A212 Embryology, Developmental Biology and Physiology of Reproduction

Parthenogenetic activation, but not electrofusion, alters developmental kinetics and hatching of mouse embryos

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Keywords: blastocyst, electrofusion, parthenogenesis.

The functioning of the trophectoderm (TE) is influenced by epigenetic modifications and requires biparental complementation. Parthenogenesis alters the epigenetic environment and affects the physiological function of embryonic cells. This work aimed to evaluate the development of murine embryos after oocyte and embryonic manipulation. Kinetics and hatching rate were evaluated in blastocysts: *i*) derived from parthenogenetic activation followed (Group EP) or not (Group PG; theoretically haploid) by electrofusion; *ii*) from electrofusion of two blastomers (Group EL; theoretically tetraploid) and; *iii*) from *in vivo* fertilization (Control Group). There was no significant difference (Chi-square or Exact Fisher's Test, $P > 0.05$) for the hatching rate between groups Control and EL (56.9 and 47.5%), but they differed from the other groups. Between the groups PG and EP, hatching rates were similar (respectively, 14.6 and 7.5%) and the lowest of all groups. The electrofusion technique (EL) itself was not deleterious to hatchability. Thus, parthenogenesis itself and/or the activation process might have negatively affected PG and EP groups. There was a clear difference in developmental kinetics between the groups. While group EL developed similarly to the control group, the embryos that underwent parthenogenetic activation were delayed, possibly due to the exclusively maternal genome. There are reports in the literature that it was possible to rescue the paternal imprinting in parthenogenetic mouse embryos, by ESC derivation (Chen *et al.*, Stem Cells, 27:2136-45, 2009), or by serial SCNT (Hikichi *et al.*, Development, 137:2841-47, 2010). The authors reported that parthenogenetic cells could constitute placenta and fetus itself, partially reverting the original imprinting. Although only morphologically evaluated, the difference observed in the embryos of EP group, compared to PG embryos, suggests that diploidy was not beneficial for parthenogenetic embryos as previously described (Liu *et al.*, Biol Reprod, 66:204-10, 2002). We infer that since diploidy on group EP was exclusively maternal, the full function of the trophectoderm was impaired, in which paternal imprinting is important. Expansion and hatching kinetics of blastocysts was used for the assessment of TE functionality. These functions arise from the capacity of TE to pump sodium ions into the blastocoele, promoting water influx. On group EP, the electrofusion apparently was not the detrimental source to the embryos as embryos from group EL had developmental kinetics and hatching rate similar to those in control group.

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Is the count of ovarian antral follicles ≥ 3 mm in diameter associated with fertility in lactating Nelore cows?

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Keywords: antral follicle, fertility, Nelore.

The follicular growth in cattle occurs in a wave pattern of 2 to 3 waves per estrous cycle, and is characterized by synchronous growth of a cohort of antral follicles, from which usually only one of these will become dominant. The amount of recruited follicles per wave is variable among animals and breeds, but is highly repeatable among individuals. This variation in ovarian follicular population can interfere with fertility by influencing oocyte competence (Ireland et al., 2007). We aimed to identify Nelore (*Bos taurus indicus*) cows with high and low antral follicle count (AFC) and to compare their pregnancy rates. We evaluated 268 multiparous Nelore cows from 40 to 70 days postpartum and body condition score between 3.5 to 4.5 (5-point scale). The cows were divided into groups according to the antral follicle count (≥ 3 mm in diameter). Hence, 33% of animals with the greater AFC were enrolled in high population group (HG, n=89, >38 follicles), while the intermediate animals (33%) were allocated in the intermediate group (IG, n=88, between 28 and 38 follicles) and animals (34%) with lower AFC were included in the low population group (LG, n=91, < 28 follicles). The animals underwent three ultrasonographic evaluations (days D-10, D0 and D28). In D0, at random day of the estrous cycle, all cows received an intravaginal device containing progesterone (1.0 g, DIB®) and estradiol benzoate (EB, 2.0 mg, IM, Estrogin®). Eight days later (D8) we administered 75 µg of D-cloprostenol (Croniben®) and the intravaginal device was removed. Twenty-four hours after DIB removal, the cows were treated with EB (1.0 mg, IM) and after 30-36 hours animals were artificially inseminated at fixed-time (FTAI). Data were analyzed using PROC GENMOD and FREQ SAS System 9.1 for Windows (2002-2003). The mean (±SD) of antral follicles in both ovaries was 32.7±17.8. There was no difference (P=0.144) in pregnancy rates between the HG, LG and IG animals (32.6, 46.6 and 42.9%, respectively). But there was a difference in the probability of becoming pregnant (P = 0.0268) decreased as the AFC in anestrus cows increased the probability of pregnancy (N = 138). Thus, we concluded that there was no difference in pregnancy rates between Nelore cows either with high or low population of ovarian antral follicles when submitted to FTAI, however, in this study the animals in anestrus with the lowest AFC were more likely to become pregnant.

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Is the count of ovarian antral follicles ≥ 3 mm in diameter associated with fertility in lactating Aberdeen Angus cows?

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Keywords: Angus, antral follicles, fertility.

