parameters neonatal weight (kg), height (cm) and thorax perimeter (cm) were, respectively, (G1: 48.06 ± 5.68, 93.33 ± 2.18 and 79.66 ± 1.66, G2: 50.27 ± 1.30, 96.43 ± 1.15 and 81.56 ± 0.67; G3: 48.50 ± 1.32, 94.00 ± 1.41 and 80.16 ± 1.19). None of the parameters showed statistical differences among age groups ($p > 0.05$). Satué et al. (2011, Polish Journal of Veterinary Sciences, 14, 173-180) reported that mares between 8 to 12 years exhibited greater development of their reproductive tracts (causing a shorter gestation) but such circumstance was not observed in our study. According to Wilsher et al. (2003, Equine Veterinary Journal, 35, 476-483), maternal age can influence the microcotiledonary density, allowing a greater materno-fetal contact area and probably a greater supply of nutrients to the foal. But this work was not possible to compare the mean of the parameters evaluated, not establishing relationships that determine the effect of maternal age on the maternal, placental and neonatal variables.

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Effects of estradiol on endometrial proteins involved in the synthesis of PGF2α in cattle

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Keywords: estradiol, luteolyse, PGF2α.

Although the release of PGF2α can be induced by estradiol (E2) in cattle, the mechanism underlying this process remains unknown. Treatment of E2 pre-exposed bovine endometrial explants or bovine endometrial cells (BEND) with calcium ionophore (CI) induces a marked increase in the release of PGF2α in comparison to specimens that were untreated or pre-treated with E2 or CI only. Our hypothesis is that E2 stimulates the expression or the activity of PKC and PLA2, which are both calcium-dependent enzymes essential for the synthesis of endometrial PGF2α. The aim of this work was to test the effect of E2 on the mRNA and protein expression of endometrial PKC and PLA2 by using western blotting and RT-qPCR. Nelore heifers (n=12) were paired on day 17 of a synchronized estrous cycle, injected with 0 (n=6) or 3 mg of 17B-estradiol (n=6), and slaughtered 2 hours after treatment. Endometrial explants were immediately isolated and stored at -80^{o}C. The extraction of proteins from endometrial tissue was performed with buffer containing 1mM EDTA (Fisher BP 120-500); 1mM EGTA (Sigma E8145); 1mM DTT (Fisher BP 172-5); 0,5mM PMSF (Sigma P-7626); 300mM NaCl; 0,5% NP-40; 50mM Tris-base pH 8.0; 10% glicerol; 10µg/mL aprotinin (Sigma A-4529); 10µg/mL leupeptin (Sigma L-2884) e 10µg/mL pepstatin (Sigma P-4265). Concentrations of protein extracts were determined by Bradford method, and 50µg of total protein per sample were loaded onto gels. Primary rabbit anti-phospho-PKC antibody [Cell Signaling, 9377S, phopho-PKC theta (Thr538), rabbit Ab] was used at a 1:500 dilution, and anti-rabbit secondary antibody (Cell Signaling, 7074, anti-rabbit IgG-HRP linked) was used at a 1:3,000 dilution. Primary goat anti-PLA2 (Santa Cruz Biotecnology, sc-55887, group V PLA2 T-20, goat polyclonal IgG) was used at a dilution 1:600, and anti-goat secondary antibody (Santa Cruz Biotecnology, sc-2020, donkey anti-goat IgG-HRP) was used at a 1:10,000 dilution. X-ray films were scanned and optical density values of the respective bands were measured and analyzed by ANOVA, using the PROC GLM procedure of the SAS software. Gene expression of endometrial tissue explants was analyzed by RT-qPCR for the following transcripts: PLA2G4A, PKCa, PKCB and PKCY. Mean ± standard error of the mean of the optical density values for PKC were 1.00 ± 0.19 and 0.96 ± 0.14 for cows untreated and treated with E2, respectively. Mean optical density values for PLA2 were 1.00 ± 0.33 and 1.25 ± 0.25 for cows untreated and treated with E2, respectively. There was no effect of E2 on PKC (p=0.9) and PLA2 (p=0.6) protein synthesis. There was no effect of E2 on the expression of the tested genes (p>0.05). Our hypothesis that E2 stimulates the synthesis of PKC and PLA2 proteins was not confirmed.

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Study of the involvement of phospholipase A2 (PLA2) in the prostaglandin F2α synthesis of endometrial cells

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Keywords: PLA2, prostaglandin F2α.

The exposure of bovine endometrial cells (BEND) to estradiol-17β (E2) and calcium ionophore (CI) stimulates the synthesis of PGF2α in the endometrium, although the mechanisms by which this event occurs are unknown, possibly the E2 stimulates the synthesis of PLA2. The hypothesis of this work is that inhibition of PLA2 activity reduces E2-stimulated synthesis of PGF2α in the endometrial cells of cows. The objective was to measure PGF2α production by BEND cells treated or not with E2 and CI, in the presence or absence of a specific PLA2 inhibitor (AACOCF3). Prior to experiments, BEND cells were grown and expanded in complete culture medium (40% HAM F-12 and 40% of Minimal Essential Medium; 200 IU of insulin/L, 10% Fetal Bovine Serum, 10% horse serum and 1% antibiotic-antimycotic solution). Cells were trypsinized, suspended and seeded in 24-well plates at a density of 4 x 10^4 cells/well in 1.5 mL of complete culture medium. Cells were cultured at 38.5°C and 5% CO_2. After reaching 90% confluency, cells were washed twice with the culture media described above, in the absence of serum (serum free, SF), and then incubated in serum free media for 24 hours. After serum starvation, cells were exposed to the following treatments: SF medium (Control group); SF medium supplemented with 20μM of AACOCF3 (PLA2 inhibitor group); SF medium supplemented with 20μM of AACOCF3, 10-6M CI and 10-13M E2 (PLA2 inhibitor + E2 + CI group), and SF medium supplemented with 10-6M CI and 10-13M E2 (E2 + CI group). Treatments were performed in triplicates and repeated 3 times, totaling nine treated wells per group. Cells were pre-incubated for 60 minutes with 1mL of SF medium with or without the AACOCF3. After pre-incubation, an 100μL aliquot of medium was collected (time 0), and immediately after that, 100μL of SF medium with or without E2 + IC was added to the remaining 900μL, for a final concentration of 10-6M and 10-13M, for IC and E2, respectively. Twelve hours later, a 100μL aliquot of medium was collected from each well. Concentrations of PGF2α in the medium were measured by RIA. The production of PGF2α within the first 12 hours (DIF 12) was obtained by subtracting the concentration of PGF2α at time 0 from the PGF2α concentration after 12 hours of treatment. Data were analyzed by ANOVA using PROC GLM from SAS. The DIF 12 was 167.16 ± 18.17 pg/mL (control group), 91.47 ± 17.69 pg/mL (PLA2 inhibitor group), 125.42 ± 14.59 pg/mL (PLA2 inhibitor + E2 + CI group) and 235.54 ± 18.61 pg/ml (E2 + CI group). There was an effect of experiment ($p < 0.001$) and inhibitor ($p < 0.05$). Cells exposed to the PLA2 inhibitor (PLA2 Inhibitor and PLA2 inhibitor + CI + E2 groups) showed a DIF 12 lower ($p < 0.05$) than unexposed cells (control and CI + E2 groups). In conclusion, our data support the hypothesis that PLA2 activity is necessary for E2-induced PGF2α synthesis by the endometrium in cows.

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Assessment of the effect of follicular wall hemi-sections (HS) co-culturing on the physical characteristics of bovine cumulus-oocyte complexes (COCS)

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Keywords: co-culture, hemi-sections, oocyte.

Physiologically, meiosis resumption occurs after LH surge at the end of the maturation process. However, early withdrawal of oocytes from the follicular environment automatically causes restart of nuclear maturation. On the other hand, when oocytes are co-cultured with hemi-sections (HS) of follicular wall, oocyte meiotic arrest is maintained. This fact makes evident that the factor(s) responsible for keeping the meiotic arrest is/are produced by the follicular cells, but the mechanism by which they exercise their activity in vitro is unknown. The aim of this experiment was to assess the effect of follicular wall HS co-culturing on the physical characteristics of cumulus-oocytes complex (COCs). Follicles measuring 3-5 mm were dissected from ovaries obtained from a slaughterhouse, then cut in half and taken to the maturation medium [200 µL of TCM 199/BSA (0,04g/mL)]. Afterwards, COCs were retrieved from the remainder ovaries and separated in three groups for culture with 0 (control), 6 and 8 HS, respectively, at 38.5°C and 5 % CO_2. Nuclear maturation stage and membrane integrity of the oocytes and cumulus cells (CCs) were evaluated with 1% orcein stain and Hoechst 33342 double staining (10 µg/mL) and propidium iodide (10 µg/mL), respectively. Furthermore the degree of cumulus cells expansion was subjectively observed. The results obtained from membrane integrity assessment were assessed through analysis of variance, and further variables through chi-square test. COCs co-cultured with 8 HS of follicular wall showed a lower proportion ($p<0.05$) of injured-membrane CCs as compared to those co-cultured with 6 HS and the control (34.33% ± 8.33 vs 60.25% ± 7.32 and 58.33 ± 5.85, respectively), whereas for membrane integrity no difference was shown among treatments (82.33% ± 3.51; 80.66% ± 2.08 e 82.66% ± 2.07) ($p>0.05$). It was observed that in the group co-cultured with 8 HS a higher amount of oocytes maintained meiotic arrest (64.75% ± 2.75 oocytes in germinal vesicle and germinal vesicle breakdown stages vs 39% ± 4,53 e 1,33% ± 1,15), and that the degree of CCs expansion in all cells was proportional to the amount of HS ($p<0.05$): control group had full expansion; the group with 6 HS had very low expansion; and the group with 8 HS was had no expansion. These results suggest that a higher quantity of HS in the medium not only intensifies the nuclear maturation inhibiting factor, but also increases CCs plasma membrane integrity and inhibits its expansion. This causes oocytes-CCs bidirectional communication to be more effective. Therefore, the factor produced by follicular wall cells that is responsible for maintaining meiotic arrest, has a greater opportunity to act in the oocytes through CCs.

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Hormonal dinamic after inhibition of prolactin and prostaglandin during luteolysis in heifers

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Keywords: luteolysis, PGFM, prolactin.

