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Brucella abortus rough strain vaccine is safe for cows at different stages of pregnancy

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Keywords: abortion, brucellosis, reproductive performance.

Field strain of *Brucella abortus*, as well as the vaccines made from portions of strain S19 lipopolysaccharides (LPS), induce the same specific antibody, which makes serological tests unable to distinguish between vaccinated and infected animals. To avoid serologic interference in diagnosis, we use a vaccine developed from a strain isolated in the 80's, whichdoes not induce agglutinating antibodies, i.e. that does not induce O-lipopolysaccharide (O-LPS) antibodies. This vaccine offers protection even in adult cows. However, some reports indicate that the rough strain is safe when inoculated in pregnant females, but only at reduced dose, which leads to potential failures of immunization programs. Thus, the aim of this study was to determine if a full dose of vaccine made from a rough strain (1.0-3.4 × 1010 colony forming units - CFU) (Brucelina Rebeccin™ – Vallée – Brazil) is safe for pregnant cows at different stages of pregnancy. The study was performed in Minas Gerais, Brazil. Ninety-six (96) crossbreed cows (Bos taurus x Bos indicus), nulliparous and multiparous, at three different pregnancy stages (initial stage: TI, middle stage: TC, and final stage: TF), were randomly divided into two groups (treated and control – T or C), leading to 6 treatments with 16 animals each. After vaccine administration, the animals were maintained together on brachialis grass pastures with mineral supplementation. Groups were compared using X2 test of the following variables: Percentage of born calves, percentage of live calves 60 days after delivery and number of seropositive cows and calves during each period, considering $P \leq 0.05$. There were no differences between treatments in TI, TC and TF or when the three gestational periods were analyzed together for the percentage of calves born alive and healthy (87.5; 87.5; 93.8; 93.8. 81.3 and 100%) or for the calves that were alive 60 days after delivery (75.0; 87.5; 87.5; 87.5. 81.3 and 93.8%) - P>0.05 for TI-C, TM-C, TF-C, TI-T, TM-T and TF-T, respectively). No animal was found to be seropositive in the usual detection tests (fast sero agglutination) at 60 days of age. It was concluded that the administration of vaccine Brucelina Rebeccin™ at any pregnancy stage is safe for cows, since it does not lead to fetal losses nor interferes with calves' feasibility after birth. Additionally, the vaccination of pregnant cows with this product does not promote the formation of antibodies, which can be detected by the usual brucellosis diagnostic tests in calves and cows.

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Effect of injectable vitamin E on incidence of retained fetal membranes and reproductive performance of dairy cows

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Keywords: dairy cow, reproduction, vitamin E.

Objectives were to evaluate the effects of injectable vitamin E supplementation during the last 3 weeks prepartum on the incidence of retained fetal membranes (RFM) and reproductive performance of dairy cows in Brazil. Eight-hundred and ninety dairy cows, 390 Holsteins (132 primiparous and 258 multiparous) and 500 crossbred Holstein x Gyr (199 primiparous and 301 multiparous) from three dairy farms in Brazil were assigned to the study from May 2012 to April 2013. In all three farms, prepartum cows were housed in pastures, and from October to March, cows had access to pasture and were managed under grazing and received 2 kg/d of a mixture of finely ground corn, soybean meal, and minerals and vitamins. From April to September, prepartum cows received a TMR composed of corn silage, finely ground corn, soybean meal, and minerals and vitamins. During the prepartum period, cows received 280 (Farm 1), 390 (Farm 2), and 480 (Farm 3) IU of vitamin E/d in the diet. Within each farm, cows were randomly assigned to remain as untreated controls or to receive three i.m. injections of 1,000 IU each of DL-α-tocopherol administered at 258 ± 3, 265 ± 3, and 272 ± 3 d of gestation (VitE). Blood was sampled from a subset of 141 cows immediately before enrollment to determine the α-tocopherol status. Blood was also analyzed for concentrations of cortisol and nonesterified fatty acids (NEFA). Statistical analysis was performed with SAS software using the GLIMMIX, MIXED, PHREG and LIFETEST procedures ($P<0.05$). The concentration of α-tocopherol in serum of cows did not differ between treatments and averaged 2.97 ± 0.10 µg/mL. A total of 53.2% of the cows had inadequate concentration of serum α-tocopherol based on the 3.0 µg/mL cut-off for adequacy. The probability of cows to have RFM decreased as the concentration of α-tocopherol in serum increased. Milk production did not differ between controls and VitE cows. Treatment with injectable α-tocopherol improved postpartum health by reducing the incidence of stillbirths, RFM, and death by 200 d postpartum. Cows receiving VitE tended to have improved pregnancy per insemination (P/AI) at first AI (30.1 vs. 36.7%) because of less pregnancy loss in the first 60 d of gestation (20.5 vs. 12.5%). Despite similar insemination rate, cows receiving VitE had 22% greater pregnancy rate than control cows, which was attributed to the improved P/AI and reduced pregnancy loss in all inseminations during the first 200 d in milk. Cows in VitE had reduced concentrations of cortisol and NEFA around calving. In summary, when cows were fed limited amounts of supplemental vitamin E, 28 to 48% of the recommendations for prepartum dry cows, supplementation with injectable α-tocopherol improved postpartum health and reproduction.

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Sexual transmission of *T. gondii* by intrauterine AI in sheep with infectted and cryopreserved semen

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Keywords: ruminants, semen, toxoplasmosis.

Reports of sexual transmission of *Toxoplasma gondii* are scarce in the literature. In sheep, transmission was confirmed experimentally by natural mating (LOPES, W.D. Vet Parasitol. v.1; 195(1-2), p.47-56, 2013). The infection of sheep by *T. gondii* by AI (artificial insemination) was also described using fresh semen infected with tachyzoites (MORAES, E.P.B.X. Animal Reproduction Science, v.122, p.36–41,2010). This study is innovative, because its goal was to evaluate the sexual transmission of *T. gondii* in sheep inseminated with tachyzoites infected semen before cryopreservation. For this, semen from a ram serologically negative for *T. gondii* by the Modified Agglutination Test (MAT) was collected and evaluated. After semen dilution, 50 doses were designed for experimental infection with $15x10^5$ RH strain tachyzoites (not cystogenic) of *T. gondii*. Another 50 doses were destined for the control group (without addition of tachyzoites). The straws were filled and submitted to the slow cooling curve of semen process. After cryopreservation, semen was evaluated by a nested-PCR reaction for SAG3 marker for detection of the parasite DNA. Besides, infectivity was assessed by bioassay in four mice. The cryopreserved semen was then used for intrauterine AI in nine sheep serologically negative for *T. gondii* (by MAT). Among them, four were inseminated with semen free from tachyzoites (control group) and five were inseminated with infected semen. After the end of the hormone treatment for estrus synchronization, two AI were performed with 100 million spermatozoa per animal. The infected semen was adjusted to a concentration of $4x10^7$ tachyzoites (MORAES, E.P.B.X. Anim Reprod Sci, v.122, p.36-41, 2010). All females were evaluated for sexual transmission (days d-14, d-7, d0, and weekly until 53 days after insemination) through MAT. A descriptive analysis of the data was used. The parasite DNA was detected in experimentally contaminated semen. Also, inoculated mice developed a response to *T. gondii* infection and died within the period of two weeks. The serum-reactivity of five female inseminated with cryopreserved semen infected with *T. gondii* was detected seven days after insemination. It was concluded that sexual transmission of toxoplasmosis via artificial insemination with experimentally infected semen occurred and that the process of sperm cryopreservation did not inhibit the infectious ability of tachyzoites of *T. gondii*.

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Manipulation of the periovulatory sex steroidal milieu affects the oviduct gene expression in Nelore cows

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Keywords: ovarian steroids, oviduct, transcriptome.