Bovine follicular growth occurs in a wave pattern of two or three waves per estrous cycle. These waves are characterized by the growth of a synchronous group of antral follicles from which usually only one becomes dominant. The number of recruited follicles per wave is variable among animals and breeds, but it shows high repeatability within individuals. Ereno et al. (2013) reported that the number of follicles recruited per wave is higher in zebu cattle when compared to taurine. This variation in the ovarian follicular population can interfere with fertility by affecting oocyte competence (Ireland et al., 2007). The objective of this study was to identify Aberdeen Angus cows (Bos t. taurus) of high and low antral follicle count (AFC) recruited per follicular wave and compare their pregnancy rate. We used multiparous cows (272) between 40 and 70 days postpartum and body condition score between 2.5 to 4.0 (5-point scale).The cows were divided into groups according to the antral follicle count (≥ 3 mm diameter). Thus, 35% of the animals with the highest AFC were included in high population group (HG, n=94, >22 follicles), while the intermediate AFC (31%) were placed in the intermediate group (IG, n=85, between 15 - 22 follicles) and animals with the lower AFC (34%) were included in the low population group (LG; n=93, ≤15 follicles). We performed three ultrasound examinations of the ovaries (D-10 days, D0 and D28). At D0, random day of the estrous cycle, all cows received an intravaginal device containing progesterone (1.0 g, DIB®) and estradiol benzoate (EB, 2.0 mg, IM, Estrogin®). Eight days later (D8) we administered 75 µg, d-cloprostenol (Croniben®) and the intravaginal device was removed. After 24 h of DIB removal, cows were treated with EB (1.0 mg, IM) and 30 to 36 h after animals were artificially inseminated at fixed time (FTAI). Data were analyzed using PROC GENMOD and FREQ - SAS System 9.1 for Windows (2002-2003). The mean (±SD) of both antral follicles in all the ovaries was 19.97±9.03. There was no difference (P=0.12) in pregnancy rate among animals HG, LG and IG (54.3, 39.8, and 43.5%, respectively). But there was a difference in the probability of becoming pregnant (P = 0.0491) as increased AFC raised the possibility of becoming pregnant. In this study, we conclude that there was no difference in pregnancy rate after FTAI between lactating Aberdeen Angus cows of high or low population of antral follicles, however, the animals of high AFC were more prospective to get pregnant.

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Accuracy of two forms of early pregnancy diagnosis in cattle by ultrasonography

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Keywords: bovine, pregnancy diagnostic, ultrasonography.

The use of ultrasound in the diagnosis of pregnancy has been an indispensable tool for good reproductive performance. Pregnancy diagnosis via ultrasonography allows early detection of non-pregnant, allow the decision of turning it pregnant quickly, thus reducing the time that these animals do not remain pregnant. There are two ways diagnostic ultrasound for pregnancy: The first is the traditional view of the fetus and heart rate and the second is the observation of the amniotic bladder, characterized by the presence of fully anechoic content and distended uterine wall considered pathognomonic signs of pregnancy in cattle. The objective of this study was to evaluate the accuracy and execution time of these two forms of early ultrasound pregnancy diagnostic in cattle. We evaluated 674 crossbred cows used as embryo recipients in the same rural property, located in southern Minas Gerais. These were distributed randomly in two treatments: T1 (N = 351), diagnosis by detecting the amniotic bladder and T2 (n = 323), by browsing the fetus and heartbeat. All animals were between 28 and 32 days of gestation. The same equipment was used for both forms of diagnosis (Mindray M5 ™) with a transrectal transducer of 5 MHz. The examination of each run time was calculated using a digital timer. The pregnant females in both tests were reassessed by ultrasound considering the characteristics of the bladder and the presence of the fetus 30 days later. The data were evaluated by ANOVA. The differences in the percentage of pregnant females between 30 and 60 were compared using Fisher's exact test. It was considered significant, differences of below that 5% probability. The initial total pregnancy rate was 49.40% (333/674), of which 172 pregnant considered using the T1 and 161, using the T2. The difference between the total number of cows pregnant between 30 and 60 days was 5.70% and 5.88% for T1 and T2, respectively ($P > 0.05$), showing that the two methods have the same accuracy efficiency for pregnancy diagnosis at 30 days. The average running time of diagnosis was lower ($P < 0.05$) in T1 to T2 (0.5 ± 0.3 vs 1.8 ± 1.6 minutes). It is concluded that the two techniques of early diagnosis of pregnancy has the same accuracy after 30 days. As the female time of manipulation is lower in T1, this method should be indicated.

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Reproductive abnormalities in prenatally androgenised male sheep

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Keywords: andronisation, changes, reproduction.

Aims: This study aimed to verify reproductive abnormalities in male sheep exposed in utero to testosterone in a model for polycystic ovary syndrome (PCOS). Materials and Methods: Animals - Adult sheep from Corriedale breed were obtained from a farmer from the state of RS after the approval of Animal Ethics Committee (CEUA – UFSM, number 009-2013). The androgenisation protocol consisted in the administration of testosterone propionate in the mothers (Androgenol®, Juatuba, Brasil) 100 mg i.m. biweekly from the day 30 to 90 of gestation. No treatments were performed in the control group. After the birth of males (controls n=5 and prenatally androgenized PA n=8), they were followed to address the scrotal perimeter and weight. For the semen analysis executed at 16 months, a microscope technique was employed. There was one case of cryptorchid testes in PA group. For comparison between variables with normal distribution, the T Student test was used. Proportions were analyzed by Fisher´s test. A significant p was considered if <0.05. Results: No differences were observed regarding the weight and scrotal perimeter between PA sheep and controls. At 16 months, the weight in control group was (mean + SD) 33.0 ± 2.5 kg while in androgenised male sheep it was 32.2 ± 3.9 kg. Similarly, the scrotal perimeter was at this time 21.3 ± 0.4 cm in controls and 21.1 ± 0.75 cm in PA male sheep (NS).There was one case of cryptorchidism with reduced weight of the testes. The analysis of the semen showed some abnormalities in the androgenized group, where 60% demonstrated vigor equal to one and 75% motility equal or inferior to 40%. However, a significant decrease in the proportion of PA male sheep with these two features together (vigor >2 and motility > 40%) was noted in comparison to controls (p =0.0476, Fisher´s test). Conclusions: Prenatal androgenisation of Corriedale male sheep did not produce differences in the scrotal perimeter although it adversely affected the quality of the semen, as similarly described in Suffolk breed (Recabarren SE et al, Endocrinology 149(12):6444; 2008).