In Heifers, pulses of prolactin (PRL) are most prominent and rhythmic during the 12 hours before the end of luteolysis and the first 12 hours of postluteolysis, being associated with pulses of PGF2α metabolite (PGFM), as described by Ginther and Beg (2011, Anim. Reprod. Sci. 128, 29-36). The aim of the present study was to evaluate the effect of circulatory PRL and PGF2α suppression on functional and structural regression of the CL. Holstein heifers were treated with a dopamine-receptor agonist (Bromocriptine; Br) and a Cox-1 and -2 inhibitor (Flunixin Meglumine; FM) to inhibit PRL and PGF2α, respectively. The Br was given when ongoing luteolysis was indicated by a 12.5% reduction in CL area (cm2) (Hour 0) from the area on Day 14 postovulation, and FM was given at Hours 0, 4, and 8. Blood samples were collected from every 8 h beginning on Day 14 until Hour 48 and hourly for Hours 0 to 12. Hormonal analyses were done for P4, PGFM, PRL and LH. Three groups of heifers in ongoing luteolysis were used: control (n = 7), Br (n = 7), and FM (n = 4). The statistical analyzes were done using SAS PROC MIXED (Version 9.2; SAS Institute Inc., Cary, NC, USA) with a repeated statement to minimize autocorrelation between sequential measurements. Differences between groups at each hour and selected comparisons of means within a group were analyzed by Student's unpaired and paired t-tests, respectively. The LSD test was used to locate differences among hours within groups or hours. Differences between groups for discrete characteristics were analyzed by ANOVA. A probability of $p \leq 0.05$ indicated significant differences. Treatment with Br decreased ($p < 0.003$) the average PRL concentrations over Hours 1 to 12. During the greatest decrease in PRL concentration (Hours 2 to 6), LH was increased. Progesterone concentrations averaged over Hours 0 to 12 were greater ($p < 0.05$) in the Br group than in the controls. In the FM group, no PGFM pulses were detected, and PRL concentrations were reduced. Concentrations of PGFM were not reduced in the Br group, despite the reduction in PRL. The present study demonstrates that a decrease (12.5%) in CL area (cm2) is more efficient in targeting ongoing luteolysis (63%) than using any day from Days 14 to ≥ 19 (efficiency/day, 10 to 24%). The hypothesis that PRL has a role in luteolysis was supported but was confounded by the known positive effect of LH on progesterone. In regard to the synchrony between PGFM and PRL pulses, a relevant finding was the observation of a positive effect of PGF2α on PRL rather than vice-versa. Further studies using inhibition of LH, shorter intervals for CL scanning, and evaluation of other structural CL characteristics such as blood flow are needed to clarify the effect of circulatory PRL on functional and structural CL regression.

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Effect of the onset of the puberty on thickness of the endometrium, ovaries and other structures in Nelore heifers

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Keywords: Bos indicus, follicular population, uterine tone.

The reproductive parameters of puberty in Nellore females have an important impact on reproductive efficiency, production and economy in the herd. The assessment of uterine development, ovarian cyclicity and follicular population in prepubertal heifers might represent an important tool to identify precocious heifers. The objective of this study was to evaluate the development of the uterus, ovaries and their structures, as well as to investigate their effect in the onset of puberty in female heifers up to 24 months. A total of 64 heifers from a herd of Institute of Animal Science (IZ Sertãozinho, SP, Brazil) were used. The reproductive tract was evaluated by transrectal ultrasonography and endometrial thickness (ET), average ovary areas (AOV) and corpus luteum (CL) diameters (when present) were measured. These measurements were taken at each 18 to 21 days, in heifers between aging 12 and 24 months (total of 18 evaluations). During the measurement (and/or counting) of follicular population, the follicles were classified into three groups: 1) small: ≤4mm; 2) middle: 4.1 to 7.9mm; 3) large: ≥8mm. Similarly, during the assessment of uterine tone, uterus was classified into three groups: 1) score 1 = modest or no tone; 2) score 2 = medium tone; and 3) score 3 = turgid tone. The animals were considered pubertal heifers after demonstrating visible CL and, concomitantly, plasma progesterone levels above 1.5ng/mL (quantified by radioimmunoassay). Statistical analysis was performed in SAS PROC MIXED using the repeated command. It was observed that 45.31% (29/64) of heifers (up to 24 months of age) reached puberty. The ET and AOV differed between pubertal (n=29) and non pubertal (n=35) heifers. The ET and AOV of pubertal heifers (PUB) were 10.51±0.10mm and 3.69±0.07cm2, respectively, and of non pubertal heifers (NPUB) were 9.89± 0.04mm and 3.39±0.07 cm2, respectively ($p<0.01$). The amount of small and middle follicles did not differ between PUB and NPUB heifers (Small follicles: PUB=36.82±1.98 and NPUB=39.8±0.74, P=0.14; Middle follicles: PUB=3.67±0.3 and NPUB=3.20±0.08, P=0.21). However, the amount of large follicles was higher ($p<0.01$) in NPUB (1.23±0.02) compared to PUB (0.64±0.11). Another interesting finding was regarding to uterus tone assessed by rectal palpation. The PUB heifers presented a score of 2.1±0.07 and NPUB presented a score of 1.31±0.02 for uterus tone ($p<0.01$). Hence, additionally to the increase in ET, the uterine tone becomes significantly different with the proximity of first ovulation. Therefore, endometrium thickness, ovary area and uterus tone can be used as parameters for selecting puberty heifers raised in pasture management systems.

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Insulin-like growth factor-I rescues heat-induced cellular damages in germinal vesicle stage bovine oocytes

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Keywords: heat shock, IGF-I, oocyte .

Germinal vesicle stage oocytes (GV) are susceptible to deleterious effects of heat shock. Heat-induced cellular damage can be manipulated by a wide range of biological factors, such as insulin-like growth factor-I (IGF-I). The objectives of this study were to 1) characterize the effects of heat shock on GV oocytes and 2) evaluate the thermoprotective effects of IGF-I in this context. For all the experiments cumulus-oocyte complexes (COCs) collected from slaughterhouse ovaries were cultured in meiotic inhibition medium (medium 199 supplemented with 50 μg/mL gentamycin, 0.2 mM sodium pyruvate, 100 μM cysteamine) containing 12.5μM butyrolactone for 14 hours. In each experiment, meiotic arrest was assessed by Hoechst 33342 (> 85% of oocytes at GV stage). For laboratorial control oocytes were in vitro matured without prior inhibition, and there was no effect of butyrolactone. Nonparametric data were subjected to Wilcoxon and Kruskal-Wallis tests and parametric data were subjected to analysis of variance using the SAS. The first experiment evaluated the effect of different IGF-I concentrations (0, 12.5, 25, 50 and 100 ng/mL) on heat-induced DNA fragmentation on bovine GV oocytes. In the absence of IGF-I, heat shock for 14 hours increased ($p < 0.05$) the incidence of TUNEL-positive oocytes from 0 to 5.7% for oocytes cultured at 38.5° and 41°C, respectively. Addition of 12.5 and 25 ng/mL IGF-I reduced (0%, $p = 0.07$) the effects of heat shock in the percentage of TUNEL-positive oocytes. Experiments 2 and 3 aimed to determine the effects of 0, 12.5 and 100 ng/mL IGF-I on group II caspase activity and developmental competence of bovine GV oocytes subjected to heat shock. Heat shock and IGF-I did not affect the percentage of high caspase activity oocytes. However, in the absence of IGF-I, heat shock reduced ($p < 0.05$) day 8 blastocyst rates from 26.4 to 15.5% for 38.5° and 41°C, respectively. The concentration of 12.5 ng/mL IGF-I reversed the detrimental effects of heat shock on development to the blastocyst stage (30%). The objective of experiment 4 was to determine the effect of 0 and 12.5 ng/mL IGF-I on GV-stage oocyte mitochondrial activity subjected to heat shock. In the absence of IGF-I, heat shock decreased mitochondrial activity as compared to oocytes cultured at 38.5°C. However, addition of 12.5 ng/mL of IGF-I, rescued heat-induced changes on oocyte mitochondrial activity. In conclusion, exposure of bovine GV oocytes to moderate heat shock at 41°C for 14 hours decreased mitochondrial activity, oocyte developmental competence and induced apoptosis. At low concentrations IGF-I exerted thermoprotective effects.

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Influence of feed intake on plasma concentrations of insulin and progesterone and expression of liver enzymes involved in metabolism of progesterone in sheep

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Keywords: liver enzymes, metabolism, progesterone.

The objective was to evaluate the influence of feed intake on plasma insulin and progesterone (P4) concentrations and the expression of the hepatic enzymes Cytochrome P450 2C (CYP2C) and Cytochrome P450 3A (CYP3A) responsible for metabolism of P4, in prepubertal sheep. Lambs (n=24) F1 Dorper x Santa Inês, 5 m old and with an average body weight (BW) of 29.4 kg were confined in individual stalls. The sheep were blocked according to the initial BW and divided into two groups: Ad libitum group (Ad), with average intake of 3.8% dry matter (DM) per kg of BW and Restriction group (R) with 2.0% DM per kg BW. The study lasted 10 weeks, with 2 weeks of a maintenance diet and 8 weeks on the experimental diets. At the end of the fourth (W4) and the eighth experimental weeks (W8), the ewes received an intravaginal P4 release device (0.33 g P4, CIDR, Pfizer), designated as D0. Dinoprost (PGF2α, 10 mg, im, Lutalyse, Pfizer) was used on D0 and D6. On D8, blood was collected at -0.5, 1, 2, 3, 4, 5, 6 and 7 h in relation to the time the diet was offered (0 h). Measurements of plasma insulin and P4 were done by radioimmunoassay. At D9, 4 h after diet introduction, a liver biopsy was performed and the sample stored in RNA later (Invitrogen, USA) for analysis of gene expression (mRNA) by quantitative real time PCR. Data were analyzed using PROC GLIMMIX of SAS and were presented as least squares means ± SE. The average daily feed intake was 1.8±0.04 and 0.6±0.01 kg/animal/d for the Ad and R Group, respectively ($p<0.001$). The Ad Group had an average weekly BW gain of 1.3±0.09 kg and the R group had no change in BW ($p<0.001$). Plasma insulin concentrations were higher ($p<0.0001$) in the Ad Group compared to the R Group at the end of W4 (8.2±0.94 vs. 1.5±0.16 μIU/mL) and W8 (12.0±1.03 vs. 2.2±0.19 μIU/mL). In general, the postprandial peak of insulin occurred between 3 and 5 h after initiation of feed intake. The Ad Group had lower ($p<0.0001$) plasma P4 than Group R both at the end of W4 (3.2±0.32 vs. 5.5±0.32 ng/mL), and W8 (2.8±0.29 vs. 5.2±0.29 ng/mL). In general, ewes had the lowest P4 concentrations after 4 h of feeding. The CYP2C enzyme was 1.8 times more expressed in the Ad group compared to the R group in W8 ($p=0.07$). However, there was no difference between treatments for both CYP2C in W4, and for CYP3A in W4 and W8 ($p>0.10$). Thus, in contrast to previous results, we did not find an inhibitory effect of elevated insulin on CYP2C or CYP3A, and there was no evidence that insulin led to decreased P4 metabolism. It seems likely that the dramatic decrease in circulating P4 after ad libitum feeding was due to elevated P4 metabolism due to a greater liver blood flow than found in ewes with feed restriction.

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Isolation, culture and characterization of mesenchymal stem cells isolated from bovine fetal anexa

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Keywords: bovine, cell culture, fetal membranes.