The oviduct provides the environment in which several reproductive processes occur: oocyte transport, sperm capacitation and early embryonic development. Alterations in the oviductal micro-environment can impact the zygote's developmental potential and the subsequent embryo quality. However, to which extent periovulatory sex steroidal milieu can impact the transcriptome profile of the oviductal tissue has not been fully elucidated so far. We aimed to evaluate if the ampulla and isthmus gene expression is affected by the peri-ovulatory endocrine milieu. Using a pre-defined progesterone (P4)/estradiol (E2) based protocol, Nelore cows were manipulated to ovulate small (SF-SCL; n=20) or large (LF-LCL; n=21) follicles and subsequently, luteal growth, plasma E2 and P4 concentrations differ between groups. On day 4, animals were slaughtered and ampulla and isthmus tissue were collected. After RNA extraction, transcriptome profiles of ampullary tissue (n=3/group) were determined using Illumina RNA sequencing (RNA-Seq) analysis. After mapping, approximately 80% of the reads where aligned and gene expression and splicing isoforms were quantified. Gene expression analysis revealed that 190 genes were significantly differentially expressed between groups (p= <0.05), being 114 up-regulated in the LF-LCL group and 76 up-regulated in the SF-SCL group. Gene Ontology analysis showed that LF-LCL terms were related to immune response, nucleotide binding, extracellular matrix and protein-lipid complex; whereas major terms in the SF-SCL were related to ion and cation chanel complex and Golgi apparatus. At the post-transcriptional level, sequencing mRNA allowed us to identify alternative splicing of the P4 and E2 receptors present in both groups. We used qRT-PCR to study differential expression of 20 genes (steroids receptors, heat shock proteins, cathepsin S, lipoprotein lipase and extra cellular matrix components) that were identified by RNA-seq analysis, 90% of the re-evaluated genes displayed the same expression profile compared to the RNAseq dataset (n=7/group). However, when gene expression was measured in both oviductal regions, isthmus gene expression was significantly different for some genes when comparing between groups and between regions. The novel transcriptome profile and the differentially expressed genes indicate that bovine oviductal tissue is modified by periovulatory endocrine milleu. Further studies are needed to clarify the molecular pathways involved in each oviductal region in response to optimal levels of E2 and P4 and how the association between these increases pregnancy success.

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Follicular concentration of IGF-I and insulin in the postpartum of Girolando crossbred cows

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Keywords: follicular wave, postpartum period, ultrasound.

The negative energy balance that occurs in the postpartum period of dairy cows determines endocrine and metabolic changes that affect follicular fluid composition and can impair the cow fertility. The aim of this study was to evaluate the follicular fluid concentrations of insulin and IGF-I in the postpartum of Girolando cows. The experiment was conducted at Embrapa Dairy Cattle Experimental Station, located in Coronel Pacheco, MG. Twenty-seven primiparous Girolando cows with 442.34 ± 2.45 kg of body weight and body condition score of 3.04 ± 0.01 (1-5 scale) were evaluated. Eight 3/4 Holstein x Gir (HG) and seven 7/8 HG were fed a high energy diet (1.93 Mcal/kg dry matter (DM) of net energy for lactation and 0.168 kg of crude protein/kg of DM) and seven 3/4 HG and six 7/8 HG were fed a low energy diet (1.69 Mcal/kg of DM of net energy for lactation and 0.170 kg of crude protein/kg of DM). The diets were based on corn silage, grounded corn and soybean meal. The fluid of the dominant follicle of each cow was collected at 19, 33, 47 and 61 days postpartum. The follicular wave was synchronized by the ablation of follicles greater than 6mm present in the ovaries. On the fifth day after the synchronization, the largest follicle was aspirated for fluid recovery (Vet® DP 2200, Mindray, Shenzhen, China) equipped with follicular aspiration guide and vacuum pump (WTA, Cravinhos , Brazil). The recovered fluid was centrifuged at 300g for 10min and immediately stored at - 20°C until analysis of the concentrations of insulin and IGF-I, using commercial radioimmunoassay kits (Immunotech®, Prague, Czech Republic). The concentrations of insulin and IGF-I were analyzed by ANOVA using proc GLM of SAS, considering the effects of diet, cross, days postpartum and their interactions. Insulin concentration was higher (P = 0.0575) in the follicular fluid of cows fed high energy diet (6.60 ± 1.01 µIU / mL) compared to those fed low energy diet (4.72 ± 0.54 µIU/mL). There was an interaction (P = 0.004) between diet and breed on the concentration of IGF-I. The 3/4 HG cows fed with high energy diet had higher follicular concentration of IGF-I (148.5 ± 12.5 ng/mL) than those fed low energy diet (87.24 ± 12.98 ng/mL) and cows 7/8 HG fed diets high (87.07 ± 8.05 ng/mL) or low energy (83.14 ± 8.92 ng/mL). Over the postpartum period, there were increased (P < 0.0001) concentrations of IGF-I between days 19 and 33 (75.11 ± 12.73 ng/mL, 77.44 ± 9.33 ng/mL, respectively) and days 47 and 61 (112.44 ± 13.20 ng/mL and 132.04 ± 11.67 ng/mL, respectively). The endocrine changes in follicular concentrations of insulin and IGF-I indicate that these hormones are important for follicle development and fertility in postpartum cows.

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Comparison between well of the well and polyester mesh systems to improve blastocyst yield in individual bovine embryo culture.

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Keywords: individual culture, polyester mesh, well of the well.

The aim of this study was to evaluate the effect of the volume of culture medium on bovine early embryo development on individual-shared medium systems compared with conventional group culture. Ovaries were obtained from slaughterhouse, COC´s were aspirated from 2-8 mm follicles and selected were those with more than three uniform and compact cumulus cells layers. Groups of 50 oocytes were matured in IVM and fertilized in F-CDM medium under maximum humidity with 5% CO2 at 38.5°C. Zygotes were cultured with CDM-1 medium with humidity saturation in 5% O2, 5% CO2 and 90% N2 in air at 39°C for 60 h. The embryos were cultured in CDM-1 in an atmosphere with 5% O2, 5% CO2 and 90% N2 at 39°C for 60 h. Groups of 20 embryos of 5-8 cells were subjected to one of three culture systems: well of the well (WOW) and polyester mesh (PM) for individual-shared medium, or conventional culture (CV) for control group. All systems were subjected to two treatments. In first treatment four-well dishes (Thermo Scientific) were used with 400 µl CDM-2-CSU medium with a layer of 400 µl mineral oil, whereas the second treatment was performed in Petri dishes 35 x 10 mm (Falcon) with microdroplets of 100 µL medium and covered with 5 mL mineral oil. Eight replicates were performed and statistical analysis was carried out with a binomial response model using PROC CATMOD SAS (SAS Institute Inc., Cary, NC, USA). Fixed effects of the culture system, the type of treatment and the interactions between them were analyzed. Contrasts were made between culture systems. There was a significant difference between the two treatments and between individual culture systems on shared medium. Treatment with 400 µL medium obtained a blastocyst percentage of 55 ± 2.2%, whereas that containing 100 µl 41 ± 2.2% ($P < 0.0001$). This result could be due to the mineral oil, which was in greater proportion within the microdrop. Mineral oil presents some negative effects concerning in vitro embryogenesis, as the release of cytotoxic factors and migration of lipophilic substances. Regarding the culture systems, WOW generated 53 ± 2.7% blastocyst and TP system 44 ± 2.7% ($P < 0.05$). In conclusion, the useof smaller amount of mineral oil in relation to the culture medium and concave surfaces can improve the production of individually cultured blastocysts.

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Relationship of maternal and placental characteristics with weight, height and thoracic perimeter in Mangalarga Paulista foals

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Keywords: chorioallantois, equine, neonate.

Studies indicate that some maternal characteristics play a major role in placental function, resulting in bigger and heavier foals (Wilsher, Equine Vet J, 35, 476-83). Therefore, the present study related maternal and placental characteristics with weight, height and thoracic perimeter in Mangalarga Paulista foals. Twenty deliveries were monitored in a breeding farm located in Piracaia- Sao Paulo. Maternal parameters including age, parity, gestation length, weight, height and thoracic perimeter post partum were collected; placental parameters were time to expel fetal membranes, fetal membranes total weight, allantochorion weight and umbilical cord length as well as foal's parameters: weight, height and thoracic perimeter at birth – D0, with 7 – D7 and 30 – D30 days after birth. Pearson Correlation test was used to correlate variables, considering $p<0.05$. Heavier mares (404-576 kg, mean: 490,28±52,85) had heavier foals (r=0,47/p=0,04) with greater thoracic perimeter (r=0.52/p=0.03). The mare's thoracic perimeter had positive correlation with the foal's weight and thoracic perimeter D0 (r=0.67/p=0.002; r=0.66/p=0.003) and D30 (r=0.56/p=0.04; r=0.59/p=0,04), however, at 7 days only correlation with thoracic perimeter (r=0.62/p=0.01) was observed. Older mares (4-19 years, mean: 9.53±4,54) had heavier foals at D0 (r=0.51/p=0.03), D7 (r=0.54/p=0.04) and D30 (r=0.58/p=0.03). Pluriparous mares had bigger and heavier foals at D0 (r=0.46/p=0.05; r=0.51/p=0.03), and heavier at D7 (r=0.54/p=0.04). Gestation length (317-404 days, mean: 333±20.85) was negatively correlated with foal's weight at D0 (r=-0.48/p=0.04) and at D7 (r=-0.54/p=0.04). Mares that had heavier fetal membranes (3.3-5.9 kg, mean: 4.71±0.75) showed positive correlation with foal's weight (r=0.54/p=0.03) and thoracic perimeter (r=0.69/p=0.006) at D0. Such mares took less time to expel fetal membranes (r=-0.53/p=0.05). Foals that were born bigger and heavier maintained this relationship at seventh days after birth (r=0.83/p=0.0002; r=0.86/p=<0.0001). Our results indicate that heavier mares, older mares, pluriparous and heavier fetal membranes have strong influence in the neonate's physical characteristics, mainly at birth. These findings suggested that these maternal characteristics provide most nutritional support for fetal development.