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Evaluation of gestational length in Criollo mares – preliminary data

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Keywords: criollos, equine, reprodutive characteristics.

The participation of Criollo horses in competitions has grown in recent years. The growing appreciation of the Criollo horses market stimulates conducting research about reproductive characteristics of the breed. Despite the growth in the use of reproductive biotechnologies, there are few studies related to pregnancy monitoring in Criollo horses. The aim of this study was to evaluate the physiological variations related to gestational length in Criollo mares. Retrospective and prospective data were collected from a Criollo Farm in the south of Brazil, during breeding seasons of 2008-2014, including 50 mares. The gestational length, age of the mare, number for deliveries, and gender of the foals were considered. The gestational length was determined by the time of the ovulation until delivery. The mares were assigned into two groups according to age: Young mares (until 7 years); and old mares (> 8 years). For the comparison of the gestational length in relation to the age of the mare and gender of the foals was performed Two Sample T test. The Pearson correlation test was performed to evaluate the relationship between the numbers of deliveries and gestational length. Data for response variables were reported as mean + SE. The means of gestational length was 334 days + 1.5 (minimum: 313 days; maximum; 371 days). It was observed shorter gestational length in young mares (331 days + 1.7) compared to old mares (338 days + 2.4). No difference was observed between the gestational length and gender of the foals. It was observed a tendency toward positive correlation between gestational age and the number of deliveries of the mares ($r=0.24$, $p=0.08$). The gestational length in mares ranged from 320 to 360 days, according to the breed and studied population, although pregnancies between 310 until 380 days can be result in healthy foals. These variations occur due to the influence of maternal, fetal and environment factors. Among the maternal factors, we include the age of the mare, nutritional condition and number of deliveries. Fetal factors include gender of the foal and environment factors involve the month of delivery, the weather and the year (Bueno, et al., III Congreso Argentino de Reproduccioón Equina. Córdoba: UniRio, 2013. p. 137-139, 2013). The means of gestational length observed in this study are in accordance with the data described for Criollo breed (Winter et al., J. Equine Vet. Sci. v.27, n.12, p.531–534, 2007). This study is the first description of relationship between the maternal and fetal factors with the gestational length in Criollo mares. We conclude that young mares present shorter gestational length than old mares, and also a positive correlation tendency between the gestational length and number of deliveries in Criollo mares.

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Metabolic evaluation of repeat breeder Holstein cows during summer and winter

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Keywords: dairy cow, metabolism, repeat breeding.

The aim was to determine the occurrence of metabolic changes in blood serum of Holstein repeat breeder cows (RB, n=67) compared to peak lactation cows (PL, n=70) and heifers (H, n=70) in summer (S) and winter (W). The metabolites studied (Automatic Biochemical Analyzer, Randox Daytona) were cholesterol (CHOL), urea, BHB, NEFA, total protein (TP), albumin (ALB), globulin (GLOB), AST, GGT, CK, triglycerides (TRIGS), DHDL, LDL, VLDL, glucose (GLU) and creatinine (CREA). Data was analyzed with GLIMMIX, SAS. There was no season-category interaction for ALB (P=0.58), AST (P=0.10), GGT (P=0.31), CK (P=0.49) and ALB/GLOB (P=0.12), CHOL (P=0.58), LDL (P=0.60), AGNEs (P=0.16), GLU (P=0.88), urea (P=0, 30) and CREA (P=0.34). Also, no category effect for CREA (H: 0.92±0.02; PL: 0.94±0.03; RB: 0.98±0.03mg/dL; P=0.34) or season effect for ALB (3.17±0.03 vs 3.16±0.03mg/dL; P=0.84), CHOL (119.5±6.0 vs 123.6±5.2mg/dL; P=0.31), LDL (78.9±4.9 vs 74.5±4.0mg/dL; P=0.49), GLU (63.5±0.8 vs 62.2±1.1mg/dL; P=0.31) and CREA (0.93±0.02 vs 0.96±0.02mg/dL; P=0.20) in W and S. In S there was greater AST (59.3±3.0 vs 67.5±1.8U/L; P=0.0005) and lower GGT (21.2±3.1 vs 17.8±2.0U/L; P=0.03), CK (140.7±44.8 vs 72.1±4.3U/L; P=0.007), ALB/GLOB (0.77±0.02 vs 0.69±0.01; P=0.002), AGNEs (0.34±0.03 vs 0.29±0.02mMol/L; P=0.03) and urea (33.5±1.3 vs 26.6±0.9mg/dL; P<0.0001), regardless of category. H had higher CK (162.8±61.9b vs 69.7±5.3a vs 80.1±11.1aU/L; P=0.0003) and lower ALB (2.97±0.03b vs 3.24±0.04a vs 3.30±0.03ag/dL; P<0.0001), AST (53.2±1.9b vs 73.4±3.7a vs 64.3±2.6aU/L; P=0.0005), GGT (8.4±0.9b vs 22.1±1.6a vs 28.2±4.9aU/L; P<0.0001), CHOL (60.6±1.5b vs 156.1±5.5a vs 149.5±5.1amg/dL; P<0.0001), LDL (30.2±1.1b vs 102.0±4.4a vs 98.5±4.5amg/dL; P<0.0001) and urea (22.8±1.0b vs 36.0±1.6a vs 30.8±1.1amg/dL; P<0.0001) than PL and RB regardless of season. RB had intermediate values of NEFA (H:0.15±0.01c; PL:0.45±0.03a; RB:0.35±0.02bmMol/L; P<0.0001) and GLU (H:69.9±1.2a; PL:56.4±0.9c; RB:62.0±0.9bmg/dL; P<0.0001). Category-season interaction was found for PT (P=0.03), GLOB (P=0.04), TRIGS (P=0.02), DHDL (P=0.009), VLDL (P=0.02) and BHB (P=0.0003). In W, PT and GLOB did not differ between categories. In S, H were lower than RB and PL for PT (7.5±0.1b vs 7.9±0.1a vs 8.1±0.1ag/dL; P<0.005) and lower than RB for GLOB (4.5±0.1b vs 4.7±0.1ab vs 4.8±0.1a; P=0.0002). For TRIGS and VLDL, H were lower than cows in W (TRIGS: 24.5±1.2a vs 16.3±0.6b vs 16.1±0.9bmg/dL; VLDL: 4.9±0.2a vs 3.3±0.1b vs 3.2±0.2bmg/dL) and higher in S (TRIGS: 27.4±1.4a vs 14.3±0.5c vs 18.0±1.0bmg/dL; VLDL: 5.5±0.3a vs 2.9±0.1c vs 3.6±0.2bmg/dL). Though metabolites values differ between season and category, they are considered within the normal range for dairy cows and are not indicative of pathological changes. Thus, differences must be related to milk production, DMI, physical activity and nutrition inherent in each category/season and cannot be considered as causes of RB.