The fetal membranes have been reported as an alternative source of mesenchymal stem cells (MSCs) having advantages over bone marrow, such as non-invasive collection, low rejection rate, the possibility of formation of storage banks and evidence that these cells retain characteristics of their original tissues, such as expression of the pluripotency marker Oct-4. MSCs have a great capacity of proliferation with little or no immunogenicity and tumorogenicidade, not forming teratomas when inoculated, which contributes to the scientific interest in these cells in several species. The aim of this study was to isolate, grow and differentiate MSCs obtained from cattle amniotic membrane (AM) and extravascular matrix of the umbilical cord (UC), as well as immunophenotypically characterize the samples of UC. The samples were collected from gravidic uterus at different stages of pregnancy and kept chilled in a refrigerator at 5˚ C until arrival at the laboratory. The samples were then washed in PBS with antibiotics, fragmented, and placed in 4% collagenase solution at 38˚ C for two hours for enzymatic digestion. The solution was centrifuged two times for complete removal of collagenase and the pellet resuspended in 5 mL of culture medium (DMEM/F12, fetal calf serum (FCS), antibiotics and antimycotics) and incubated at 38º C in 5% CO_2 in air. The medium change was performed every 24 hours, and after the second passage an aliquot was separated to perform the osteogenic, adipogenic and chondrogenic differentiation. Additionally, an aliquot was plated in 24 wells to performe the immunocytochemistry with the Oct-4 and CD44 markers. The remaining cells were maintained in culture up to the fourth passage and then the cells were frozen in 90% FCS + 10% DMSO. Cells showed adherence to plastic, and fibroblastoid morphology after 12 hours in culture and reached approximately 80% confluence after 24 hours, demonstrating large proliferation potential. Cells from UC and AM demonstrated ability to differentiate in the osteogenic, adipogenic and chondrogenic strains, as revealed by matrix deposition of extracellular calcium, deposition of glycosaminoglycans and intracytoplasmic lipid droplets, respectively. Despite the negative immunostaining for Oct-4, UC samples were positive for CD44, showing their mesenchymal origin. From these results, we concluded that a rapidly isolation and culture of MSCs from bovine fetal membranes is possible, making them an excelent alternative source for cellular therapy in veterinary medicine.

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The peri-ovulatory endocrine milieu regulates the expression of genes associated with chemotaxis and leukocyte infiltration in the bovine endometrium

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Keywords: endometrium, immune response, progesterone.

The timely combination of progesterone (P4) and estradiol production has been linked to positive regulation of uterine protein expression and secretion, concept development and pregnancy success. Similarly, the recruitment of specific classes of leukocytes to the endometrium and the switch in the profile of the local immune cell population and cytokine production is intimately associated with pregnancy status. Our aim was to determine the gene expression response of the endometrium to the manipulation of the pre-ovulatory follicle diameter and P4 concentrations during diestrus (i.e. periovulatory endocrine milieu). Specifically, genes associated with chemotaxis (CXCL16, PLA2G7 and CCL16), infiltration (PLA2G10, TIMP3 and ICAM1) and migration of leukocytes (STAP2, ANGPT4 and CFB) were investigated. Twenty-two cyclic and non-lactating Nelore cows received a P4 device along with estradiol benzoate on day -10 (D-10). Animals were divided to receive cloprostenol (PGF; high P4 group; HP N=11) or not (low P4 group; LP; N=11) on D-10. Progesterone devices were withdrawn and PGF injected on D-2.5 for cows of the HP group and on D-1.5 for cows of the LP group. Ovulation was induced with GnRH on D0. Plasma P4 concentrations were measured daily from D0 until the day animals were slaughtered and endometrial fragments collected (D7). Gene expression assessment was performed by quantitative PCR (qPCR). Comparison of mean expressions rates between groups was performed by student's t test and linear regression analyzes were executed by the Minitab 16 software. Expression for genes CCL16, PLA2G7, TIMP3, STAP2, ANGPT4 and CFB was not different between groups HP and LP, whereas ICAM1 expression increased 34% in HP ($p=0.07$). Furthermore, cows from HP group increased in 65% endometrial expression of CXCL16 and decreased expression of PLA2G10 by 32% ($p<0.05$). Assessment of the relationship between relative expression rates of each of the tested genes and P4 concentrations on D7 post-estrus, regardless of group assignment, revealed significant linear regressions for genes CXCL16, PLA2G7, ICAM1, ANGPT4 and PLA2G10 ($p<0.05$). Except for a negative correlation for PLA2G10 (-0.25), a positive correlation was observed for the other 4 genes (0.23, 0.31, 0.16 and 0.25, respectively). Along with CXCL16, genes identified only by their positive correlation with P4 concentrations on D7 are also known to stimulate leukocyte chemotaxis, macrophage infiltration and cell migration, whereas PLA2G10 inhibits macrophage infiltration. These results support the positive regulation of immune cell chemotaxis, migration and infiltration into the endometrium by increased P4 concentrations on D7 post-estrus in cows. This pattern of immune cell local regulation in the endometrium may be associated with higher probability of conception and improved embryonic growth rates previously linked to increased concentrations of P4 on early pregnancy.

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Inhibition of prostaglandin biosynthesis during postluteolysis and effects on corpus luteum regression, prolactin, and ovulation in Holstein heifers

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Keywords: corpus luteum, follicle, prostaglandin F2α.

Secretion of prostaglandin F2α (PGF2α) by the endometrium on about 17 days postovulation is fundamental for luteolysis in cattle. After diminishing during postluteolysis, the secretion of prostaglandins increases late in the preovulatory period and plays a role in the ovulatory process. Pulses of a metabolite of PGF2α (PGFM) around the beginning of postluteolysis are temporally associated with an increase in prolactin (PRL) concentrations in heifers (Ginther OJ. Anim Reprod Sci; 128:29–36) and mares (Ginther OJ. Theriogenology; 77:99–107). The aim of this study was to evaluate the effects of prostaglandin inhibition during postluteolysis on corpus luteum (CL) regression, PRL, and ovulation in heifers. The beginning of postluteolysis (progesterone < 1 ng/mL) was targeted by using 8 h after ultrasonic detection of a 25% decrease in CL area (cm2) in Holstein heifers and was designated hour 0. Flunixin meglumine (FM; n = 10) to inhibit PGF2α secretion or vehicle (n = 9) were given intramuscularly at hours 0, 4, 8, 16, 24, 32, and 40. The FM was at a dose of 2.5 mg/kg at each treatment. Blood sampling and measurement of the CL and dominant follicle were done every 8 h. Blood samples for detection of PGFM and PRL pulses were obtained hourly for 24 h beginning at hour 0. The SAS PROC MIXED procedure (Version 9.2; SAS Institute) was used for analysis with a repeated statement to account for the autocorrelation between sequential measurements. The Least Significant Difference (LSD) was used for comparisons between hours within a group. Discrete or single-point data for characteristics and intervals for PGFM and PRL pulses were analyzed for differences between groups by Student's unpaired t-test. Fisher's exact test was used for comparisons of frequency data. Pulse concentrations of both PGFM and PRL were lower in the FM group than in the vehicle group ($p<0.05$). Concentration of PRL was greatest at the peak of a PGFM pulse ($p<0.05$). Neither CL area nor progesterone concentration differed between groups during hours 0 to 48 (postluteolysis). Ovulation occurred in nine of nine heifers in the vehicle group and in three of 10 heifers in the FM group ($p<0.003$). The anovulatory follicles in the FM group grew to 36.2 ± 2.9 mm, and the wall became thickened from apparent luteinization. The hypothesis that PGF2α was involved in the continued P4 decrease and structural CL regression during postluteolysis was not supported. The hypotheses that pulses of PGFM and PRL were temporally related and that systemic FM treatment induced an anovulatory follicle were supported.

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Technique for fetal sex diagnosis in horses by Doppler ultrasonography of the gonads

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Keywords: fetus, mare, pregnancy.

The Doppler ultrasonography is an emerging technology, practical and efficient on the non invasive and in real-time evaluation of the blood flow of the reproductive system of large animals. The detection of the fetal sex in horses was not routinely used due the difficulties found in locating the genital tubercle between 55 and 70 days of gestation. However, recently, the fetal sexing begun to be performed, more frequently, trough the evaluation of the gonads and external fetal genitalia, between 100 and 160 days of gestation. During this period, the gonads that undergo a hypertrophy, occupies most part of the fetal abdominal cavity, facilitating its visualization and consequently allowing a more accurate examination. The present study aimed to describe a diagnosis technique of the fetal sex in horses by Doppler ultrasonography trough the evaluation of the gonads and its vascularization. The exams were done by via transrectal in 75 mares in the period of 90 to 160 days of gestation, between the months of December 2011 and February 2012. The colored Doppler My Lab Five (Esaote, Nutricell, Campinas-SP, Brazil) was used. First in B mode (conventional) the transducer was placed ventro-dorsal in relation to the fetus. After locating the head, the transducer was directed on the cranio-caudal direction observing the following organs: heart, stomach and liver. Then, the exam proceeded to the location and identification of the gonads at the posterior third of the abdomen. Posteriorly the Doppler color mode was activated, and the approximately 60 mm^2 colored box positioned over the gonad image. Of the 75 animals the gonads of 58 fetuses (77%) were possible to be observe at first examination, being 24 females and 34 males. On color Doppler ultrasonography, in all the females, a vascular ring at the cortex-medulla interface was observed. And in all the males the pampiniform plexus vascularization was possible to be observed, however the testicular vein vascularization was detected only in 85% (29/34) of the fetuses. From the above results it is possible to conclude that the color Doppler ultrasonography is a useful tool on the fetal sex diagnosis, which from the identification patterns of gonadal vascularization became an adjuvant technique to improve the efficacy of fetal sex determination in horses.

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Effectiveness of parthenote and fertilized embryo aggregation in mouse

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Keywords: chimerism by aggregation, Mus musculus, parthenogenetic activation.

Embryonic chimerism, i.e., mixing of cells originated from at least two different fertilizations, has been used as a tool for stem cell pluripotency diagnosis, transgenic rodent production and organogenesis studies. Additionally, parthenotes are used for studies related to gene imprinting and the ability of their cells to compose the placenta and/or an adult animal. The aim of this work was to validate the production of parthenogenetic embryos (PGE), obtained from C57BL/6 EGFP mice (EGFP), to determine PGE chimerism potential, and to localize PGE cells on the produced blastocyst. To aggregation were used pre compaction embryos produced either by in vivo (Swiss Webster strain; SW) or by in vitro (EGFP) and after in vivo fertilization (IVvF) or parthenogenetic activation (PGA), respectively. To PGA was used strontium chloride hydrate (5 mM for 6 h). Two experiments were outlined in order to: i) evaluate the development from IVvF (DIV; n=53 embryos) and from PGA (DPG; n=409 embryos) techniques; ii) evaluate the aggregation between pairs of control embryos (C; n=20, IVvF from SW) and IVvF/SW plus PGA/EGFP embryos (PG; n=40). After manipulation (e.g., removal of the zona pellucida and approximation of pairs, for C and PG groups), all the embryos were kept in vitro culture (37ºC, 5% CO2 and saturated humidity) during 48 to 60 h (C and PG), or up to blastocyst stage (DIV and DPG). Chimerism rate and PGE fluorescence (pre and post culture) were evaluated under an inverted microscope with epifluorescence source and digital images were captured (Eclipse Ti and NIS-Elements, Nikon, Tokyo, Japan, respectively), by merging visible and UV light images. The rate of PGA (EGFP oocytes) assessed by the presence of at least one pronucleus was 54.5% (66/121). The rate of blastocyst production from PGA (oocytes) or 2 cell embryos (IVvF) was significantly different ($p<0.001$; Fisher's exact test) between DIV and DPG (71.4 and 12.9%, respectively). When developmental kinetic was evaluated there was observed a difference in the average time to the majority of embryos reach blastocyst stage for DIV (48 h) and DPG (120 h). Chimerism rate differed ($p=0.006$; Fisher's exact test) between C (55.0%; 11/20) and PG (17.5%; 7/40) groups, after assessment from 48 to 60 h of culture. In PG group, was observed a random incorporation of EGFP cells in the obtained chimeras. It was concluded that PGE have a slower developmental kinetic (until 120 h of culture) than embryos derived from IVvF. In one of the chimeras obtained, was verified unequivocally the capacity of parthenogenetic cells be incorporated in the inner cell mass of the blastocyst.