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Profile of H3K27 epigenetic marks in porcine embryos during in vitro development

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Keywords: epigenetics, histone, methylation.

Changes in epigenetic marks on the lysine 27 of histone H3 (H3K27) appear to play a key role in lineage determination of mammalian embryos. Genes associated to organogenesis, morphogenesis and embryonic development are temporarily suppressed by trimethylation of H3K27 (H3K27me3) in pluripotent cells. However, the complete profile of epigenetic marks in the H3K27 during early stages of porcine embryo development is not entirely known. The aim of this study was to evaluate the acetylation (H3K27 ac) and methylation (mono-, di- and trimethyl; H3K27 me1, me2 and me3) profile of H3K27 during early development of in vitro-produced (IVP) porcine embryos. Oocytes/embryos (approximately 6 for each mark and developmental stages) were fixed at the following developmental stages: germinal vesicle oocytes (GV), metaphase II oocytes (MII), embryos at pronuclear stage (PN), two cells (2C), four cells (4C), eight cells (8C), D6 blastocysts (D6), and D8 blastocysts (D8). Oocytes/embryos were subjected to an immunofluorescence protocol using rabbit anti-H3K27 ac and anti-H3K27 me1, me2 and me3 primary antibodies (1:1000), and goat anti-rabbit Alexa Flour 488 as a secondary antibody (1:1000). Nuclei were stained with DAPI (10 µg/mL). The fluorescent signal for H3K27 ac was intensive in all developmental stages. H3K27 me1 signal was strong in GV, MII and in only one pronucleus, but the fluorescent signal was weak in 2C, 4C and 8C stage embryos. In D6 and D8 embryos, the H3K27 me1 signal became strong again. The signal for the H3K27 me2 mark was strong in GV and MII stage oocytes, but absent in PN. In embryos at 2C, 4C and 8C stages, the H3K27 me2 signal was very weak or absent, but it was detected again in D6 and D8 embryos. H3K27 me3 was intensely detected in GV, MII and in only one pronucleus. In 2C, 4C, 8C and D6 embryos, the H3K27 me3 signal was very weak or absent. Additionally, 29 D8 blastocysts were subjected to immunofluorescence for detection of H3K27 me3. Of these, 7 did not present any sign of fluorescence, 19 had some or all cells marked and 3 showed H3K27 me3 only in the inner cell mass. The results of this study indicate that the epigenetic marks on the H3K27 are highly dynamic during porcine embryogenesis, which suggests that H3K27 plays an important role in the regulation of gene expression during early embryo development.

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PAR6C polarity protein expression pattern in male and female bovine embryos

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Keywords: female bovine embryos, PAR6C, polarity.

Despite the unique importance of blastomere polarization during preimplantation development, little is known about this phenomenon in bovine embryos. Polarization transforms symmetrical spherical blastomeres into highly polarized radial cells, and once established, polarity domains distinguish embryonic inner and outer cells. Cells exhibiting outer polarized domains cannot depolarize, and will contribute to trophectoderm lineage. Bovine embryos develop slower than mouse embryos, and at lower rates. Also, gender influences some characteristics in bovine embryos, including cell numbers and apoptosis. Therefore, the aim of this study was to characterize PAR6c, a polarity marker well established in mouse embryos, in bovine embryos, and investigate if its expression is similar in male and female counterparts. In experiment 1, bovine embryos (n= 52) were produced by IVF and embryo culture at standard conditions, in two replicates. Samples were collected at several stages of development (zygote, 2-, 4-, 8-, 16- morula and blastocyst), and immunofluorescence (IF) for PAR6c protein was performed. In experiment 2, male and female embryos (n=192) produced in three replicates using sexed semen from the same bull were cultured separately, and morphological embryo development assessment was performed daily from 24-144 h.p.i. Developmental rates were compared between groups at each evaluated time point using Chi-Square Test. In experiment 3, male and female embryos were collected (n=46) at critical stages (late 8; early 16; mid 16; late 16; morula) for PAR6c IF. Mouse embryos were used as positive controls for all PAR6c IF reactions. Negative control reactions were also performed. We observed PAR6c consistent apical expression from 16-cell embryos onwards; although about half embryos (9/17) exhibited very low expression already at 8-cell stage. Before 8-cell stage, no expression was detected. In experiment 2, we observed a difference in 8 to 16-cell embryo percentage between male and female embryos at 48h.p.i. (M=20%; F=34%*) and 72h.p.i (M=64%; F=43%*). In experiment 3, we observed that PAR6c expression and stage of compaction were similar in both groups, and were first evidently detected at early 16-cell stage. At late 8-cell stage, 60% male embryos and 80% female embryos had at least one blastomere expressing PAR6c, but at very low levels. We concluded that PAR6c asymmetric protein expression seems to be consistently established at 16-cell stage, one cell cycle after occurs in mouse embryos. This result suggests that cell fate decisions in bovine could also be started before blastocyst stage. We found that 48-72 h.p.i. is a critical stage for male and female embryos comparative analysis in our system. However, at this stage male and female embryos display similar pattern of PAR6c polarization and compaction, suggesting those events are not related to phenotypic differences previously reported between those groups.

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The influence of GDF-9 gene and season on the follicular dynamics in Santa Inês sheep

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Keywords: follicular dynamics, prolificacy, ultrasonography.

This study aimed to characterize follicular dynamics in Santa Inês sheep under the influence of different genotypes of the GDF-9 gene: homozygous mutant (EE), heterozygous (EW) and wild homozygous (WW), for FecGE mutation. The follicular development and the luteal phase during the estrous cycle were evaluated. In addition, two different periods of the year in Federal District were also evaluated. Santa Inês ewes (n = 26), WW (n=10), EW (n=11) and EE (n=5) were synchronized with two PGF2α injections 7 days apart. At 48 hours after the second dose, ultrasound (US-MyLab™30Gold, Esaote 6-8 MHz) evaluation has started, daily from the first ovulation until the next one (approximately 17 days between ovulations). The second ovulation was confirmed later by detection of corpus luteum (CL), seven days after the disappearance of the greatest follicle. A total of 30 and 40 complete cycles was evaluated in periods of drought and rainfall, respectively. Data were analyzed by Generalized Linear Models. The duration of the estrous cycle was greater ($P<0.05$) in the dry season, averaging 17.27 ± 0.79 versus 16.78 ± 0.98 days in the rainy season. The follicular phase was longer during the dry period 7.41 ± 1.35 ($P<0.05$). Sheep had a pattern of 3 and 4 follicular waves. Ovulation rate was higher in the rainy season - 1.83 ± 0.36 ($P<0.05$) compared to 1.43 ± 0.52 in the dry one. It was also observed that sheep EE recessive genotype had a higher frequency of ovulation compared to other genotypes 2.00 ± 0.33, 1.85 ± 0.26 and 1.69 ± 0.43 ($P<0.05$), respectively. The observations revealed that there was an increased ovulation rate ($P<0.5$) in the left ovary ($P<0.05$). The total volume of CLs from a single ovulation (884.74 mm^3) was greater ($P<0.05$), compared to CL of multiple ovulation ewes (710.06 mm^3). We conclude that the homozygous mutant animals (EE) must be more prolific than others (EW and WW). Another conclusion was that during the rainy season, characterized by increased forage supply, the frequency of observations of multiple ovulations is higher than in the dry season. Moreover, it was observed that the diameter of the ovulatory follicle and the total volume of CL are inversely proportional to the number of ovulations per estrous cycle.

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Raman spectroscopy is a rapid and nondestructive method for evaluation of culture media of in vitro produced bovine embryos

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UFABC.

Keywords: embryo, kinetic, raman spectroscopy.