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A219 Embryology, Developmental Biology and Physiology of Reproduction

Pharmacological blocking of h3k27 trimethylation alters the expression of polycomb repressive complex 2 genes in bovine blastocysts produced *in vitro*

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Keywords: embryo development, h3k27me3, polycomb repressive complex 2.

Trimethylation of histone H3 on lysine 27 (H3K27me3) is established by Polycomb Repressive Complex 2 (PRC2) and it is associated with stable and heritable gene silencing. In pluripotent cells, genes associated with development and cell differentiation are maintained repressed by H3K27me3. However, this process is not fully understood. The AdoHcy hydrolase inhibitor 3-Deazaneplanocin A (DZNep) can block the action of the PRC2enzymes and thereby inhibit H3K27me3. In this study, we evaluated the effect of treating bovine embryos during *in vitro* development with DZNep on the expression of genes encoding PRC2 enzymes (EZH2, EED and SUZ12), and transcription factors regulating cell pluripotency (OCT4 and NANOG) and trophoblast differentiation (CDX2). Oocytes obtained from slaughterhouse ovaries were subjected to *in vitro* maturation (IVM) for 24 h at 38.5°C, with 5% CO_2 in air and saturated humidity. *In vitro* fertilization (IVF) was performed with a previously tested frozen-thawed semen from a single Nellore bull. The oocytes and spermatozoa remained in coculture for 22 h under the same conditions of IVM. In D3 (considering the day of IVF as D0), the cleaved embryos were randomly allocated into four groups and exposed to 5 µM DZNep from: a) D3 to D5 (DZNep D3-D5); b) D3 to D8 (DZNep D3-D8); c) D5 to D8 (DZNep D5-D8); or d) without DZNep (Control Group). Embryos that developed to the blastocyst stage on D8 were collected for RNA extraction followed by qRT-PCR to assess abundance of transcripts. The experiment was repeated three times and all samples were analyzed in duplicate using 30 embryos per group. Total RNA was extracted using the PicoPure RNA isolation Kit (Life Technologies) and cDNA was synthesized using the SuperScript VILO cDNA Synthesis Kit (Life Technologies). Relative mRNA abundance was normalized to the levels of two reference genes (beta actin and 18S ribosomal). Data were analyzed using ANOVA and the means were compared by Dunnett's test. DZNep treatment did not alter mRNA levels of SUZ12, NANOG, OCT4 and CDX2 in embryos that developed to the blastocyst stage. However, exposure to DZNep from day 3 to 8 increased mRNA levels of genes encoding the Polycomb enzymes EZH2 and EED. Findings from our previous studies confirmed that exposure of bovine embryos to DZNep during these periods of culture reduced blastocyst formation. These findings indicate that inhibition of H3K27me3 alters the regulation of Polycomb enzymes EZH2 and EED in early developing embryos, which suggests that these enzymes are involved in cell proliferation and blastocyst formation in the bovine embryo.

A220 Embryology, Developmental Biology and Physiology of Reproduction

Meiosis blockage in bovine oocytes with cordicepim: kinetics of maturation and embryo production

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Keywords: meiotic blocker, oocyte maturation, pre-maturation.

The meiosis blockage may be an alternative to improve oocyte maturation *in vitro*. This study aimed to investigate the effects of cordicepim blocker used for the pre maturation of bovine oocytes. To that, three experiments assessed the kinetics of nuclear maturation just after 6 h blocking (experiment 1); after a maturation period of 20 or 24 h (experiment 2), and the in vitro embryo development (experiment 3). Data were analyzed by the chi-square test with 5% of significance level. In experiment 1, 456 oocytes were incubated for 6 h in one of the following treatments: standard maturation medium (containing the additives serum and gonadotropins) without (IVM/CONT) or containing 79.6 nM / mL cordicepim (IVM/CORD), or in TCM-199 medium (without the additives) (TCM/CONT) or in (TCM/CORD) with cordicepim added. Oocytes were fixed for assessment of nuclear status. Cordicepim in the absence of additives (TCM/CORD) blocked significantly more oocytes (67.0%) in VG/VGBD, than the treatments IVM/CONT (52.5%), IVM/CORD (47.7%) and TCM/CONT (45.7%). In the experiment 2, 504 oocytes were submitted to IVM/CONT and TCM/CORD treatments for 6 h, followed by maturation for either 20 or 24 hours. At the end of maturation, oocytes were fixed for nuclear status assessment. The oocytes treated with cordicepim in the absence of additives showed a significant reduction in MII rates (irreversible blockage) after 20 (TCM/CORD+20, 47.3%) or 24 h of maturation (TCM/CORD+24, 64.8%), in comparison to the treatments with additives: IVM/CONT+20 (98.8%) and IVM/CONT+24 (100%). In the experiment 3, 1527 oocytes went through IVM/CONT+20h, IVM/CONT+24, TCM/CONT+20h and TCM/CORD+24 treatments, in order to assess embryo development rates (cleavage and blastocyst) after parthenogenetic activation. Cordicepim significantly reduced the cleavage rates after 20 (42.0 vs. 56.4%) or 24 hours of maturation (44.3 vs. 54.4%). When it comes to the blastocyst rates, cordicepim significantly reduced (12.1 vs. 24.8%) after 20 h of maturation. However, when maturation length was increased to 24 h, blastocyst rates were no longer affected (22.9 vs. 25.0%). We conclude that in the absence of serum and gonadotropins, cordicepim effectively blocks oocytes in an irreversible manner. Conversely, when these additives are present, the blockage does not occur. Furthermore, increasing the maturation length from 20 to 24 h prevents the detrimental effect of cordicepim in the blastocyst rates.