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Mouse blastocyst reconstruction by heterologous aggregation of inner cell mass with trophectoderm

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Keywords: blastocyst, chimeric, mouse.

Aiming to decrease fetal losses of bovine clones (Meirelles et al., 2010.Rep Fert Dev.22:88-97) it was proposed the replacement of trophectoderm (TE; Muramaki et al., 2006.Clon St Cells.8:51-61) by microinjection and immunosurgically inner cell mass (ICM) isolation. Zheng et al. (2005.Zygote13:73-7) reported that both techniques could damage the ICM and/or TE. An alternative to solve that problem would be the technique of cell aggregation by approximation, which had been used only in pre-compaction embryos. The aims of this study were to assess the chimerism effectiveness of post-compaction embryos and to evaluate the feasibility of to reconstruct blastocysts by ICM and TE approximation. Mice (Swiss Webster; SW and C57BL/6/EGFP; EGFP strains) were superovulated and embryos were obtained from 2.5 to 3.5 days post-coitus. In all the control groups (CG) the zona pellucida were removed from whole embryos (from eight-cell to pre-compaction morula stages) before aggregation. The bisections were performed with a blade under a micromanipulator control to produce two fragments according each experiment requirements. The fragments/embryos were put in close contact and were in vitro cultured by 24 h in KSOMaa medium. Aggregation rate (AR) of the fragments/embryos was morphologically evaluated by the presence of a single, cohesive cell mass in the well. Furthermore, in 25% of the aggregation attempts it was used segregating phenotype embryos (EGFP-SW) and aggregations were evaluated under UV light. In Experiment 1, three sorts of aggregation were tested to assess embryonic stage effectiveness: G1 (n=28), 2 demi-blastocysts; G2 (n=20), demi-morula and demi-blastocyst; and CG (n=25), control. In Experiment 2, phytohemagglutinin (PHA-adhesive agent) and the increasing from two to four fragments were used to the groups: G1 (n=24), 2 demi-blastocysts; G2 (n=22), 4 demi-blastocysts; and CG (n=22). After the validation of the aggregations (Experiments 1 and 2), it was tested blastocystic reconstruction by the approximation of ICM and TE fragments (in the PHA presence) on the groups: G1 (n=48), 1 TE fragment and 1 ICM; and G2 (n=17), 2 TE fragments and 1 ICM. The results were analyzed using chi-square and Fisher's exact test (significance when $p<0.05$). In both experiments 1 (G1=3.6%; G2=15.0%; and CG=60.0%) and 2 (G1=8.3%; G2=36.4%; and CG=77.3%) AR groups differed among each other. In Experiment 3 there was no difference on the AR between G1 (27.1%) and G2 (29.4%). Despite the low adhesive potential of the embryonic cells from blastocysts, it was feasible the production of chimeric embryos by aggregation. We could infer that agglutinant agent (PHA) increased AR by preventing loss of contact among the fragments.

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Concentration of serum prolactin and follicle development of postpartum Santa Ines sheep under different systems of suckling

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Keywords: early weaning, ewe, follicular diameter.

In ewe, suckling management may influence prolactin blood concentration, exerting negative feedback on gonadotropin release and, consequently, interfering in postpartum follicular development. The objective of this study was to evaluate the effect of different suckling regimens on serum prolactin and follicular development in postpartum Santa Ines ewes. 32 ewes were used, divided into blocks according to parity, number of lambs and body weight and subjected to three treatments: continuous suckling (n = 11), controlled suckling (n = 10), two suckling per day for an hour from the 10th day, or early weaning (n = 11), with total separation of the lambs on the 10th day. The controlled feeding ewes could hear the lambs but they could not see them and the ones subjected to early weaning did not maintain visual or auditory contact with their lambs. Blood samples for prolactin measurement were performed on the day of birth (day zero) and thereafter at intervals of six days until the first estrus manifestation or until the 30th day postpartum. Ovaries ultrassonographic exam were performed every three days from the 12th day after delivery until the first detection of estrus or until the 60th day after delivery to assess the interval from calving to first follicle with a diameter greater than or equal to 5 mm and the diameter of the first follicles greater than or equal to 5mm. The prolactin concentration was analyzed by the MIXED procedure (SAS, 2008). The interval from calving to the visualization of follicles with a diameter greater than or equal to 5mm was analyzed by generalized linear models, considering the Poisson distribution, using the procedure GENMOD. Follicular diameter was analyzed using the GLM procedure, by analysis of variance and F test. The concentration of prolactin in ewes continued suckling, controlled suckling or early weaning (mean ± standard error of mean, 138.12 ± 10.95; 113.27 ± 9.91; 84.74 ± 5.60 ng / mL), the delivery interval of the first follicle having a diameter greater than or equal to 5 mm (38.00 ± 3.59; 35.57 ± 3.93; 26.55 ± 3.25 days), the diameter of the first follicle equal or higher to 5 mm (5.73 ± 0.05; 5.89 ± 0.07; 5.65 ± 0.06 mm) did not differ among the ewes that received different treatments ($p > 0.05$). In conclusion, the management of suckling did not influence the concentration of prolactin and follicular development in postpartum Santa Ines ewes.

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Gene expression profile during *in vitro* maturation of bovine oocytes

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Keywords: gene expression, maturation. Oocyte.

During oogenesis mRNA transcription and accumulation is important for acquisition of oocyte competence. The formation of these mRNA stocks should occur before germinal vesicle (VG) break down, since, after meiosis resumption, the RNA polymerase activity transcription is almost zero. The establishment of the expression profile of genes linked to epigenetic and apoptosis pathways, during in vitro maturation (IVM), may contribute to understand the mechanisms involved in acquisition of competence and oocyte degeneration. The aim of this study was to evaluate the expression pattern of genes linked to some epigenetic pathways such DNA methylation (DNMT-1), histone acetylation and methylation (HDAC-2 and SUV39H1), also, evaluate apoptosis gene expressions (CASPASE 3 and P-53) during IVM. CCO's were obtained from slaughterhouse ovaries and after selection were in vitro matured for 0, 8, 22 and 24 hours. At each time point, oocytes were removed from culture, denuded and stored in RNA later ® (Life Technologies) at -20 °C until the analysis. To assess the gene expression, four pools of 20 oocytes were used for each group. RNA was extracted using the Micro Plus kit RNeasy Kit® (Qiagen) and cDNA synthesis performed using Oligo-DT12-18primer (® Invitrogen) and enzyme SuperScript III (Invitrogen ®). Relative gene expression was evaluated by qPCR Power using SYBR ® Green PCR Master Mix® (Applied Biosystems). GAPDH was used as endogenous control. Relative expression of target gene was calculated by the ΔΔCT method with efficiency correction by Pfaffl method. Comparison of gene expression among different time points was performed using ANOVA and Tukey's test. For HDAC 2 and P-53 gene no changes in mRNA relative abundance were observed during IVM ($P>0.05$). However, mRNA levels for SUV39H1 gene showed a tendency to be increased ($P<0.10$) on the 8 hours group. DNMT-1 and CASPASE 3 genes showed a different pattern of expression during IVM than the others genes, showing an increase ($P<0.05$) in transcript level at 22 hours of maturation. At 24 hours, mRNA levels were similar ($P>0.05$) to those observed in 8 hours for the DNMT-1 gene. In 24 hours group, CASPASE 3 gene expression were similar ($P>0.05$) to 0 and 8 hours group. Although some genes of both pathways have not presented any changes in their expression during IVM, CASPASE 3 and DNMT-1 showed an increase in mRNA abundance. Even though, that is not the general pattern of expression for this period, these results suggest that transcription occurs after meiosis resumption in bovine oocytes.

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Early development of *in vitro* produced bovine embryos after low level laser irradiation

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Keywords: bovine, embryos, laser.

Although well established, the in vitro production of bovine embryos still does not reach its maximum efficiency. It occurs mainly due to the inefficiency of mimicking the in vivo process, so the rates of blastocyst remain around 40%. An alternative to improve the IVP system is the low level laser irradiation (LLLI) of the cells with. The LLLI is capable to biomodulate several cell types, including mitotic and metabolic responses, depending on the wavelength used. In a previous study, we found that the LLLI during IVM was able to induce, in cumulus cells, increased mitochondrial activity and the number of cells committed to the cycle, with no changes in viability. On this basis, the objective of the present study was to evaluate the effect of LLLI (λ 633nm HeNe 1J/cm2 and fluency) during the early stages of IVF on cleavage and blastocyst rates, as well as the percentage of total and viable cells of embryos. For that, COCs were aspirated from follicles of slaughterhoused cows and, after selection, they were randomly divided into three groups: IVF Control (C), experiment Control (FCIV) and Laser (LFIV). IVF (D0) was performed after 22-23h from the beginning of IVM, and right after insemination LFIV group was irradiated with LLL. Group C was placed in the incubator after insemination and the FCIV group remained under the same conditions as LFIV but in the absence of light. The cleavage and the blastocyst rates were assessed at 72hpi (D3) and 168hpi (D7), respectively. In D7, embryos were removed from culture and double stained for evaluation of the total number of cells and the number of viable cells. The rate of cleavage, blastocyst, total cell number and number of viable cells were analyzed by ANOVA with Tukey post test (Prism 5 (GraphPad Inc.). There was no difference among groups in rates of cleavage (C: 64,73 ± 5,50%; FCIV: 69,9 ± 4.14%; LFIV: 65.18 ± 9.19%) and blastocyst (C: 30,24 ± 5,97%; FCIV: 32.22 ± 4.13%; LFIV: 32.92 ± 5.26%) and the number of viable cells from embryos (C: 74,82 ± 11,34 cells; FCIV: 60.5 ± 11.14 cells; LFIV; 57.83 ± 10.61 cells). These results suggest that the irradiation conditions used in this experiment, at the beginning of IVF did not have a significant effect on the parameters evaluated.

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In vitro development and DNA fragmentation of parthenogenetic cat embryos subjected to insulin-transferrin-selenium and low oxygen tension

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Keywords: DNA fragmentation, feline, parthenogenesis.

The establishment of effective maturation and culture conditions is essential for the success of any assisted reproductive techniques. The study of these techniques in the domestic cat could be very useful to be applied in endangered wild felids for sustaining genetic biodiversity. In the present study, we compared the development of parthenogenetic cat embryos using insulin-transferrin-selenium (ITS) during oocyte maturation together with 5%O_2 during embryo culture (antioxidant conditions), respect to standard maturation medium (SM) and 21%O_2. Ovaries were recovered from domestic cats subjected to ovariectomy and transported to the laboratory within 2 h. The cumulus-oocyte-complexes (COCs) were released from the follicle by repeatedly puncturing and scraping the ovaries. The selected COCs were in vitro matured in TCM 199 containing 1 IU/ml HCG, 10 ng/ml ECG, 2.2 mM calcium lactate, 0.3 mM pyruvate, 0.3% BSA, 3% antibiotic-antimycotic, and ITS when it is specified. Matured oocytes were denuded of cumulus cells after 22h of culture. They were activated with 5 uM ionomycin in TALP-H for 4 min and subsequently treated with 1.9 mM 6-DMAP for 3h. The culture medium used was SOF and the treatments were analyzed by Fisher test ($p<0.05$). The group matured with ITS (ITS-PA-5%O_2 group, n=119) was cultured in 5%O_2 and the group matured in SM (SM-PA-21%O_2 group, n=121) was cultured in 21%O_2. The cleavage rates (day 2) did not differ between the groups (68.9% and 66.2%, respectively), whereas the blastocyst rates (day 8) were higher when ITS and 5%O_2 were used, 38.7% vs. 17.4%. In order to determine the quality of the blastocysts generated, we evaluated total cell number and DNA fragmented nuclei, by TUNEL assay. Differences in total cell number were analyzed using SAS Proc Mixed and the proportion of fragmented nuclei over total cell number was analyzed by "Difference of proportions test". The mean of total cell number was higher in the ITS-PA-5%O_2 blastocysts 161±34.7 (n=6) vs. 86.7±11.4 (n=11), and the proportion of TUNEL positive cells was statistically higher in the same group, 73% vs. 40%. We concluded that antioxidant conditions improve blastocyst formation, even in those embryos with high levels of DNA fragmentation. We consider that these results are important to be considered in cloning to assist wild felid reproduction.