Several technologies have been explored to evidence metabolic differences among embryos to identifying those most able to generate pregnancy. Thus, spectroscopy techniques allow a rapid and nondestructive analysis of various types of biological samples, including culture media. The objective of this study was to determine, by Raman spectroscopy, molecular biomarkers in culture media of in vitro produced bovine embryos that may be related to embryo kinetics. In addition, to identify spectroscopic profiles of these embryos, allowing a rapid and nondestructive characterization of embryo diversity, contributing to the improvement of in vitro production of bovine embryos. For that, bovine embryos were produced in vitro by conventional protocols. The cumulus-oocyte complexes were aspirated from slaughterhouse ovaries, selected (grade 1 and 2), placed in 90 µL IVM drops (M-199 + HEPES; 20 oocytes/drop) and cultured for 22 hours at 38.5°C and 5% CO2. The matured oocytes were in vitro fertilized in 90 µL droplets of IVF medium (Parrish et al., 1988 Biol. Reprod. 38th, 1171-1180) in an atmosphere of 5% CO2 in air at 38.5°C and high humidity for 18 hours. Subsequently, the zygotes were cultured individually in an incubator with atmosphere with 5% CO2 in air and high humidity at 38.5°C for 7 days. The embryos were classified in fast (4 cells 40 hpi) and slow (2 cells 40 hpi) and the culture media were collected at cleavage, morulae and blastocyst stages. The culture media were transferred to cryovials and remained frozen at -80°C until analysis. For Raman spectroscopy analysis, drops of culture media covered with mineral oil were scanned by a T64000 triple Raman system from Horiba Jobin Yvon SAS, with CCD detector 1024x256. The data were normalized (Fityk software) and analyzed for Principal Component (PCA) and clustering (Minitab 16). At cleavage, morulae and blastocyst stages it was possible to evidence different spectra profiles, characteristics for fast and slow embryos. Based on our results, we can conclude that RAMAN spectroscopy analysis of culture media is a powerful tool to characterize different spectroscopic profiles of IVP bovine embryos.

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Analysis of embryotoxic effects of nanocapsules used in the in vitro maturation of bovine oocytes

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Keywords: embryotoxicity, in vitro maturation, nanocapsules.

Nanocapsules (NC) and liposomes highlight among the possible uses of nanotechnology in controlled release systems.These systems of carrying and releasing drugs have advantages like the ability to gradually release the substances that were adsorbed or incorporated in the lipid or polymer-particle. Hence, the combination of nanotechnology into the context of reproductive biotechnologies allows for potential innovative attempts to improve production systems. The objective of this study was to evaluate the embryotoxicity of NC (the vehicle without any incorporation) when added to IVM medium. Cumulus cell expansion after maturation and embryo development were evaluated morphologically. Cumulus-oocyte complexes (COCs) from follicles between 3 and 8 mm were obtained from bovine ovaries from abattoir. Only good quality COCs (grade I and II) were selected for the experiment. For IVM, 10 to 20 COCs were washed in HEPES TCM199 and subsequently washed 6 times in drops containing the specific media for each group. Six groups (G1, G2, G3, G4, G5 and G6; 5 replicates, n=76 oocytes/group) were defined: G1: negative control [1mL TCM199 bicarbonate, 5µL amikacin (16.67 mg/µL), 2µL pyruvate (0.011 g/mL)], G2: experimental control [5 mL of TCM199 bicarbonate, 0.030 g of BSA, 5µL of FSH (0.1 mg/mL), 25µL amikacin (16.67 mg/µL), 10µL pyruvate (0.011 g/mL)], G3: laboratory control [0.9 mL of G2 media, 100µL FCS,10µLLH (50 µg/mL) and 1µL E2] and G4 to G6 groups containing 0.9 mLof G2 media plus 10% (approximately 0.1 g), 1% and 0.1% v/v of NC, respectively for G4, G5 and G6. The NC were produced by the coacervation method, containing a mix of grape seed oil, propylene glycol, isopropyl myristate and Tween 20 added to the aqueous phase containing atelocollagenand xanthan gum. NC were produced without the active compounds and sonicated (to decrease the size to manometric level). There was a clear difference in cumulus cells morphology (expansion) observed after IVM. In G1 no expansion was observed, while cumulus cells from G2 and G3 expanded as expected. In G4 (10% NC) only partial expansion was observed while in G5 and G6 the expansion was similar to G2 and G3. The percentage of cleaved embryos (D3) was similar to G1, G2 and G3 (58.8, 72.1 and 74.7% respectively, $P > 0.1$), but different between G4, G5 and G6 (32.1, 59.3 and 69.5% respectively, $P < 0.001$). The production of morula and/or blastocysts (D7) was evaluated and although the G4 (5.1% compared to presumptive zygotes) and G4 and G6 (respectively 16.7 and 14.8% compared to cleaved) have produced smaller percentages of embryos, there was no difference between groups ($P > 0.1$).

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Effect of diet energy on the relationship of follicular fluid estrogen and progesterone concentration in postpartum crossbred Holstein x Gir cows

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Keywords: ovum pick-up, steroidogenesis, ultrasound.

The reduction in reproductive performance in postpartum dairy cows is associated with negative energy balance, as well as changes in the composition of follicular fluid. This study aimed to evaluate the composition of follicular fluid in the postpartum of crossbred Girolando dairy cows fed high or low energy diets. The experiment was conducted at Embrapa Dairy Cattle Experimental Station, located in Coronel Pacheco, MG. Twenty-seven primiparous Girolando cows with 442.34 ± 2.45kg of body weight and 3.04 ± 0.01 of body condition score (1-5 scale) were evaluated. Eight 3/4 Holstein x Gir (HG) and seven 7/8 HG were fed a high energy diet (1.93 Mcal/kg of dry matter (DM) of net energy for lactation and 0.168 kg of crude protein/kg of DM) and seven 3/4 HG and six 7/8 HG were fed a low energy diet (1.69 Mcal/kg of dry matter (DM) of net energy for lactation and 0.170 kg of crude protein/kg of DM). The diets were based on corn silage, grounded corn and soybean meal. The fluid of the dominant follicle of each cow was collected at 19, 33, 47 and 61 days postpartum. The follicular wave was synchronized by the ablation of follicles greater than 6 mm present in the ovaries. On the fifth day after the synchronization, the follicle diameter was measured and the aspiration of the dominant follicle fluid was proceeded using ultrasound (Vet® DP 2200, Mindray, Shenzhen, China) equipped with follicular aspiration guide and vacuum pump (WTA, Cravinhos, Brazil). The recovered fluid was centrifuged at 300 g for 10 min and immediately stored at -20°C until analysis of the concentrations of estrogen (E) and progesterone (P), using commercial radioimmunoassay kits (Immunotech®, Prague, Czech Republic). Data from follicular diameter on the fifth day after the synchronization of the wave, the concentrations of estrogen and progesterone and the E:P ratio were analyzed by ANOVA using proc GLM of SAS, considering the effects of diet, cross, days postpartum and their interactions. Cows fed high energy diet showed a smaller (P = 0.0204) dominant follicle (10.44 ± 0.32 mm) than cows fed low energy diet (11.34 ± 0.29 mm). There was effect of the cross for follicular estrogen concentration (P = 0.0216) and the E:P (P = 0.0035). The 3/4 HG cows showed higher concentration of estrogen (267.58 ± 14.38 ng/mL) and higher E:P ratio (5.99 ± 0.47) than cows 7/8 HG (223.49 ± 13.99 ng/mL and 4.16 ± 0.27 , respectively). There was interaction between diet and cross (P = 0.0151) in the concentration of progesterone. The 7/8 HG cows fed low energy diet had higher concentration of progesterone (75.32 ± 7.98 ng/mL) than those fed high energy diet (49.02 ± 4.87 ng/mL) and those 3/4 HG fed low and high energy (48.63 ± 4.94 and 50.98 ± 4.15 ng/mL, respectively). Differences in follicular concentrations of estrogen and progesterone and the E:P may be associated with differences in negative energy balance between evaluated crosses and diets.

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Kisspeptin stimulates LH release in buffalo cows in the breeding and nonbreeding season

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Keywords: fertility, kisspeptin, ovulation.