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Characterization of lipidic profile from Piau swine breed

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Keywords: embryo, mass spectrometry, pig.

Piau breed is one of the locally adapted swine breed that is in constant decline of its population, due to absorbent cross with more premature and economically profitable breeds. In this regard, it is important to maintain this genetic source, which can be done by gametes and embryos cryopreservation. However, appropriate techniques for successful cryopreservation of swine germplasm is not established. In order to propose protocols to increase the efficiency of these techniques, it is necessary to know the characteristics of the material to be cryopreserved. Therefore, the aim of this study was to characterize the lipidic profile of Piau embryos. Therefore, Piau gilts had their estrus observed twice a day, and were naturally bred 12 and 24 hours after estrus detection. Six days after the, the embryos was collected by laparotomy. The embryos (expanded blastocyst, grade 1; n = 8) were stored in methanol at -80°C. To determine the profile of phospholipids, spectrums were obtained by MALDI-TOF mass spectrometry. Mass spectrums were acquired in frequency between 700-90 m/z, in positive/reflected mode in an AutoFlex Speed MALDI-TOF/TOF (Bruker Daltonics, Germany) mass spectrometer. For ionization, each embryo was individually allocated in a well of the MALDI plate and covered with a acid 2,5-dihydroxybenzoic acid (DHB) matrix. Sixteen lipids were found. Among the phospholipids, phosphatidyl cholines [PC (32:0) + H]+, [PC (34:1) + H]+ and [PC (36:4) + H]+, represented by 734.5; 760.5 and 782.5 m/z ions, showed high intensity. Some triglycerides were also found: PPL (50:2) + Na+, PPO (50:1) + Na+, PLO (52:3) + Na+ and POO (52:2) + Na+, represented by 753.5; 755.7; 879.7 and 881.6 m/z ions. The lipidic profile found is similar to the embryos and human oocytes spectrums (Ferreira et al Journal of Lipid Research, v.51, p. p.1218-1227, 2010). However, the triglycerides profile observed is different from bovine embryos but similar to bovine oocytes. These results characterize the lipidic profile from Piau and this knowledge can be used to optimize the cryopreservation of Piau embryos, an essential process for the conservation of this genetic material.

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Morphological aspects from placentitis lesions of equine placenta

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Keywords: equine, morphologic aspects, placenta.

Conditions affecting uteroplacental contact and placental efficiency may profoundly influence fetal well-being, development, and survival. In equine, placentitis is the most common condition of placental failure (BUCCA, Vet Clin Equine Pract, 752, 2006). The aim of this study was to evaluate the morphological aspects from placentitis lesions of placenta in Thoroughbreds mares at foaling. The parturition of 188 Thoroughbred mares was assisted during years 2011 to 2013, of which 40 cases of placentitis were identified in histologic evaluation at foaling. The placentas were submited to gross evaluation immediately after expulsion, and fragments were collected from nine placental points (cervical star, uterine body, gravid horn, non-gravid horn, bifurcation, amnion and three points of umbilical cord). The placental fragments were fixated in 10% formalin, to perform histologic slide confection. The slides were evaluated through light microscopic. In 82.5% (33/40) placentas, the gross evaluation were compatible with histologic findings of placentitis, showed areas devoid of villi, edema and suppurated material on the chorionic surface. It was observed that 52.5% (21/40) of placentas showed severe suppurative inflammation throughout the chorioallantoic membrane, with the predominance of neutrophils, necrosis and eosinophilic material consisting of cellular debris present between the chorionic villi. These findings featuring acute placentitis are frequently associated to bacterial infection. In others 47.5% (19/40) placentas the inflammatory infiltrate were formed by mononuclear cells, with a prevalence of macrophages and lymphocytes, mild to moderate necrosis of villi and edema in choriallantois membrane, demonstrate chronic placentitis. Lesions with morphological distribution of ascending placentitis were identified in 72.5% (29/40), these lesions were present in cervical star and uterine body. Focal lesions were observed in 12.5% (5/40) placentas on the regions of gravid horn, non-gravid horn and bifurcation. These morphological characteristics are frequently associated with fungical infection, despite this agent has not been identified on the histologic evaluation. In 15% (6/40), the lesions distribution was diffuse, morphologic characteristic associated with hematogenous infection. The hematogenous placentitis diagnosis can be difficult to perform during gestation, and is frequently observed only in post-partum evaluation, since many mares did not show clinical sings (WILLIAMS, Proc. 22 Workshop on the Equine Placenta, 90, 2004). We concluded that a higher incidence of ascending placentitis in the morphological aspects of lesions was observed. The morphologic characteristics of focal and diffuse lesions were 28%, therefore, more studies are necessary to identified clinical sings and etiologic environment in these cases of placentitis.