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Inhibition of histone H3 lysine 27 trimethylation affects porcine embryo development

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Keywords: differentiation, H3K27, polycomb.

In the early embryonic stages, genes required for development and cell differentiation are held in repressive state in pluripotent cells by Polycomb group proteins (PcGs). This effect occurs by trimethylation of the lysine 27 of histones H3 (H3K27me3) that are associated with these genes. The 3-Deazaneplanocin A (DZNep) is capable of inhibiting the action of the PcGs, preventing H3K27me. The aims of this study were to: 1) assess the global profile of H3K27 acetylation and methylation (mono-, di- and tri-methyl) during early development of porcine embryos; and 2) investigate the effect of DZNep treatment on cultured embryos. In Experiment 1, oocytes / embryos were fixed at different stages of development, from immature oocytes to D8 embryos. The structures were subjected to an immunofluorescence protocol with primary antibodies anti-acetylated, mono-, di- and trimethylated H3K27. In Experiment 2, a total of 564 IVF and 221 parthenogenetic embryos were cultured with 0 (control group) or 5 μM DZNep (treated group) starting at D2 or D4 of culture. Embryos were fixed at D8 and subjected to cell counting, and means were subjected to analysis of variance and compared by t test. Acetylated H3K27 was detected from immature oocytes until D8 blastocysts. Monomethylated H3K27 was observed in GV and MII oocytes and pronuclei; with low intensity in 2-8 cell embryos, and signal was intense again in D6 and D8 embryos. Dimethylated H3K27 signal was observed in GV and MII oocytes, absent in pronuclei and increased until D6 and D8 blastocyst. Trimethylated H3K27 was detected in GV and MII oocytes, and in one pronucleus. Signals were weak or absent from the 2-cell stage to D6 blastocysts, and re-emerged in some D8 blastocysts. In Experiment 2, IVF embryos exposed to DZNep at D2 and D4 had lower blastocyst rates (3.89 and 7.25%, respectively) than control group (19.01%). The average cell number was also higher in control group (48.75) compared to those exposed to DZNep, either at D2 (17.0) and D4 (21.14). In parthenogenetic embryos, control group showed a blastocyst rate of 55.29%, higher than group DZNep D4 (37.14%), but similar to group DZNep D2 (39.39%). Control group showed a higher number of cells (46.21) than groups DZNep D2 (17.33) and DZNep D4 (21.0). These findings suggest that the Polycomb group proteins play an important role in the regulation of porcine embryonic development, since their inhibition result in lower blastocyst rates and embryos with lower cell number.

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Preimplantation developmental capacity of parthenogenetic or *in vitro*-fertilized bovine embryos

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Keywords: embryo, *in vitro* fertilization, parthenogenesis.

Parthenogenesis has been shown as a potential method to avoid ethical limitations with research involving fertilized human embryos, as studies dealing with embryonic stem cells (Paffoni et al., Placenta 29:5121-5125, 2008). Nevertheless, several biological aspects of parthenogenetic embryos are still unknown. This study aimed to use the bovine model to compare the developmental capacity during the preimplantation period of parthenogenetic embryos with in-vitro fertilized counterparts. Oocytes (n=1541) obtained from slaughtered cows were in vitro matured and parthenogenetically activated (4.62 µM ionomycin for 5 min followed by 2 mM 6-DMAP for 4h) or in vitro-fertilized (2 x 106 sperm/mL for 20h, with semen from only one batch). Seventy two hours post-activation or fertilization (hpaf), embryos were allocated in groups according to their developmental capacity as follow: PART≥8 – parthenogenetic embryos with 8 or more cells (with high developmental capacity); PART<8 – embryos with less than 8 cells (with low developmental capacity); FIV≥8 – in vitro-fertilized embryos with 8 or more cells; and FIV<8 – embryos with less than 8 cells. All embryos were cultured in CR2aa medium supplemented with 2.5% FCS under 5% CO_2, 5% O_2, 90% N_2 at 38.5ºC and blastocyst rates were evaluated at 168 hpaf (D7) and 196 hpaf (D8) as well as the coefficient of variation (CV) among replicates (n=7). Data was compared by analysis of variance and means by Student Newman Keuls test. Values are shown as mean±SEM. Embryos with 8 or more cells produced higher ($p<0.05$) blastocyst rates at D7 and D8 than embryos with less than 8 cells, indicating their greater potential to develop until blastocyst stage, regardless whether they were activated parthenogenetically or in vitro-fertilized. Embryos from PART≥8 had higher ($p<0.05$) blastocyst rate at D7 (63.6±3.4%) than those from FIV≥8 (45.3±8.9%), however, the blastocyst rate at D8 was similar between those groups (56.7±3.0% and 44.2±8.9% for PART≥8 and FIV≥8, respectively; $p>0.05$). The CV for blastocyst rate in the PART≥8 was lower (14.1% and 14.2% for D7 and D8 respectively) than that in the FIV≥8 (52.1% and 53.4% for D7 and D8 respectively) group. There was no difference ($p>0.05$) regarding to blastocyst rate at D7 and D8 for embryos with less than 8 cells obtained from parthenogenesis or in vitro fertilization. In conclusion, parthenogenetic bovine embryos have developmental abilities similar to in vitro-fertilized counterparts during the pre-implantation period, but with lower variation among replicates. Nevertheless, comparative studies of cellular and molecular features are needed.

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Biochemical and physiological profiles in the maternal and fetal plasma of cloned, *in vitro*-derived and control bovine pregnancies on day 225 of gestation

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Keywords: biochemistry, clone, IVF.

Pre- and post-natal developmental abnormalities are usually seen in cattle after in vitro manipulations, such as in the in vitro production (IVP) of embryos by in vitro fertilization (IVF) or cloning by somatic cell nuclear transfer (SCNT) procedures, in a set of symptoms known as the Abnormal Offspring Syndrome. The aim of this study was to evaluate phenotypic features and biochemical profile of Nelore cattle concepti produced by SCNT (n=6) and IVF (n=4), in comparison to control pregnancies (n=4). Pregnant recipients were slaughtered on Day 225 of gestation. Maternal blood samples were collected 30 min prior to (PLVM00) and after slaughter (t=30 min, PLVM30), whereas fetal blood samples were collected after the removal of the fetus (t=30 min, PLVF); plasma samples were frozen at -196^{o}C. Concepti were also morphologically evaluated. Plasma samples were subjected to biochemical and physiological analyses of blood parameters for liver and kidney functions, and energy, nitrogen, hemoglobin, and calcium and phosphorus metabolisms. Data were analyzed by ANOVA and simple correlation ($p<0.05$). SCNT-derived fetuses were significantly larger and heavier (24.2 ± 2.0 kg) than fetuses from the IVF (14.1 ± 3.2 kg) and control (12.5 ± 2.3 kg) groups. Plasma concentrations for direct bilirubin in maternal plasma of clone-bearing pregnancies, and for indirect and total bilirubin in fetal plasma of the SCNT and IVF groups were higher than in the control group, indicating a possible higher erythrocyte clearance. Parameters for liver (LDH, AST, ALT, GGT, alkaline phosphatase) and kidney (creatinine, uric acid) functions were similar between groups. Nevertheless, urea levels were higher in plasma of clones (36.7 ± 2.0 mg/dL) than in IVF (15.8 ± 2.4 mg/dL) and control (13.6 ± 2.4 mg/dL) animals, indicating an increased activity of nitrogen metabolism in animals carrying clone pregnancies and their concepti. No difference was observed between groups for glucose, lactate, cholesterol, triglycerides, HDL, calcium, phosphorus, chloride, and total protein plasma concentrations. However, the SCNT and the IVF groups had higher VLDL and LDL plasma concentrations, respectively, than the control group. In general, glucose, total protein, and parameters for lipid metabolism and/or liver function were strongly correlated with one another ($r>0.700$; $p<0.0001$). In summary, bovine cloned concepti were larger and heavier on Day 225 of pregnancy than IVF-derived and control counterparts, with pieces of evidence for differences in energy metabolism, but not in systemic homeostasis, between cloned, IVF and control concepti.

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Functional characterization of SOX2 in bovine preimplantation embryos

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Keywords: blastocyst, bovine, SOX2.

Establishment of pluripotent embryonic stem cells from bovine embryos has been so far, unsuccessful. The lack of a reliable marker for pluripotency in bovine is still hampering efforts to derive these cells. We previously aimed to find genes upregulated in the inner cell mass (ICM) of bovine blastocysts and after quantitative PCR analysis, we chose SOX2 for further characterization in bovine embryos. We hypothesize that SOX2 is a marker for ICM and required for its establishment in bovine embryos. Spatial and temporal localization of SOX2 protein was verified by immunocytochemistry and revealed that its expression starts at the 16-cell stage and is then restricted to the ICM of blastocysts. To study the role of SOX2 during bovine early embryo development, we designed and co-injected siRNA targeting SOX2 mRNA and Dextran-TRITC. Non-injected (CTRL) or scramble-siRNA (SCR) injected embryos were used as controls. Embryo development rates and cell allocation data were analyzed by ANOVA using PROC GLM and PROC MIXED of SAS 9.3 respectively, both followed by Tukey's adjustment for comparison of means ($p<0.05$). Quantitative PCR data was analyzed using PROC MIXED of SAS 9.3 software as described previously (Steibel et al. 2009, Genomics, 94, 146-52.) At first, zygotes were injected and blastocyst rate declined when compared to controls. To determine whether SOX2 had a role in cell allocation, we injected one blastomere of a two-cell embryo with SOX2 siRNA-Dextran-TRITC. Development to blastocyst was not different from the control groups and daughter cells of the injected blastomere, tracked by TRITC fluorescence, were able to contribute to the ICM. Moreover, these embryos displayed cells in the ICM that lacked SOX2. No changes were observed in cell allocation to ICM or trophectoderm. Gene expression analysis revealed a decrease in SOX2 and Nanog gene expression in siRNA injected embryos; however OCT4 expression was unchanged when compared to control groups. Overall, we identified SOX2 as an ICM marker of bovine blastocysts; however our data indicates that SOX2 is not required for ICM formation in the bovine embryo.

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Cellular proliferation and apoptosis in endometrial tissue of cows with different periovulatory endocrine profiles

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Keywords: cattle, endometrium, progesterone.