This study aimed to evaluate the kisspeptin (Kp) capacity to induce release of LH as well as the curve profile in breeding (EF) and nonbreeding (ENF) season in buffalo cows. In a 2x2 factorial with two replicates (15 days apart) and crossover where cows did not receive the same treatment more than once between replicates, six ovariectomized buffalos were allotted in two groups: KP (n = 6) – i.m. administration of Kp diluted in NaCl 0.9% solution (10 µg Kp/Kg ~ 4mL); GnRH (n = 6) –10 µg of buserelin acetate (Sincroforte®, Ourofino, Brazil). Blood was sampled (8mL) at -20, 0 (trat), 20, 40, 60, 80, 100, 120, 140, 160, 180, 210 e 270 min. Tubes were immediately placed in an iced cooler for posterior centrifuging (1500 x G/20 min, 4ºC). Plasma was aliquoted and frozen at -20ºC until determination of LH and P4 concentration by RIA. The intra and inter-assay variation coefficient for LH and P4 was respectively 8.7 and 13.0%; 4,3 and 4.6%. The LH profile was analyzed by GLIMMIX proc for repeated measures (SAS v. 9.2). The area under the curve (AUC), beginning (IP), moment (MP), final (FP) and amplitude of the LH peak were calculated by GraphPad Prism 5.0 software and analyzed by GLIMMIX proc in SAS. Data are presented by mean ± SEM and significance was considered when $P<0.05$. The P4 concentration in the females at the moment of the treatments was basal (0.26 ± 0.05 ng/mL), showing the ovariectomy efficiency and not affecting the results by this steroid. Animals presented LH release under positive control (GnRH) stimulus, validating experimental model. There was neither breeding season effect on LH release (P = 0.3827) nor breeding season and treatment interaction (P = 0.1284). Thus, the Kp response is independent of the season of the year. However, the profile of LH curve was different between treatments over time (P = 0.0071). In this way, as there was not treatment and time interaction it was not possible to calculate the mean of LH released by group. AUC was not influenced by season (P = 0.5341); however, the treatment type define the amount of LH released expressed by AUC (KP = 405.42 ± 88.43 ng2; GnRH = 1173.69 ± 206.72 ng2; P = 0.0047). Kp anticipates the IP (3.43 ± 0.59 min vs. 11.47 ± 8.46 min; P = 0.0497) and MP (20.00 ± 0.00 min vs. 118.89 ± 19.32 min; P = 0.0001) compared with GnRH. However, Kp during ENF induces a short LH peak observed by FP (P = 0.0436), which may be due to the blockade of GnRH containing neurons by low melatonin levels. The amplitude of LH peak is higher in GnRH (11.72 ± 1.73 ng/mL) than in KP (7.89 ± 0.70; P = 0.0132). The buserelin acetate is a potent analogue of GnRH and worked in this experiment only as positive control. This study, presents that i.m. Kp administration is efficient to induce LH release in buffalo cows not only in the breeding season but also in nonbreeding.

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Inflammatory response and uterine hemodynamics of mares inseminated with frozen semen of different qualities

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Keywords: Doppler, endometrial cytology, fluorescent probes.

The aim of the present study was to examine the uterine hemodynamics of mares, through color and spectral Doppler ultrasonography and compare it with endometrial cytology after AI with frozen semen. Eight ejaculates from one stallion were used. Semen was collected, analyzed and cryopreserved. Two frozen semen straws of each batch were thawed and analyzed for motility by the Computer-Assisted Sperm Analysis (CASA), and integrity of plasma and acrosomal membrane and mitochondrial potential by the association of fluorescent probes PI-H342, FITC-PSA and JC-1 (CELEGHINI et al., Braz Arch Biol Tech, v.53, p.1285, 2010) defining this way the percentage of intact plasma membrane, intact acrosome and high mitochondrial cells (IPIAH). Fifteen mares were used and had their estrous cycles randomly distributed in four groups: group C (control): no insemination, only mimic of the technique of AI (n=7); group D: intrauterine infusion of skim milk based extender (n=7); group A: inseminated with High quality semen (30% sptz IPIAH, total of 300 x 10^6sptz IPIAH /dose of 1 x 10^9 sptz) (n=7) and Group B: inseminated with Low quality semen (15% sptz IPIAH, total of 150 x 10^6sptz IPIAH /dose of 1 x 10^9 sptz) (n=7). For group B, the cells were damaged by flash frozen. Ovarian follicular dynamics were monitored daily by transrectal palpation and ultrasound examination. Ovulation was induced using hCG (2500IU) IV and 30 hours later the mares were examined every 6 hours until ovulation, for post-ovulation AI. Uterine hemodynamics was analyzed through Doppler ultrasonography by Spectral and Color-flow modes on seven moments: immediately before hCG, immediately before AI, 2, 6, 12, 24 and 48 hours after AI. The numerical values of resistance index (RI, 0-1) of uterine arteries and vascularity scores (VS, 1-4) of uterine horns were considered (SILVA et al, Biol Reprod, v.72, p.755, 2005). Endometrial cytology was done 6 hours after AI, using cytobrush. The slides were prepared and stained using Panotic Stain Method. The percentage of polymorphs was determined by the count of 300 cells/slide, by microscopy (400x magnification). The data was analyzed for treatment and time effect, and interaction treatment x time, by SAS PROC MIXED (Version 9.3). No difference ($p>0.05$) was found between the groups at any moment for RI an VS values. For cytology, group B (76.41±5.89%) presented a more intense inflammatory process ($p<0.05$) when compared to group D (20.12±6.57%), and both did not differ ($p>0.05$) from group C (35.58±6.36%) and A (47.87±10.59%). The highest percentage of IPIAH spermatic cells deposited in the reproductive tract of the mare results in an increase of inflammatory cells, caused by the inflammatory response to semen, but it does not affect uterine blood flow.

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Use of constant doses of PGF2α during the periovulatory period in buffaloes submitted to the superovulatory protocol

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Keywords: embryo structures, osmotic pump, prostaglandin.

The low embryo recovery rate reported in buffaloes may be related to the failure of oocytes to enter the oviduct after superstimulation of follicular growth. In rabbits, the administration of subsequent doses of PGF2α during the periovulatory period stimulates contraction of oviduct smooth muscles, allowing the activation of the oviduct fimbriae to capture the oocytes. Based on these observations, the hypothesis was that constant doses of PGF2α (provided by intra-muscular administration or slow release via osmotic pump) during the periovulatory period, increase the embryo recovery rate of buffaloes submitted to the superovulation protocol. In order to verify this hypothesis, on a random day of the estrous cycle (D0, pm), 66 buffaloes were randomly divided into three groups: Control Group (GCont, n=22), PGF injection Group (GPGFinj, n=22) and Osmotic Pump Group (GPGFpu, n=22) in a cross-over experimental design. Follicular wave emergence was synchronized with an intravaginal progesterone device (P4, Sincrogest®, OuroFino, Brazil) and an injection of 2mg im of Estradiol Benzoate (Sincrodiol®, OuroFino, Brazil). From D4, all buffaloes received 200mg im of FSH (Foltropin-V®, Bioniche, Canada) twice-daily, in 8 applications of decreasing doses. A dose of 0.53mg im of PGF2α (Sincrocio®, OuroFino, Brazil) was given on D6 (pm) and D7 (am), and the P4 device was removed on D7 (pm). On D8 (pm), all buffaloes received 20μg im of GnRH (Sincroforte®, OuroFino, Brazil) and 12 and 24 h after were performed the artificial inseminations. The buffaloes in GPGFinj received four extra doses of PGF2α (2.12mg, Sincrocio®, OuroFino, Brazil) from D8 (pm) to D10 (am), while animals of GPGFpu received at D8 (pm) subcutaneous administration of a mini-osmotic pump (Alzet Osmotic Pumps, ALZA Corporation, California, USA) containing 2.12mg of PGF2α (Sincrocio®, OuroFino, Brazil), which was removed in D10 (pm). All animals were subjected to ultrasonographic evaluations (Mindray DP2200Vet, China) performed on D0 to verify ovarian activity, on D8 to assess the superstimulatory response and on D14 for accounting of ovulations. Embryonic structures were collected by nonsurgical method in D14 (pm). Statistical analysis was performed by the GLIMMIX procedure of SAS®. There was no difference between experimental groups (GCont vs. GPGFinj vs. GPGFpu, $P>0.05$) for the following variables: follicles ≥ 8mm at D8 (15.3±3.1 vs. 13.7±2.8 vs. 13.6±2.3) and D14 (4.7±1.4 vs. 3.4±0 7 vs. 4.6±1.1) and the embryonic structures recovery rate (20.3 vs. 21.1 vs. 16.6 %). However, differences were found in ovulation rates (67.8 vs. 71.2 vs. 61.6 %, $P=0.04$). It is concluded that constant doses of PGF2α during the periovulatory period do not increase the embryonic structures recovery rate of buffaloes submitted to the superovulatory protocol.

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Optmization of in vitro development of mouse embryos using mesenchymal stem cells as feeder layers

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Keywords: co-culture, embryonic development, mesenchymal stem-cells.