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Involuntary culling of dairy cows due to reproductive disorders

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Keywords: dairy cattle, involuntary culling, reproductive problems.

The involuntary culling of dairy cows is a complex practice that depends on the production goals of the farm (Silva et al. Rev Bras Saúde Prod Anim 5(1): 9-17, 2004). This study evaluated the main causes of waste on dairy farms in the central-northwest region of Rio Grande do Sul/Brazil. The data were obtained in a period of five years (2010-2014) from 2861 lactating cows from five farms (A, B, C, D, and E) which a mean of 114.4 ± 17.6 lactating cows/herd. The main causes of involuntary culling related to reproductive, mammary gland or locomotor system were evaluated. The variables analyzed were the MEANS, GLIMMIX PROC, PROC CORR, and PROC REG of SAS (SAS 9.3, USA, 2003). The average milk production/herd during the study period was 23.8 ± 0.6 kg/cow/day (A = 23.5 ± 0.8, B = 23.5 ± 0.7, C = 24.4 ± 0.9, D = 27.6 ± 0.9, E = 20.2 ± 0.7 kg/cow/day) (P < 0.0001). The mean pregnancy rate/AI (P/AI) was 41.5 ± 1.8 (A = 38.3 ± 5.0, B = 42.7 ± 4, C = 42 ± 4.5, D = 35.2 ± 1 6, E = 49.5 ± 3.1% P/AI) (P = 0.1247). Significant negative correlation was found between milk production and pregnancy rate/AI (P < 0.0001; r = -0.79). During the study period 22.2% cows in production activity (i.e. overall discard rate) (634/2861), were discarded. From this group, 77.3% (490/634) were discarded by involuntary culling reason, and 22.7% (144/634) for voluntary discarding. It´s remarkable that the reproductive problems represented the most important cause of culling in herds: 39.4% (250/634), and as well as among the involuntary culling: 51.1% (250/490) (P = 0.001). Diseases of the mammary gland and musculoskeletal system were detected in 38.9% (191/490) and 10% (49/490), respectively. Among the reproductive causes, repeat-breeder cows represented 40.8% (80/196), seropositive for Neospora caninum cows that had abortions: 27.5% (54/196), metritis/ postpartum endometritis: 20.4% (40/196), and 11.2% (22/196) of the cows had others reproductive causes (ovarian cysts, dystocia and obstetric surgery). No differences were observed between farms at the percentage of involuntary culling by reproductive causes (A = 44.2, B = 45.7; C = 48.4 D = 33.1; E = 35.6%) (P = 0.2131). However, the total percentage of discard from farms A and B were smaller (15.7% and 14.6%, respectively); while the farms C, D, and E showed bigger culling rates 24.4%, 25.7% and 24.6%, respectively (P = 0.0129). We concluded that reproductive problems are the leading cause to involuntary culling in the dairy farms of this study. Management improvements and monitoring system for involuntary cutting should be carefully adopted to reduce economic losses.

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Reproductive performance of *Bos indicus* and *Bos indicus* x *Bos taurus* heifers: Effect of eCG and P4 level during TAI programs

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Keywords: *Bos indicus*, *Bos taurus* x *Bos indicus*, TAI.

The aim of this study was to evaluate the effects of P4 level and eCG treatment in the TAI programs for *Bos indicus* (Nellore) and *Bos indicus* x *Bos taurus* (crossbred) heifers. Heifers used in the study (n = 1989) included Nellore (n = 992) and Crossbred (n = 997) that were 14-24 mo of age (BCS: 3.08 ± 0.01, BW: 329.09 ± 0.66 kg). Ovarian ultrasonography was performed twice (7 days apart) on all heifers at the start of the study to identify heifers with a CL present. Heifers with a CL were submitted to a TAI program. Heifers without a CL at either ultrasonography were submitted to a puberty induction protocol (Rodrigues, Theriogenology, 82, 760, 2014). Only heifers with a CL that was detectable by ultrasonography 12 days after puberty induction remained in the study. The TAI program that all heifers received was as follows: D0 – Insertion of an intravaginal P4 device (CIDR 1.9g; 1st and 2nd use = High P4; 3rd and 4th use = Low P4; Zoetis, Sao Paulo, Brazil) and 2 mg (i.m.) of estradiol benzoate (Gonadiol; Zoetis); D7 – 12.5 mg (i.m.) of dinoprost tromethamine (Lutalyse; Zoetis); D9 – CIDR withdrawal and 0.5 mg (i.m.) of ECP (ECP; Zoetis). At this moment heifers were randomly assigned to receive either 0 (Control; 994) or 200 IU (eCG; 995) of eCG (Novormon; Zoetis); D11 – TAI was performed, 48h after CIDR withdrawal. On Days 9 and 11, a subgroup of heifers was evaluated by US in order to record the largest follicular diameter (Ø). Continuous variables were analyzed using the PROC MIXED and the binomial variables using the PROC GLIMMIX, both from SAS. Included in the models were effects of breed, group, eCG and P4 level. Differences were significant when $P < 0.05$. The follicular Ø on D9 was greater for crossbred heifers (10.8 ± 0.01 mm) than Nellore heifers (9.9 ± 0.02) and in heifers from Low P4 (10.7 ± 0.01) when compared to heifers from High P4 (9.9 ± 0.01). The follicular Ø on D11 was greater for heifers from Low P4 (11.8 ± 0.01) when compared to heifers from High P4 (11.4 ± 0.01) and Crossbred heifers tended to have a greater follicular Ø on D11 than Nellore heifers (11.8 ± 0.01 and 11.4 ± 0.01, respectively). Ovulation rate was greater for Nellore compared to Crossbred heifers (91.1% vs 88.0%, respectively). Crossbred heifers (63.0%) had greater conception rate than Nellore (57.8%). Crossbred heifers (58.4%) tended to have greater pregnancy rate than Nellore (54.1%). Furthermore, there was an interaction between P4 level and eCG on pregnancy rate. The High P4 heifers receiving 0 IU eCG (51.9%) had lower pregnancy rate than High P4 heifers receiving 200 IU eCG (62.4%). However, in Low P4 heifers, eCG treatments did not differ (0 IU: 56.6%, 200 IU: 54.1%). Differences between Crossbred and Nellore heifers synchronized with the same TAI program were observed and regardless breed, the eCG treatment increased pregnancy rate in heifers that received high P4.