In cattle, elevated progesterone (P4) concentrations during the first week post-estrus has been associated with a higher pregnancy success. It is believed that this may be due to the positive influence of P4 on the endometrial tissue cells, specifically regulating the cell's density and hence, the tissue's secretory capacity. The objective was to investigate the relationship between the P4 concentrations and the endometrial tissue proliferative and apoptotic activity measured on Day 7 post-estrus. Cycling, non-lactating Nelore cows received a P4 device along with a shot of estradiol benzoate on day -10 (D-10). Animals were divided to receive an injection of prostaglandin analog (PGF; HP group; N=11) or not (LP group; N=11) on D-10. The devices were removed and PGF administered on D-2.5 on cows in the HP group and on D-1.5 on cows in the LP group. Ovulation was induced with GnRH on D0. It was expected that cows in the HP would ovulate larger follicles and develop larger CLs than LP cows. Plasma P4 concentrations were measured daily from D0 to D7. On D7 endometrium was collected. Subsequently, RNA was extracted, cDNA synthesized and gene expression measured by qPCR using cyclophilin as the endogenous control. Proliferative activity was estimated by measurement of the Proliferating Cell Nuclear Antigen (PCNA) gene expression, while apoptotic activity by BAX and BCL2 gene expression. Variables in the HP group in relation to the LP group ($p<0.05$) was observed: Maximum diameter of the preovulatory follicle (mean ± SEM; 12.8±0.4 vs. 11.1±0.4 mm), CL weight on D7 (3.2±0.5 vs. 2.1±0.2 g), CL volume on D7 (3045.2±522.1 vs. 1656.2±164.4 mm3), P4 concentration on D7 (4.5±0.3 vs. 3.4±0.3 ng/mL) and rate of increase in the P4 concentrations from D2 to D4 (7.3 ± 0.5 vs. 2.8 ± 0.4 times) were greater in the HP group in relation to the LP group ($p<0.05$). Although BAX and BCL2 gene expression did not differ between groups, there was an increase in PCNA gene expression in the HP group, as well as a significant positive correlation ($r=0.26$) between PCNA expression and the increase in P4 concentrations from D2 to D4 ($p<0.05$). It is suggested that higher P4 concentrations on D7 post-estrus induce greater endometrial tissue cell proliferation rates without changing the level of cellular apoptosis. It is also suggested that an increased PCNA gene expression corresponds to a higher endometrial tissue cell density, potentially increasing its secretory capacity.

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In vitro production of bovine embryos in the absence of FBS under different oxygen tensions: implications in embryonic development and levels of intracellular reactive oxygen species - preliminary results

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Keywords: gaseous atmosphere, protein supplementation, ROS.

FBS and BSA, in different proportions and combinations, are used routinely as protein source in embryo developmental medium. However, reports have shown that FBS may increase the lipid content, and alter the morphology and gene expression in embryos, as well as introduce toxic and pathogenic elements in the system. The use of high O_2 tension during IVC induces oxidative stress by increasing intracellular levels of reactive oxygen species (ROS), causes loss in embryonic development and quality. Thus, embryos produced in FBS-free system and under low O_2 tension would possess better quality and cryotolerance, due to lower lipidic content. So, aiming the establishment of a IVP system under the conditions described above, this study was conducted to evaluate the effect of supplementation of maturation and culture medium with BSA or FBS under different oxygen tensions (20% or 5%) on embryonic development and production of intracellular ROS. COCs (n = 1601) were matured in B199 (TCM-199 with bicarbonate and hormones) supplemented with 10% FBS or 6mg/ml BSA-FAF (fatty acid free). After IVF, zygotes were IVC in SOFaa with 5mg/ml BSA-FAF + 2.5% FBS or 8mg/ml BSA-FAF in the two atmospheres proposed (5% CO_2 in air - ATM1; or gaseous mixture of 5% CO_2, 7% O_2 and 88% N_2 - ATM 2). Cleavage was evaluated at 48hpi and blastocysts rates at 168hpi when they were stained with 5μM fluorescent probe H2DCFDA (Molecular Probes, Inivitrogen, Oregon, USA) to measure ROS. Embryos were evaluated in an epifluorescent microscope (excitation 495 nm and emission 530 nm) by 508 ms irradiated by once, and analyzed by Q-Pro Image Capture software to determinate the fluorescent intensity. Data were analyzed by ANOVA ($p<0.05$). The cleavage rates was 80.4%^{a} (SFB - ATM1), 79.8%^{a} (BSA - ATM 1), 78.9%^{a} (SFB – ATM 2) and 82.1%^{a} (BSA – ATM 2). Blastocysts rates were 44.5%^{a}, 13%^{c}, 27.9%^{b} and 7.3%^{c}, respectively. The fluorescent intensity were similar ($p>0.05$) among groups (0.9±0.1 to 1.0±0.1). Embryos produced with FBS in 5% CO_2 in air atmosphere showed the best rate of embryonic development. The use of BSA in IVM and IVC, independently of the atmosphere used in IVC, resulted in lower embryos production when compared to that produced in presence of FBS during IVM and IVC. These preliminary data suggest that the use of low O_2 tension did not alter the amount of ROS produced, independently of the protein supplementation used. The total removal of FBS from the system of embryo production decreased the production rates, indicating that the semi-defined media requires further formulation to meet the needs of the various embryonic beneficial factors presents in FBS.

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Effects of *in vitro* pre-maturation of bovine oocytes with cAMP modulators on nuclear progression

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Keywords: cAMP, oocytes, pre-maturation.

Oocyte maturation is a key procedure in IVP of embryos, and has a major effect on embryonic development. A novel and promising approach is the supplementation with adenylate cyclase (AC) activators and phosphodiesterases (PDEs) inhibitors (ALBUZ et al., Hum. Reprod., 25(12): 2999, 2010), aiming higher levels of cAMP to maintain the meiotic block during the called pre-maturation. This study was designed to assess the meiotic progression after induction of bovine oocytes pre-maturation with cAMP modulators agents, during in vitro maturation (IVM) in serum-free medium. Oocytes were cultured in TCM 199 supplemented with 6 µg/mL BSA, 1.0 µg/mL FSH, 50 µg/mL hCG, 1.0 µg/mL estradiol, 0.20 mM sodium pyruvate and 83.4 µg/mL amikacin. In treated groups, 100 µM (T100) or 150 µM (T150) of forskolin (AC activator) and 750 µM IBMX (nonspecific PDE inhibitor) were applied in the first 2 h of culture. Subsequently, oocytes were transferred to IVM base medium supplemented with 20 µM cilostamide (oocyte-specific PDE inhibitor) in groups of 15-20, at 38.5° C in 5% CO_2 in air atmosphere. Samples were obtained 20, 24 and 28 h after maturation treatments and oocytes were stripped from cumulus cells with hyaluronidase (2 mg/mL), stained with 10 µg/mL Hoechst 33342 for 15 minutes and evaluated regarding nuclear progression. Three replicates were performed, and nuclear maturation rates were assessed by chi-square (χ^2) or, when appropriate, by Fisher's exact test, in SAS v.8.2. At initial evaluation after 20 h IVM, even though the control group displayed 82.6% of oocytes (57/69) in metaphase II with extrusion of the 1st polar body, lower rates ($p < 0.05$) were observed in T100 (26/84 - 30.9%) and T150 (23/79 - 29.1%) groups. No difference was detected between T100 and T150 groups. After 24 h IVM, lower maturation rates were also observed in treated groups (control: 68/77 - 88.3%; T100: 57/83 - 68.7%, T150: 56/89 - 62.9%). Nonetheless, 28 h after IVM, results were similar ($p > 0.05$) among all groups (control: 51/56 - 91.1%, T100: 65/78 - 83.3%; T150: 62/69 to 89.8%). Therefore, we concluded that the use of cAMP modulators led to slower nuclear progression during IVM, in a similar way between concentrations 100 and 150µM forskolin. Despite the attenuation caused by these modulators, satisfactory rates of nuclear maturation were obtained after 28 h of IVM and this probably reflects on the optimal time for in vitro fertilization of oocytes. Further experiments to evaluate the effect of this pre-maturation system on embryo development are required, to better characterize the potential benefits caused by increased levels of cAMP and slower nuclear progression during maturation.

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Effect of cortisol on *in vitro* maturation and *in vitro* culture on bovine embryo

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Keywords: bovine; cortisol, embryo.

There is evidence of cortisol metabolism in oocytes and in placenta cells, indicating that this hormone plays a role on the processes of ovulation and fetal development, respectively. Therefore this study aims to evaluate the effect of the hormone cortisol in the stages of oocyte maturation and embryo development in vitro. The Experiment 1 evaluated the effect of addiction of different concentrations of cortisol on oocyte maturation. In this groups 20 COCs were selected and incubated in medium with or without cortisol in IVM according to the following experimental groups: control (0 µg / mL), Group IVM 0.01 µg/mL, Group IVM 0.1 µg/mL and Group IVM 1.0 µg/mL, at 38.5°C and high humidity for 18 hours. For IVF, the sperm of a single bull was thawed and the sperm were deposited with COCs. After 24 hours of incubation, the presumptive zygotes were cultured in SOF supplemented with 6 mg /mL BSA and 2.5% SFB. In Experiment 2 the effect of this hormone in in vitro culture of bovine embryos was evaluated. For this COCs were selected and incubated for IVM for 18 hours, then IVF was performed as described in experiment 1. After 24 hours of incubation, 80 probable zygotes were subjected to repeat pipetting for removal of remaining cumulus cells, and then randomly distributed among the following groups: control (0 ug/mL cortisol), Group IVC 0.01 ug/mL, Group IVC 0.1 ug/mL, Group IVC 1.0 ug/mL. For each experiment four replications were performed, and in each one the rates of cleavage (Day 2 of culture) and blastocyst (day 7) were analyzed. The results were evaluated by ANOVA test, adopting $p < 0.05$. In experiment 1 there was no statistical difference in cleavage rates between the groups ($p > 0.05$) Control (69 ± 9.8); Group IVM 0.01 ug/mL (85 ± 10.2); Group IVM 0.1 ug/ml (80 ± 5.5) and Group IVM 1.0 ug/ml (84 ± 8.7), but in relation to the embryonic development, the Group 0.1 ug/ml (38 , 10 ± 4) showed an increased rate of blastocyst when compared to the control group (20.8 ± 1.4) ($p < 0.05$). In the second experiment the cleavage and blastocyst rates were similar between groups ($p > 0.05$) Control (67±11 and 32±7.7, respectively), Group IVC 0.01 ug/ml (66±6 and 30±10, respectively), Group IVC 0.1 ug/ml (70±5 and 34±7, respectively) and Group IVC 1.0 ug/ml (70±11 e 25±12, respectively). Thus, the partial results presented here suggests that the cortisol during oocyte maturation has a positive effect on embryo development, increasing rates of embryo development. The same was not observed during embryo culture.