Despite the advances in assisted reproduction techniques, the poor quality and failures in in vitro embryo development remain as a drawback resulting in low pregnancy rate. Bone marrow mesenchymal cells (MSCs) have emerged as a novel therapeutic option due to their unique properties of releasing bioactive factors and supporting others cell growth. In addition, murine embryonic fibroblasts (MEFs) have been widely used as a feeder layer to support embryonic stem cells due to their release of growth factors. In the present study we have compared the role of MSCs and MEFs in supporting C57Black6 mouse early embryo development. MSCs and MEFs were isolated from mice and cultured in DMEM-F12 with 10% fetal bovine serum up to the third passage. All the embryos were obtained in approximately 42 hours (2nd day) after mating and were randomly distributed in the following groups: CTRL - cultured in control culture medium; iMSC - co-cultured with MSCs inactivated to arrest proliferation; and iMEF - co-cultured with MEFs inactivated to arrest proliferation. Inactivation was performed using mitomycin C. Embryo development was evaluated daily for 5 days (7th day after mating). Immunocitochemistry, diameter and total cell number of blastocysts were measured at the 3rd day after culture. The statistical analysis was performed by non-parametric Kruskal-Wallis test with Dunns post-test and $p<0.05$ was considered as statistically significant. We observed at 2nd day after mating (day of embryo acquisition) the proportion of following development stages: 87.0% at 2-cell, 6.5% at 3-cell, 3.7% at 4-cell and 2.8% at 5-8-cell. After the 3rd day in culture, the embryos co-cultured with iMSC or iMEF showed a greater development when compared with the CTRL group. On the 5th day in culture the rate of hatched blastocysts in iMSC (84.1±5.8%) and iMEF (90.3±4.2%) groups were higher than CTRL group (49.2±8.8%). We did not observe any difference in the proliferation or apoptosis among the groups, however, the blastocysts co-cultured with iMSC presented a significant higher number of inner cell mass (26.1±1.6%) and a lower number of trophoblast cells (73.9±1.6%) when compared to the CTRL group (20.4±1.5% and 79.6±1.5%, respectively). The iMSC and iMEF groups presented a higher cell number (70.9±2.5 and 74.5±2.7, respectively) and diameter (133.7±2.53 and 139±2.3 μm, respectively) when compared to the CTRL group (cell number: 60.3±2.14; and diameter: 123.8±2 μm). In summary, our data suggests that co-culture with inactivated MSCs or MEFs greatly supports and improves the early embryonic development in vitro.

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Methylation pattern of IGF2 gene in trophoblast cells from d14 bovine embryos: effect of in vitro culture

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Keywords: DNA, epigenetic, mutilation.

In mammals, a correct DNA methylation reprogramming and the maintenance of genomic imprinting after fertilization are essential for embryo development and pregnancy. One important imprinted gene, related with embryo development and placentation, is the IGF2 gene. Many studies have related changes in the IGF2 methylation pattern with assisted reproductive technology. The objective of this study was to evaluate the influence of in vitro culture until day (D) 7 of development on methylation pattern of the differentially methylated regions (DMR) located into the exon 10 of the IGF2 gene, of bovine embryos on day D14 of the development. In vitro embryos were produced from oocytes obtained by follicular aspiration of slaughterhouse ovaries. On D 7 after in vitro fertilization only grade I blastocysts were selected and, in number of 10, were transferred non-surgically to the uteruses of recipients previously synchronized (VT/VV group). As a control, in vivo produced D7 embryos (VV/VV) at the same stage and quality of the VT/VV group were also transferred to synchronized recipient. All groups were collected on Day 14 and a portion of each trophoblast layer was stored for sex determination, and another portion was used to determine the methylation status of the IGF2 gene. After sex determination only genomic DNA from male embryos was used for DNA methylation using bisulfite sequencing. The methylation pattern was performed individually, and each embryo (VT/VV=4 and VV/VV=5) was considered as a replicate. At least 8 clones were evaluated per embryo and the sequences were analyzed using BiQAnalyser software having the Genbank sequence NM_174087.3 as reference. The methylation pattern of the different groups was compared using Kruskal-Wallis test ($P<0.05$). No differences in DNA methylation were found between VV/VV (28.3±3.2%, n=29 clones) and VT/VV (22.7±2.9%, n=27 clones). As already known the region studied is hypermethylated in sperm and hypomethylated in oocytes, and in somatic cell types it is expected to be around 50% methylated, considering an imprinted region. Although we found a lower percentage of methylation than that expected for an imprinted region, this pattern may be the physiological pattern for trophoblast cells, being similar for both the in vitro and the in vivo produced embryos. This is the first report describing the methylation pattern of this region of the IGF2 gene on Day 14 bovine embryos.It can be concluded that in vitro production and culture of embryos did not affect their posterior methylation status of the intragenic DMR on exon 10 of IGF2 gene, when evaluated on Day 14 of development.

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Diameter of the preovulatory follicle of the mare

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Keywords: follicular diameter, mares, ovulation.

The mares ovulate follicles between 40-45 mm in diameter, dependingon breed, size, and nutritional status of the mare as well as breeding season. Thus, we aimed to identify the average diameter of the preovulatory follicle of mares belonging to genetic group "Baixadeiro". Three mares raised at the Unit for Research and Conservation of Baixadeiro Horse, located in the Farm School São Bento were used - MA (UEMA). They were evaluated for body condition score (BCS) and monitored daily by rectal palpation and ultrasonography using the equipment VET CHISON 600®.When a follicle of 35 mm was identified, its diameters were evaluated daily until ovulation. The mean BCS found was 4.0, which, according to Henneke (Equine Veterinary Journal, v.15, p.371-372, 1983) indicates moderate thinness and may influence reproductive activity according to this author. Gastal et al. (Biology of Reproduction, v.62, p.222, 2000) claim that mares with low BCS ovulate smaller follicules compared with mares in better body condition. Nevertheless, mares showed the following individual mean diameter of the preovulatory follicle: 38.12 ± 2.44; 40.23 ± 3.93; 41.10 ± 4.96. The overall mean was 39.88 ± 3.98, which corresponds to the expected values for the species. According to Murdoch et al. (Biology of Reproduction, v.35, p.1187-94, 1986) preovulatory follicle can reach a diameter of ± 40 mm; Blanchard (Manual of equine reproduction Missouri: Mosby 1998) claims that this value can reach between 40 - 45 mm; Ginther et al. (Theriogenology, v.69, p.583-590, 2008) found that the pre-ovulatory follicle has a diameter of 41 mm reaching a plateau before ovulation. It can be concluded that the mare's genetic group "Baixadeiro" showed mean values of follicular diameter in the range expected for the equine species.

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Vascular perfusion and progesterone concentrations in mares treated with dinoprost trometamine on corpus luteum luteogenesis and maintenance

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Keywords: corpus luteum, luteolysis, vascular perfusion.

The aim of the present work is to evaluate the administration effect of Dinoprost trometamine (natural PGF2α; Lutalyse®, Pfizer Animal Health, Guarulhos, SP) over progesterone plasmatic concentrations and vascular perfusion of the corpus luteum (CL), in two different moments of the CL development: D2 (luteogenic phase; D0 = ovulation) and D8 (CL maintenance phase). Mares were randomly assigned into four experimental groups. During luteogenesis (D2-Saline group, n=6) or maintenance (D8-Saline group, n=7), as control groups, 2mL of saline solution i.m. were administered. On treated groups, 10mg (2mL) of PGF2α i.m. were administered during luteogenesis (D2-PGF group, n=9) or maintenance (D8-PGF group, n=7). Data collection was performed immediately before treatment (H0) and continued every 6h until 48h after treatment on both phases. Vascular subjective (PVS) or objective (PVO) perfusions were measured by color Doppler ultrassonography as described by Silva et al. (Biology of Reproduction, v.72, p.755-76, 2005). For the CL functionality verification, blood samples were collected at the same moments from which plasmatic progesterone (P4) was measured by radioimmuno assay. Luteolysis was considered when P4 < 1ng/mL. Models for repeated measures were used to compare mean P4, PVS and PVO. Tukey's method was used to adjust the values of P resulting from multiple comparisons. Simple linear regression models were used to assess associations (PVS x PVO; P4 x PVS, P4 x PVO). It was observed that mares treated during luteogenesis showed a slightly decrease in P4 and CL vascular perfusions (PVS and PVO) when compared to control ($P>0.05$) which indicates insignificant effect of the luteolytic at this stage. At D8, a reduction of P4 from H18 when compared to control ($P<0.05$), and luteolysis at H30 were detected. A reduction of Cl vascularitywas detected from H24, and PVS showed to be more sensible to detect the decrease in vascular perfusion and strong correlation with P4 ($p=0.001$ and $r=0.71$), which indicates that Doppler ultrasonography is a rapid and practical technique for CL functionality evaluation. The use of PGF2α during luteogenesis did not influence CL vascular perfusion and P4, however, the slight reduction on the treated mares indicate the existence of a small percentage of luteinic cells responsive to the luteolytic.

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Pharmacological inhibition of H3K27 trimethylation in bovine IVP embryos

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Keywords: DZNEP, epigenetics, histone.