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Development of bovine embryos *in vitro* in co-culture with mesenchymal stem cells and murine embryonic fibroblasts

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Keywords: in vitro bovine embryo production, mesenchymal stem cells, murine embryonic fibroblasts.

Mouse embryonic fibroblasts (MEFs) have been widely used as a feeder layer to support embryonic stem cells due to their capacity to release growth factors. Mesenchymal stem cells (MSCs) also release bioactive factors which support cell growth. This study aims to investigate the effect of co-culture of MSC from rat bone marrow or MEF as a feeder layer for *in vitro* production of bovine embryos. Oocytes from slaughterhouse were collected and matured (TCM 199 medium in incubator with a temperature of 38°C, 5% CO2 concentration and 95% relative humidity) in control condition (CTRL) or in co-culture with previously inactivated MSC or MEF with 10ug/mL of mitomycin C (Sigma-Aldrich). Fertilization was performed in CTRL condition for all groups, and the embryos were cultured from fourth day in CTRL, or in co-culture with inactivate MSC or MEF, thus the following groups were performed in IVM/IVF: (CTRL/CTRL) - maturation and embryonic culture in CTRL condition; (CTRL/MSC) - maturation in CTRL condition and embryonic culture with MSC; (CTRL/MEF) - maturation in CTRL and embryonic culture with MEF; (MSC/CTRL) - maturation with MSC and embryonic culture in CTRL condition; (MSC/MSC) - maturation and embryonic culture with MSC; (MEF/CTRL) - maturation with MEF and embryonic culture in CTRL condition and (MEF/MEF) - maturation and embryonic culture with MEF. Cell inactivation was performed using mitomycin C. The data was analyzed by chi-square test for oocytes and Kruskal-Wallis nonparametric with Dunn's post-test for embryos. No significant difference in oocytes metaphase II and apoptosis rates and in embryo cleavage rate at 4th day after the beginning of the in vitro culture was found among the oocytes matured in CTRL, MSC or MEF conditions. The rates of blastocyst formation, expanded, hatched and the total of blastocysts did not differ among experimental groups ($P > 0.05$) at 7th day of embryo development. At 8th day of embryo culture we observed a difference ($P < 0.05$) in hatched blastocyst rate which was higher in the CTRL/CTRL group (14.3±1.9%) when compared to MSC/MSC group (3.6±1.4%), however, the proportion of blastocyst, expanded and total blastocysts were not different ($P > 0.05$) among the groups. The number of cells in the inner cell mass, trophoblast cells, apoptotic cells and total cells were similar ($P > 0.05$) in the embryos cultivated at all experimental groups. We conclude that the co-culture in IVM or IVC with MSC or MEF did not affect the bovine embryos development.

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In vitro embryo development and gene expression in granulosa and cumulus cells from *Bos indicus* cows with different numbers of antral follicles

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Keywords: antral follicle population, embryo development, gene expression.

The objective of this study was to investigate if *in vitro* embryo development and gene expression in granulosa cells is affected by the antral follicular population (AFP) in Nelore cows (*Bos indicus*). The average number of AFP was determined in ovaries (n = 336) from 168 Nelore cows, and the ovaries of each cow were identified and kept separately. The mean number of antral follicles was 61.14 ± 30.43 per cow. Ovaries were then separated in three groups as follows: G-High, ≥92 antral follicles; G-Intermediate, 46-76 antral follicles; and G-Low, ≤31 antral follicles. *In vitro* embryo development was assessed using oocytes collected from 752 ovaries of 356 cows in 9 replicates. Oocytes were matured in vitro under standard conditions in groups of 15 oocytes in 100 mL of maturation of medium. Rates of cleavage, development to the blastocyst stage and embryo hatching were compared between groups. Statistical analysis was performed by logistic regression at $P < 0.05$. The cleavage and blastocyst rates did not differ between groups (76.6% [473/617] and 40.6% [251/617] in G-High; 77.5% [457/590] and 36.3% [214/590] in G-Intermediate; 79.5% [418/526] and 38.6% [203/526] in G-Low). The hatching rate was higher in G-High (16.5% [102/617]) compared with G-Intermediate (11.5% [68/590]; $p = 0.0129$) and G-Low (11.6% [61/526]; $p = 0.0179$). Total RNA was purified from granullosa and cumulus cells using Trizol. Quantitation and estimation of RNA purity was performed using a Nano-Drop spectrophotometer, and then 200 ng RNA per sample was reverse transcribed using iScript cDNA synthesis kit (BioRad, ON, Canada). Quantitative Real time PCR was performed on a CFX384TM Real-Time System (BioRad) using iQ SYBR Green Supermix (BioRad). Transcript abundance was normalized to average of the internal control genes RP18S and Cyclophilin. Data were submitted to ANOVA and the averages compared by Tukey's HSD test. There was no significant difference in transcript levels of genes encoding steroidogenic enzymes (CYP19, StAR), cell proliferation and differentiation factors (TGFB1, LIFRa and BMPR2), hormones (AMH), and hormone receptors (FSHr, PGr). These findings suggested that the antral follicular population doesn't affect in vitro embryo development in Nelore cows, as well as the expression profile of genes involved in cell proliferation and follicular growth. However, the higher hatching rate suggests that embryos from high AFP cows have superior quality.