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Biochemical evaluation of equine amniotic fluid collected in different moments of pregnancy and at delivery

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Keywords: equine, amniotic fluid, biochemical evaluation

The viability and fetal maturity can be estimated by biochemical evaluation of the fetal fluids of so many species. However, the composition of amniotic fluid during pregnancy is not fully defined for the equine. The aim of this study was to establish the biochemical profile of amniotic fluid in different moments of pregnancy and at delivery, in order to explain better the peculiarities of the physiology of pregnancy in mares. It was evaluated values founded for pH, osmolality (mOsm / L), glucose (mg / dL), urea (mg / dL), creatinine (mg / dL), gamma-GT (IU / L), Sodium (mEq / L) , potassium (mEq / L), chloride (mEq / L) and total protein (mg / dL) in amniotic fluid collected from 122 mares comparing the results between the initial-third (IT - 9 samples), medium-third (MT - 28 samples) and final-third (FT - 30 samples) and at delivery (D - 55 samples). The gestational period samples are colected at slaughterhouses. Gestational moments were defined by measuring the fetal's craniocaudal distance. Were used commercial kits for kynect biochemical's evaluation (Katal/Bioclin). For estatistic evaluation were used test of Kruskal-Wallis, test of Wilcoxon and the method of Bonferroni, used to adjust p-value resultant of the multiples comparations. The pH and osmolality of equine amniotic fluid did not change significantly during the stages of pregnancy and at delivery (pH - IT: MT 7.81: 8.1 FT: 7.86 D: 8.0. Osmolality - IT: 255.5 MT: 293.56 FT: 334.63, D: 298.91). The values found for glucose were significantly lower during late pregnancy and at delivery (IT: 12.4a MT: 11.86a FT: 2.40b D: 3.91b). Values of urea tended to be statistically different in at least one of the groups (IT: 32.88 MT: 34.22 FT: 33.14 D: 36.64) and observed a pattern significant increase in creatinine values during the initial-third (0,42a), medium-third (1,31b) and the final-third of pregnancy (6.57c), and the value found at delivery remained equal to final-third (5.33b). Values for Gamma GT differed only between FT (6.20ab) and D (3.70ac) groups and more studies should be conducted about its role in the amniotic fluid of domestic species. For the sodium and chloride ions, they were not significantly differences between the stages studied, and the potassium ion was significantly different only between FT (4.04ab) and D (2.11ac) reflecting the maintenance of electrolyte balance of the amniotic fluid during equine pregnancy. Total protein concentrations were different between groups, but all the groups showed low concentrations (IT: 41.52^{a} MT: 47.02^{ab} TF: 53.56^{ac} D: 29.06^{abd}). We conclude that the values of the parameters studied were different in relation to the few studies conducted for the equine specie, demonstrating the need of more works to establish the biochemical profile of equine amniotic fluid during pregnancy and at delivery.

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Kit ligand (KL) stimulates meiosis progression and is regulated by bone morphogenetic protein 15 (BMP15) and fibroblast growth factor 10 (FGF10) in cattle

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Keywords: FGF10, kit ligand, oocyte

The oocyte and cumulus cells (CC) secrete paracrine factors that regulate cumulus-oocyte complex (COC) differentiation. Oocyte derived BMP15 and FGF10 bind to receptors in CC, which in turn secrete KL that signals to the oocyte. KL was shown to enhance nuclear maturation in rodents (Ye et al., 2009. Reprod Biol Endocrinol, v.7, p.26). The aim of this study was to determine the effects of KL on oocyte maturation in cattle and to assess the effects of BMP15, FGF10 and the interaction of on KL1 mRNA expression in bovine CC at different moments of COC IVM. Groups of 20 immature COCs (grades 1 and 2) from 3-8 mm follicles obtained from abattoir ovaries were used throughout the study. In experiment 1, immature COCs were cultured with grading doses of KL (0, 10, 50 and 100 ng/mL; n=9) for 22 hours, after which the oocytes were separated from CC and stained with Hoescht 33342 for visual assessment of the maturation stage, classified as metaphase 1 or 2. In experiment 2, imature COCs were cultured in the absence of growth factors (control) or in the presence of FGF10 (10 ng/mL), BMP15 (100 ng/mL), or FGF10 (10 ng/mL) plus BMP15 (100 ng/mL), for 4, 12 e 22 hours (n=4 per time point). Cumulus cells were then mechanically separated from oocyte, total RNA was extracted using RNeasy (Qiagen), and 100 ng of RNA was reverse-transcribed. Expression of KL1 mRNA was assessed by real time RT-PCR and normalized by Cyclophilin. Maturation data were transformed to arcsine, effects of treatments were tested by ANOVA, and means were compared with the Tukey-Kramer HSD test. KL at 50 ng/mL increased the proportion of oocytes reaching metaphase 2 (66%) regarding control (54%) while reducing the proportion of oocytes in metaphase 1 (34%) also in relation to control (46%). FGF10 alone did not affect KL1 mRNA expression, while BMP15 increased KL1 mRNA abundance at 22 hours of culture. The combination of FGF10 and BMP15 stimulated KL1 mRNA expression at 12 hours of culture, but did not further increase it in relation to the effect of BMP15 alone at 22 hours of culture. In conclusion, the present data suggest that KL stimulates meiosis progression in cattle. In addition, we propose that the effects of KL may be under the control of BMP15 and FGF10 as BMP15 was capable of stimulating KL1 mRNA expression late in culture, while it synergized with FGF10 to exert the same effect earlier.

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Dose-dependent effect of insulin-like growth factor-I on developmental competence of bovine oocytes

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Keywords: IGF-I, in vitro maturation, oocyte.

Mammalian oocyte maturation involves a well-orchestrated cascade of hormonal, autocrine and paracrine signaling. Among these signals insulin-like growth factor-I (IGF-I) has been shown to play a major role on follicular and oocyte regulation. The role of IGF-I on bovine oocyte maturation remains controversial. Therefore, the objective of the current study was to evaluate different IGF-I concentrations under standard in vitro bovine oocyte maturation conditions. Cumulus-oocyte complexes (COCs) collected from slaughterhouse ovaries were maturated in TCM-199 containing 10% (v/v) fetal calf serum, 0.5 μg/mL FSH, 7 IU/mL LH and 1 μg/mL estradiol in the presence of 0, 6.25, 12.5, 25, 50, 100 or 200 ng/ml IGF-I for 22-24 hours. Oocytes were subjected to in vitro fertilization and culture. The percentage of oocytes that cleaved and developed to the blastocyst stage was evaluated on days 3 and 7 after fertilization, respectively. This experiment was replicated 5 times using 140-163 oocytes/treatment. Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS. Addition of IGF-I to standard maturation medium increased ($p< 0.01$) the percentage of oocytes that cleaved from 56.5 + 0.02 to 70.0 + 0.02 and 68.2 + 0.02% for 0, 12.5 and 25 ng/ml IGF-I, respectively. There was also a beneficial effect of 25 ng/mL IGF-I on blastocyst development. The percentage of oocytes that developed to blastocyst stage increased ($p< 0.07$) from 18.1 + 0.03 to 27.4 + 0.03% in the presence of 0 and 25 ng/ml IGF-I, respectively. In conclusion, addition of IGF-I to standard in vitro oocyte maturation conditions exerted a beneficial effect on cleavage and blastocyst development in a dose-dependent manner. Lower IGF-I doses such as 25 ng/mL IGF-I stimulated oocyte competence.

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The influence of fat supplementation on the age of first ovulation in Nelore heifers

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Keywords: puberty, fat, Nelore

The aim of this study was to determine whether the administration of protected fat prior to weaning would anticipate first ovulation in Nelore heifers (Bos indicus). Thirty heifers were used with an average weight of 167±13 kg, sorted into 3 groups fed with or without protected fat (Megalac-E®) and a control group; fat group (FG, n=10) was fed sugar cane bagasse, 4.2 kg of concentrate and 200 g of protected fat; excess group (EG, n=10) received the same amount of sugar cane bagasse and concentrate plus 500 g of ground corn and 200 g of protected fat; and the control group (CG, n=10) received the same diet as the excess group but no protected fat. All diets were divided into two periods. After 4 months of adaptation period, treatments were administered and heifers were in average 12 months old. Ovarian ultrasound evaluation was performed 17 times in a daily basis on specific ages (10, 12, 14 and 16 months old) and spaced by 4 days between 9 and 18 and spaced by 7 days between 18 and 20 months of age. Data were analyzed with GLM procedures of SAS. During this experiment, 7 heifers became pubertal and ovulated as follow: 1 heifer in CG 20 months old (359 kg), 2 heifers in FG, 19 months and 20 months of age with 294 kg and 4 heifers in EG being 14, 18, 20, 20 months old with 246, 329, 300 and 353 kg, respectively. Heifers fed fat had lower ($p < 0.05$) number of follicles (FG= 6.94±1.56 and GE= 6.55±1.66) than the control (CG=8.12±1.68). During the 17 daily ultrasound scanning after treatments were performed, CG had greater ($p <0.05$) number of follicles (8.16 ± 1.52) compared to EG (6.52± 1.64), FG being intermediary (6.86± 1.60). In addition, fat decreased the number of follicles, but not the diameter of the largest follicle. We conclude that protected fat interferes in first ovulation in Nelore heifers.

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Evaluation of protein profile on bitches with pyometra

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Keywords: bitches, protein profile, pyometra.

The pyometra is characterized by uterine bacterial colonization associated with cystic endometrial hyperplasia, affects bitches of all ages, usually occurs during diestrus and biochemical changes as hyperproteinemia, hypoalbuminemia and hyperglobulinemia are expected. The aim of this study was to evaluate the serum protein profile of bitches with pyometra by electrophoresis in agarose gel and associate the results with the duration of hospitalization after surgery. Were evaluated 30 bitches with pyometra, which were divided into two groups according to the each patient clinical course. The group 1 (G1) consisted of bitches that were released within 48 hours after surgery and the group 2 (G2) of bitches that required hospitalization over 48 hours. Blood samples were collected on patients' admission by jugular vein puncture and placed in tubes without anticoagulant, centrifuged at 12,000 rpm / 5 min, and serum was frozen at -20 ° C until analysis. After thawing, at 5°C, concentrations of total protein was determined in an automatic equipment spectrometry (BS120® of Mindray, China) by the biuret method using commercial kit (Dialab, Austria) and one-dimensional electrophoresis was performed using a commercial kit general agarose gel (CELM) stained with Starch black 0.2% dye. The electrophoretic allowed the differentiation of five segments protein: albumin, alpha1, alpha2, beta and gamma globulin. Differences between concentrations (X±SD) were determined by t Test and values considered significant at $p \leq 0.05$. The means and standard deviations found in G1 versus G2 were: Total protein (9.95±2.0 versus 9.15±1.9 g/dL), albumin (2.45±1.16 versus 1.87±0.77 g/dL), alpha 1 (0.35±0.12 versus 0.43±0.58 g/dL), alpha 2 (1.12±0.85 versus 0.8±0.77 g/dL), beta (1.86±0.96 versus 1.45±0.65 g/dL) and gamma globulin (4.17±2.31 versus 4.6±2.31 g/dL). There were no statistically significant differences between groups. The presence of hyperglobulinemia and hyperproteinemia, due to the humoral response in bicthes with pyometra was confirmed in this study, in both groups. However, the serum protein profile could not be used as a prognostic marker of morbidity on bitches with pyometra treated surgically.

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Characterization of the L-methionine metabolic pathway in the endometrium of cows with distinct peri-ovulatory endocrine profiles

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Keywords: DNA methylation, polyamines, uterus.