Genes associated to organogenesis, morphogenesis and embryonic development are temporarily suppressed in pluripotent cells of mammalian embryos by the trimethylation of lysine 27 of histone H3 (H3K27 me3). H3K27 methylation is catalyzed by enzymes of the Polycomb Repressive Complex 2 (PRC2); however, this process is not entirely known. The AdoHcy hydrolase inhibitor 3-Deazaneplanocin A (DZNep) can prevent the action of the PRC2 enzymes, therefore inhibiting H3K27 me3. Nevertheless, there are no reports of the action of DZNep in embryos. The aim of this study was to evaluate the blastocyst rate and the cell number of bovine embryos cultured with 5 µM DZNep from day 3 (D3) or day 5 (D5) of embryonic development. Oocytes from Nelore females obtained from slaughterhouse ovaries were subjected to in vitro maturation (IVM) for 24 h at 38.5 °C, with 5% CO2 in air and saturated humidity. In vitro fertilization (IVF) was performed with a previously tested frozen-thawed semen from a Nelore bull. Oocytes and spermatozoa remained in coculture for 22 h under the same conditions of IVM. At D3 (considering the day of IVF as D0), the cleaved embryos were randomly separated into 3 groups: exposed to 5 mM DZNep from D3 (DZNep D3; n = 80), exposed to 5 mM DZNep from D5 (DZNep D5; n = 81) or without the addition of DZNep (control group; n = 82). Four replicates were performed, and the blastocyst rates were assessed at D8. All fertilized structures (not only the blastocysts) were fixed in 4% paraformaldehyde and the cell nuclei were stained with DAPI to estimate the number of cells. Blastocyst rates were compared between the treatments using a logistic regression test and the average number of cells by analysis of variance followed by Tukey test, with 5% of significance level. The blastocyst rate obtained with the control group was 34.1%, higher than the ones obtained with groups DZNep D3 (11.2%) and DZNep D5 (7.4%), which did not differ. The average cell number of the control group was 38.7, higher than the average cell number of groups DZNep D3 (7.5) and DZNep D5 (6.4), which did not differ. Based on these results, it can be concluded that H3K27 me3 plays an important role in embryonic gene regulation because its inhibition results in decreased blastocyst rates and the produced embryos have fewer cells.

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Metabolic factors associated with the incidence of uterine disease and fertility in dairy cows in winter and summer

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Keywords: metabolite, transition period, uterine diseases.

Due to the asynchrony between metabolic demand and dry matter intake in late pregnancy and in early postpartum period, dairy cows undergo a period of negative nutrient balance (NEB). Endocrine and physiological changes during NEB, affect the immunological system and the organic defenses. During transition period, dairy cows are more vulnerable to health problems, like infections or metabolic diseases. The aim of present study was to examine the associations between metabolic variables with uterine health in dairy cows, in winter and summer, and also, verify if these variables can be used as predictors of health and fertility programs in tropical conditions. The experiment was conducted on a commercial dairy farm (Fazenda Colorado, Araras - SP). We used 245 multiparous Holstein cows, for this experiment. The cows were distributed, retrospectively, according to average milk production at lactation peak (4 to 8 weeks). The average milk production at peak lactation was 45.9 kg / milk / day. Thereafter, cows were allocated within the experimental groups (High Production (AP), ≥ 45.9 to 65kg / milk / day at peak lactation; Medium production (MP), between 30 and 45.8 kg / milk at peak lactation). According to pregnancy groups, differences in the variables: early birth (P = 0.03), aid delivery (P = 0.05), retained placenta (P = 0.009), metritis (P = 0.04), and trends in effect creates weight (P = 0.07), displaced abomasum (P = 0.06), ketosis (P = 0.08) and endometritis (P = 0.08) were observed. Metabolic factors evaluated in D - 21 were significant predictors (P < 0.05) of the uterine diseases occurrence during transition period: serum levels of AST above 90 U / L , glucose below 52.4 mg / dL , NEFA above 0.4 mmol / L, BHBA above 0.7 mmol / L, and SOD below 3037.6 U / g Hb. High milk production was not a risk factor for the majority of puerperal disorders studied in animal models of this study; being the parameters of glucose, NEFA, BHBA, BUN and AST, monitored on day 21 before parturition and early postpartum, significant predictors of disease in the postpartum period and fertility, respectively.

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Effect of Fibroblast Growth Factor 10 (FGF-10) during IVM and pre IVM on in vitro production of bovine embryos.

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Keywords: embryo, growth factor, retention.

The oocytes used in assisted reproduction techniques are usually taken from 3 - 8mm follicles, which are less competent for development that in vivo matured. When removed from follicles, those oocytes resume meiosis spontaneously, being deprived of follicular events that occur in pre-ovulatory period. Those events are well known to be essential for acquisition of competence. Therefore, alternatives to improve the quality of oocytes should be studied to enhance the efficiency of IVP. FGF-10 is a major growth factor secreted by the oocyte. Moreover is produced by theca cells, which are absent during IVM. Benefits in cumulus cells expansion, quantity and quality of bovine embryos have been reported when the FGF-10 was added during IVM (Zhang et al., 2010, Reproduction, 140:815-26). The present study aimed to evaluate the effect of FGF-10 (0.5 ng/mL) in pre-IVM and IVM in bovine IVP embryos. The pre-IVM medium contained BSA 0.2%, Cilostamide 10μM and FSHrh 10-4 IU/mL, the MIV 0.4 % BSA and FSHrh 10-1 IU/mL. Initially, the effect of FGF-10 in IVM was evaluated. For this, cumulus oocyte complexes (COCs) were aspirated from slaughterhouse ovaries and after selection were divided into 2 groups: T1: control with oocytes matured in IVM medium for 22h (n = 124); T2: treatment with COCs matured for 22h in IVM medium with FGF-10 (n = 124). Subsequently, the effect of FGF-10 in pre-IVM (22 h) followed by IVM in embryo production was evaluated. COCs were divided into 5 groups: C: control, with oocytes matured for 22h (n = 116); PCMC: pre-matured oocytes for 22h after matured for 22h (n = 119); PCMFGF: pre-matured oocytes for 22h after matured for 22h with FGF-10 (n = 119); PFGFMC: pre-matured oocytes with FGF-10 for 22h after matured for 22h (n = 111). PFGFMFGF: pre-matured oocytes for 22h with FGF-10 after matured for 22h with FGF - 10 (n = 113). After maturation, oocytes of all groups were fertilized and cultured until day 7 (D7) of development. Cleavage (D2) and blastocyst rates (D7) were evaluated. Data were analyzed by Chi-square test ($P < 0.05$). There was no statistical difference on D2 (87.9 and 91.9 %) and D7 (50 and 47.5 %) between T1 and T2. When the effect of FGF-10 in pre-IVM was analysed, the PGFGMFGF group was similar to control in D2 (84.4 and 82.3%) and D7 (56.8 and 46.9%). The other groups were lower than control and similar to each other in D2 and D7 (PCMC: 73.1 and 43.6 %; PCMFGF: 78.1 and 42.8%; PGFGMC: 79.2 and 41.4%). The results suggest that FGF-10 did not improve embryo production when added during IVM, but when added at pre- IVM and IVM decreases the detrimental effect of meiotic block in PIVE.

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Characterization of the peri-ovulatory endocrine impact on the transcript abundance of estradiol, progesterone and oxitocin receptors in the bovine endometrium

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Keywords: cattle, gene expression, transcriptome.

Establishment of pregnancy depends on a well-balanced interplay between the endocrine profiles and the maternal reproductive tract. The bovine endometrium is a dynamic tissue that undergoes spatiotemporal functional changes directed by ovarian hormones, estrogen (E2) and progesterone (P4). Endocrine actions are primarily mediated by binding to specific receptors and subsequent activation of transcription of steroid responsive genes. We aimed to characterize the effects of different peri-ovulatory endocrine milieus on the endometrial transcript abundance of steroid receptor genes. Nelore cows were pharmacologically manipulated to ovulate small (SF–SCL) or large (LF–LCL) follicles and to result, subsequently, in greater proestrus concentrations of E2 and diestrus concentrations of P4 for the LF-LCL group. Groups were associated with smaller or greater pregnancy success, respectively. In three independent experiments, endometrial tissue was collected by transvaginal biopsy on day zero (D0; n=3 and 4), and post-mortem on D4 (n=8 and 8) or D7 (n=8 and 9) after GnRH-induced ovulation for SF-SCL and LF-LCL cows, respectively. Relative transcript abundance of nuclear progesterone receptor isoform A (PR-A), isoform B (PR-B) and isoform C (PR-C); membrane progesterone receptor (PRMC); nuclear estradiol receptor alpha (ESR1) and beta (ESR2); membrane estradiol receptor (GPER) and oxytocin receptor (OXTR) was measured by qPCR. Cyclophilin A, beta-actin and GAPDH were used as reference genes. For each experiment, data were analyzed by one-way ANOVA for the effect of treatment (using SAS software). The pre-ovulatory follicle, subsequent CL size, circulating E2 and P4 concentrations were significantly greater in the LF–LCL group ($P<0.05$). On D0, the abundance of transcripts coding for ESR1, PR-A, PR-B and PR-C genes was significantly up-regulated in the endometrial tissue from LF–LCL cows compared to the SF–SCL counterparts ($P<0.03$). On D4, gene expression of PR-A, PR-C, PRMC and OXTR was significantly down-regulated in the LF–LCL group ($P<0.03$). Similarly, on D7, expression of PR-B, PR-C and OXTR were significantly down-regulated in the LF–LCL cows ($P<0.02$). In contrast, expression of ESR2 and PRMC was significantly increased in the LF–LCL day 7 endometrial tissue ($P<0.03$). In conclusion, these results confirm that differential peri-ovulatory endocrine profiles impact the transcription of sex steroid-responsive genes in a time-specific manner. Complex modulation of receptor expression by proestrus E2 and early diestrus P4 may fine-tune downstream expression of target genes in the endometrium to regulate uterine receptivity to the developing embryo.