In cattle, higher progesterone (P4) concentrations post-estrus are associated with higher probability of pregnancy success. The underlying molecular mechanisms of this relationship are poorly understood and may involve the polyamine synthetic machinery in the endometrium. In ruminants, the amino acid L-methionine is metabolized by enzymes with methionine adenosyltransferase (MAT) activity, generating S-adenosyl methionine (SAM). S-adenosyl methionine can be used as the substrate of two distinct cellular pathways, polyamine synthesis and DNA methylation. Previous evaluations indicated a reduction in gene expression of the SAM decarboxylase enzyme (AMD1) in the endometrium of cows with higher P4 concentrations during the first week post-estrus (HP group), in comparison to cows with lower P4 concentrations (LP group). These results suggest that under this endocrine context there is a reduction in the use of SAM as a precursor of polyamine synthesis and a concurrent increase in its use as a methyl donor during the DNA methylation process. Our aim was to evaluate whether the periovulatory endocrine environment regulates gene expression of enzymes associated with the synthesis of SAM and DNA methylation. Twenty-two cyclic and non-lactating Nelore cows received a P4-releasing device along with estradiol benzoate on day -10 (D-10). Animals were divided to receive prostaglandin analog (PGF; HP group, N=11) or not (LP group; N=11) on D-10. Progesterone devices were withdrawn and PGF injected on D-2.5 for cows of the HP group and on D-1.5 for cows of the LP group. Ovulation was induced with GnRH on D0. Plasma P4 concentrations were measured daily from D0 to D7, when endometrium was collected. Gene expression of MAT1a and MAT2b, and DNA methyltransferases DNMT1, DNMT3a and DNMT3b was assessed by qPCR and compared between groups HP and LP by student's t test. Maximum diameter of the pre-ovulatory follicle (means ± SEM; 12.8±0.4 mm vs. 11.1±0.4 mm) was larger in HP vs. LP ($p<0.01$). Rate of increase in P4 concentrations from D1 to D7 was also higher in HP vs. LP (53.6 ± 27.7-fold vs. 15.3 ± 6.4-fold, respectively; $p<0.05$). Gene expression of the transcripts MAT1a, MAT2b, DNMT1, DNMT3a and DNMT3b was not different between HP and LP ($p>0.05$). Given that higher P4 concentrations did not alter SAM synthetic pathway (MAT1a e MAT2b) but possibly reduced its availability for polyamine synthesis (reduced AMD1 gene expression), we propose that SAM was used as a donor of methyl groups for DNA methylation. We speculate that protein expression or enzymatic activity of the DNMTs may be induced in the HP group. Regulation of the DNA methylation in the endometrium may be associated with the higher fertility of cows with higher P4 concentrations post-estrus.

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Effect of hCG application in different days of estrous cycle on uterine and ovarian blood flow and uterine and cervical tone in mares

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Keywords: corpus luteum, equine, human corionic gonadotrophin.

The use of drugs during diestrus in order to improve conception rates in inseminated and recipient mares has been the focus of several research groups. Studies using hCG found promising results regarding pregnancy rates and recipient uterine characteristics on the Day of the transfer and in serum progesterone concentration. The present study aimed to evaluate the effect of hCG application in different days of the oestrous cycle on uterine and ovarian blood flow and uterine and cervical tone. Sixteen cycling mares were divided in groups of four animals each, and the treatments consisted of: G1: intravenous injection of 2500UI hCG to induce ovulation (follicle >35mm, with endometrial edema); G2: intravenous injection of 2500UI hCG on the Day of ovulation (Day 0); G3: intravenous injection of 2500UI hCG on day 5 postovulation; G4: control group, hCG was not injected at any time of the cycle. Daily exams consisted of rectal palpation to evaluate uterine and cervical tone, Color Doppler ultrasonography scoring endometrial, mesometrial, corpus luteum and ovarian pedicle vascularization, mode B ultrasonography measuring corpus luteum area and diameter and uterine morphoechogenicity from day 0 to day 15 postovulation. Parametric data were examined for normality using Kolmogorov- Smirnov test, and then transformed to natural logarithms. Non Parametric data were analyzed with Glimmix procedure for SASin order to determine main effects and interactions. When Day effect was detected or interaction was significant, differences aiming days were examined using t Student test. Results showed that ovarian pedicule vascularization in group 2 was greater than the other groups ($p<0,04$), and lower for G4 that hCG injected on day 5. Corpus luteum vascularization was numerically greater from Day 5 postovulation and statistically higher for G2 compared to G1, G3 and G4 on days 11 and 15 postovulation ($p<0,01$). Uterine tone showed a tendency of greater tone in groups that received hCG compared to control ($p<0,05$). All other evaluated characteristics only had day effect ($p<0,01$), treatment effect was not detected ($p>0,05$). The results on uterine tone with the use of hCG could have been due to an increase in progesterone concentration, offering an interesting alternative for treating embryo recipient mares. The greater corpus luteum vascularization for the group receiving hCG on day 0 can be useful because it can provide an increased supply of substrates for progesterone production and better distribution of progesterone to circulation. Therefore, using hCG in mares intending to augment uterine tone and improve corpus luteum formation seems beneficial, specially when given on the day of ovulation.

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Detection of antibodies anti-*Toxoplasma gondii* in mares in reproductive season in Rio de Janeiro

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Keywords: mares, reproductive season, *Toxoplasma gondii*.

Toxoplasma gondii is a zoonotic pathogen that causes abortion and congenital disease in several species of intermediate hosts. Felids, in particular cats, act as definitive hosts and all warm-blooded animals, including horses, may be intermediate hosts. The infectivity and pathogenicity, vary depending on the host species and strain of the parasite. Horses are considered less susceptible to the infection, but when infected it is observed association with encephalomyelitis and reproductive problems (Dubey et al, Academic press, New York. p. 1–17, 2007; Tassi P.; Parasitologia, 49:7-15, 2007; Dubey J.P, Toxoplasmosis of animals and humans -Taylors & Francis group. second edition, p. 177-178, 2010). Taking into account the economic losses caused by reproductive problems in horses and that reports of infection in mares in the State of Rio de Janeiro (RJ) are scarce in the literature, the present study aimed to detect antibodies anti-T. gondii in equine females during the breeding season. Blood was collected from 163 mixed breed mares, aged 2-18 years and in good health, for the detection of anti-T. gondii antibodies. The mares were from two farms located in the city of Cachoeiras de Macacú, State of Rio de Janeiro, which are intended for reproduction. Serological tests were performed using the Modified Agglutination Test (MAT) at a dilution of 1:25 in accordance with the protocol established by Dubey and Desmonts (Equine Vet J, 19, 337- 339,1987). Anti-T gondii antibodies was detected in 38 of 163 mares, represented 23.9%. These values were higher compared to other studies conducted in Brazil (12.1% in MinasGerais, 12.4% in Paraná and 4.4% in Rio de Janeiro) and other countries (13.1% in Argentina, 10.5% in Spain, 17.7% in Tunisia and 2.6% in South Korea) Thus, this study demonstrated that mares destined to animal reproduction in the studied city were infected with T. gondii. Parasite control programs should be made so that the fetuses are not infected by transplacental via minimizing potential losses due to abortions.

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Analysis of the PAT family proteins (perilipin, adipophilin and tip 47) presence on *in vitro* produced bovine embryos

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Keywords: immunocytochemistry, lipids, PAT proteins.

Despite advances on in vitro produced bovine embryos cryopreservation, the technique still has limitations due to the high sensitivity of the embryos to cryopreservation and the large amount of intracellular lipid. PAT family proteins (Perilipin, Adipophilin and TIP47) are found in the phospolipid monolayer on the surface of the lipid droplets of various cell types, regulating its formation and degradation. Thus, the objective of this study was to verify the presence of these proteins on in vitro bovine blastocysts. For this purpose, oocytes were obtained from a slaughterhouse and matured in vitro in TCM 199 with hormones and 10% FBS in incubator with 5% CO2 at 38.5°C for 18 hours. The mature oocytes were fertilized in vitro with semen from a single bull (Bos indicus) for 27 hours. After fertilization, presumptive zygotes were cultured in SOF medium supplemented with BSA, 10% FBS, amino acids and antibiotics for 7 days. The blastocysts obtained were fixed in 4% paraformaldehyde and groups of 36 embryos were subjected immunocytochemistry for detection of Perilipin, Adipophilin and TIP47 proteins. Embryos were permeabilized with 0.2% Tween 20 for 15 minutes and blocked with 1% BSA for 30 minutes. Embryos were incubated in a solution containing the primary antibody (1:50) overnight and then with the secondary antibody (1:100) conjugated to FITC (for Perilipin) or Texas Red (Adipophilin and TIP-47) for 1 hour at room temperature and analyzed by fluorescence microscopy. Immunodetection of Perilipin was not observed in any analyzed embryos, whereas Adipophilin was stained circumventing embryonic lipid droplets, similar to what is observed for other cell types. TIP47 protein was also detected, however it was found around small lipid droplets dispersed throughout the cytoplasm. This paper reports, for the first time, the presence of Adipophilin and TIP47 proteins on in vitro bovine embryos, thereby generating basic knowledge about the biology of intracellular lipid in pre-implantation bovine embryos, for its better understanding and, perhaps, future manipulation.

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The effects of medroxyprogesterone acetate (MAP) suplementation on pregnancy rates in reipient ewes

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Keywords: embryo, progesterone, sheep

Embryonic death during early pregnancy (to 50 days) represents a major obstacle to commercial embryo production programs in sheep. Low concentrations of progesterone have been shown to be related to subfertility and embryonic losses. We hypothesized that higher levels of circulating progesterone would be reflected in higher rates of pregnancy maintenance. The objective of this study was to determine the effect of medroxyprogesterone acetate (MAP), administered via vaginal sponges for 55 days on pregnancy rates in recipient ewes receiving embryos in a commercial embryo transfer program. A total of sixteen Dorper ewes served as embryo donors. A total of 101 crossbred Santa Inês ewes were selected as recipients. The estrus of the recipients ewes was synchronized through an intravaginal sponge impregnated with 60mg of MAP (progespon®) for a period from 11 days plus an application of 300 i.u. equine gonadotropin (eCG). 5 days after estrus from the donor the embryos were collected and transfered for the control and treatement group. Only the embryos from quality grades 1 and 2 were transfered. The pregnancy diagnoses were performed at 21 and 55 day after the embryos transfer. The Control group recipients (n= 48) received no exogenous progesterone replacement; the Treatment group (n = 53) received an intravaginal sponge impregnated with 60mg of MAP (Novormon®), which was replaced every 11 days, totaling 55 days of progestin exposure. The results of the groups were compared statistically by the chi-square test. Significant differences between the Treatment and Control Group were observed (Group 1: Recipient with MAP = 85,7%^{a} x without MAP = 55,5%^{b} Pregnancy; Group 2: Recipient with MAP = 71,4%^{c} x without MAP = 37,5%^{d} Pregnancy; Group 3: Recipient with MAP = 85,7%^{e} x without MAP = 50,0,5%^{d} Pregnancy; Group 4: Recipient with MAP = 83,3%^{g} x without MAP = 50,0,5%^{h} Pregnancy). Values in the same group with different superscripts (a-b, c-d, e-f, g-h) differ significantly; $p < 0.01$. This study demonstrated the effectiveness of hormonal supplementation via vaginal sponge impregnated with 60mg MAP, replaced every 11 days, in the first 55 days of gestation in sheep recipients. The administration of exogenous progesterone in recipients of fresh embryos increased by more than 30% the pregnancy rate. In conclusion, pregnancy rates were higher in MAP-supplemented ewes suggesting that this treatment may have reduced embryo/fetal losses.