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Evaluation of physiological parameters of dogs during uterine lymphatic mapping using patent blue vital dye

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Keywords: bitches, hypersensitivity, vital dye.

The evaluation of sentinel lymph nodes in cases of uterine disorders in bitches might assist in early diagnosis and better prognosis. The use of patent blue is usual in human medicine and there are several reports of hypersensitivity reactions (Hunting, Allergy, 65:117-123, 2010). The objective of this study was identify possible complications during surgery with the use of patent blue dye (Bleu Patente V®, Delpharm Tours, Chambray Lês Tours - France), in bitches during uterine lymphatic mapping. The experiment was performed in 14 mongrel dogs without any reproductive disease, 1 to 6 years, weighting between 7 and 15 kg, randomly assigned into two equal groups: G-PBV- uterine infusion dye and ovariohysterectomy (OHE) and G-Control - OHE only. The animals were submitted to preoperative exams (total protein, albumin, ALT, ALP, creatinine). The anesthetic protocol was: sedation and analgesia - morphine (0.5mg/kg, IM) and acepromazine (0.03mg/kg, IM); induction - propofol (5mg/kg, IV), maintenance - inalatory isoflurane; analgesia pre-clamping of the ovarian pedicles - fentanyl (5µg/kg, IV). For lymphatic mapping, 0.4 mL of patent blue V was injected in the muscular layer of uterine body and horns, 10 minutes before OEH. Systolic blood pressure was monitored throughout the procedure and arterial blood gas analysis was performed immediate pre and postoperatively. All animals were evaluated in seven days, when the preoperative tests were repeated. Comparisons between the pre- and postoperative parameters within the same animal were performed using the Wilcoxon-Mann-Whitney test. The parameters evaluated between the pre- and postoperative periods between the control and lymphatic uterine mapping groups were compared by the Mann-Whitney test, with a level of statistical significance of 5%.The mean duration of the surgery was 42.9 minutes in the G-Control (only OHE) versus 54.3 minutes in the G-PBV (uterine lymphatic mapping and OHE). All of the biochemical parameters evaluated were within the normal range, although differences were observed in the G-PBV in both the total protein ($p=0.03$) and the albumin serum ($p=0,02$) between the pre- and postoperative evaluations, with higher values in the pre assessment when compared with postoperative. There were no extreme changes in the heart rate and systolic blood pressure. There was also a difference in the partial pressure of oxygen between the pre- and postoperative evaluations in both groups ($p=0.04$ and $p=0.02$). The oxygen saturation differed ($p=0.02$) between the pre- and postoperative evaluations in the G-Control. In both cases, higher values were observed in the preoperative evaluation compared with the postoperative, probably due to the time of blood collection (pre - animals receiving 100% oxygen; Post - extubated animals). There were no signs of adverse reactions to the patent blue V dye in all healthy bitches submitted to uterine lymphatic mapping, showing that the patent blue V dye is safe for this purpose.

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Seasonal redox profile of antioxidant enzymes and lipid peroxidation in bovine follicular fluid

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Keywords: antioxidant enzymes, bovine, follicular fluid.

Heat stress promotes a series of cellular and physiological alterations in the bovine reproductive tract microenvironment, compromising follicular dynamics, oocyte growth and maturation as well as preimplantation embryonic development. It has been shown that heat stress alters cellular and embryonic redox state. However, little is known about the effects of high ambient temperatures in the redox state of the oocyte microenvironment such as follicular fluid (FF). Therefore, the objective of this study was to evaluate seasonal changes in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity and lipid peroxidation in the bovine FF. Slaughterhouse ovaries were transported to the laboratory in 0.9% NaCl solution containing 100 units/mL penicillin-G and 100 ng/mL streptomycin at 4°C. The FF was aspirated with a needle and syringe, centrifuged for 30 minutes at 4°C and the supernatant was stored at -20°C. Samples were collected monthly throughout the year. Dry bulb temperature (DBT) and relative humidity (RH) data were collected from Pirassununga Meteorological Station to calculate the temperature humidity index (THI) according to Armstrong, 1994 (J Dairy Sci., 77:2044- 2050, 1994). Samples were selected from the three months with the highest and lowest THI. Superoxide dismutase activity was determined based on cytochrome c reduction by anion superoxide, generated by xanthine-xanthine oxidase system. Glutathione peroxidase activity was determined based on NADPH consumption and lipid peroxidation by formation of species that reacts with thiobarbituric acid (TBARS). The enzymatic activity and TBARS analysis were conducted in spectrophotometer according to Nichi, et al. 2006 (Theriogenology, 66: 822-828, 2006). The THI indicated that animals were under moderate heat stress during summer and absence of stress during winter months. Follicular fluid SOD activity was not affected by season. However, GPx activity was lower (p=0.05) in FF collected during summer (129.93 ±11.4 U/mL) as compared to winter (167.29 ± 11.4 U/mL). There was no effect of season on TBARS. In conclusion, summer reduction on FF GPx activity suggests that hydrogen peroxide was not efficiently converted to water leading to buildup of this reactive oxygen species. The THI indicated that seasonal heat stress was moderate as was the antioxidant unbalance, since SOD activity and TBARS were not affected by season.

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Nutritional density of the diet and chromium supplementation on serum concentrations of glucose, insulin and non-esterified fatty acids in lactating dairy cows

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Keywords: chromium, dairy cows, insulin resistance.

The objective of this study was to evaluate the influence of energy density of the diet and chromium supplementation on serum concentrations of glucose, insulin and non-esterified fatty acids (NEFA) in lactating dairy cows. Eighteen crossbred Holstein x Gir cows, allocated in two pastures and receiving free-choice of corn silage and water, were grouped by parturition (primiparous x multiparous) and milk yield, and randomly assigned to one of three treatments on d0 of the experiment: 1) CON: diet formulated to reach 100% of maintenance and lactation requirements, without chromium supplementation (n=6); 2) BPSC: diet formulated to reach 100% and 160% of the requirements for lactation and maintenance, respectively, without chromium supplementation (n=6); 3) BPCC: diet formulated to meet 100% and 160% of the requirements for lactation and maintenance, respectively, with chromium supplementation (n=6; 2.5g of KemTRACETM Chromium Propionate 0.4% - Kemin Industries, Inc. - added in 97.5g of corn meal/animal/day). Blood samples were collected weekly for serum glucose, insulin and NEFA concentrations during the experiment (d0 to d210). Glucose Tolerance Tests (GTT) were performed each 42 days (d0, d42, d84, d126, d168, d210). During each GTT cows were infused (i.v.) with 0.5g of glucose/kg of body weight. Blood samples were collected at -15, 0, 10, 20, 30, 45, 60, 90 and 120 min relative to infusion. Ovarian pick-up was performed for oocyte quality and embryo production evaluation. There was no difference (P=0.74) in body weight during the study. Cows from the BPSC and BPCC groups had greater (P=0.02) Body Condition Score gain when compared to cows from the CON group. There was no difference (P=0.92) in milk yield between groups. Animals in the CON group had greater (P=0.04) serum NEFA concentrations when compared to the ones in the BPSC and BPCC groups. A treatment x day interaction (P<0.01) was detected for serum insulin concentrations since d175 for primiparous cows and since d28 for multiparous cows. A treatment x day interaction was detected (P<0.01) for insulin:glucose ratio for both primiparous and multiparous cows. During GTT serum insulin concentrations (P<0.05) were greater for animals in the BPCC when compared to animals in BPSC and CON groups. There was no difference for serum glucose concentrations in any of the parameters analyzed. These data allow to conclude that dairy cows receiving a high energy density diet develop a lower insulin sensitivity, when compared to the animals in the control group, which characterize an insulin resistance status. Also, chromium was capable to minimize this condition when supplemented to cows receiving high energy diets. The I:G index and the GTT detected insulin resistant cows. Multiparous cows in the BPCC group had greater (P=0.03) oocytes production comparing to cows in the BPSC and CON groups.