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Different associations between GnRH analogue and prostaglandin for treatments of ovarian cysts in dairy cows

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Keywords: bovine, pathologies, reproductive efficiency.

Ovarian cysts are common in dairy cattle. Successful treatment of the cyst is the regression of the structure and corpus luteum formation (CL) and rapid return to regular estrous cycles. The literature mentions occurrence of cystic structures with luteinized wall, in dairy cows, and the possibility of a beneficial effect on treatment with prostaglandin analogues associated with GnRH. The objective of this study was to evaluate the effectiveness of an analogue of GnRH, the gonadorelin (GO), associated or not with a prostaglandin analogue, sodium cloprostenol (CS), in different protocols for treating cysts. We used 36 Holstein cows with ovarian cysts belonging to four dairy farms located in the southern state of Minas Gerais in which animals were managed in semi confinement. The diagnosis was confirmed by ultrasonography (Mindray® M5), whereas ovarian cysts as anecogenic structure of more than 20 mm in diameter. The animals were randomly divided into five treatments: G1 (n = 16): 2 mL of saline solution (control group), G2 (n = 31), 0.5 mg of GO (Fertagyl® MSD-Brazil); G3 (n = 28): 0.5 mg of GO and 10 days, 0.53mg CS (MSD-Ciosin® Brazil), G4 (n = 29) 0.5mg GO and 0.53mg CS at the same time, and G5 (n = 32): 0.5mg GO and two doses (0.53mg) of CS, the first together with GO and the second 10 days after. The application of cloprostenol along with the GnRH analogue (G4 and G5) aimed to improve the effectiveness of the treatment, mainly for luteinized cysts. The application of cloprostenol 10 days later (G3 and G5) aimed to cause lysis of possible CL formed with pretreatment and acceleration of returning to reproduction. The Treatment was effective when the second evaluation did not detect a cystic structure and luteal tissue mass was found. The Data were assessed for normality. The efficiency of treatments was compared by X2. The average of the different variables were submitted to ANOVA and compared between treatments by Tukey test at 5% significance level. There was no farm effect ($P > 0.05$). The efficiency of treatments was: 18.75%^{a}, 54.84%^{b}, 53.51%^{b}, 79.31%^{c}, 81.25%^{c}, the treatment interval to the first service was: 61.22 ± 17.87^{a}, 44.54 ± 16.44^{b}, 30.87 ± 12.63^{c}, 26.19 ± 14,25^{c}, 18.33 ± 10.18^{d} days and the treatment interval to conception: 71.87 ± 21.85^{a}, 60.76 ± 19.38^{b}, 48.34 ± 16.96^{c}, 46.12 ± 15.61^{c}, 35.07 ± 14.32^{d} days, for groups 1 to 5, respectively. The association between GO to CS at the start of treatment was efficient ($P < 0.05$) to improve the cure rate of the cysts (G4 and G5). The application of prostaglandin analogue 10 days after the start of treatment (G3 and G5) led to faster return of the reproductive activity and conception ($P < 0.05$). It is concluded that the combination of cloprostenol gonadorelin is beneficial in the treatment of ovarian cysts in both situations, i.e. when applied along with the GnRH analogue and also 10 days later.

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Distribution of gonadotropin-releasing hormone (GnRH) neurons in the preoptic area and hypothalamus of cow

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Keywords: gonadotropin-releasing hormone, hypothalamus, neuroendocrinology.

GnRH is the pivotal hormone to control of mammalian reproduction. It is synthesized by hypothalamic neurons and packaged in storage granules that are transported through the axons to the external zone of the median eminence (Seeburg et al., 1987, Rec Prog Horm Res. 43, 69–98). The objective of this study was to characterize the distribution and number of GnRH neurons in the preoptic area and hypothalamus of cattle. Beef cow heads (n=2) were collected from a local slaughterhouse and were perfused through the carotid arteries with 4 liters of heparinized saline solution (5 Units/mL), and 2 liters of 4% paraformaldehyde within 30 min of death. The hypothalamus and preoptic area (25x30x30 mm) of brain were dissected at the Reproduction Research Laboratory of the University of Saskatchewan and fixation was continued 72 hrs by immersion in 4% paraformaldehyde at 4°C. Samples were sequentially dehydrated in 10%, 20%, and 30% of sucrose in Phosphate Buffered Saline (PBS). After each sample sank in the 30% hypertonic solution of sucrose (7 days), tissue block was frozen at - 80°C and sectioned at 50µm thickness using a cryostat microtome from the preoptic area to mammillary area (410 sections). Each section was placed into cryoprotectant solution (30% ethylene glycol and 30% sucrose in PBS with 0.1% of sodium azide), and they were stored in -20°C until further use. Every 20th free-floating section was immunostained for GnRH by incubation in 1:2500 dilution of mouse anti-GnRH monoclonal antibody (EMD Millipore Corporation, Telemuca, USA) for 72 hours at 4°C followed by 1:250 of HRP-tagged goat anti mouse IgG (EMD Millipore Corporation, Telemuca, USA) for 24 hours. Immunoreaction was revealed by using 3,3' diaminobenzidine tetrahydrochloride (DAB, SurModics, Eden Priarie, USA). Specificity of the staining was verified by omitting the primary antibody. Presence of GnRH reactive neuron cell bodies (perikaryon region) and axons was recorded in different regions of the preoptic area and hypothalamus with 10x and 20x objective lens on a Zeiss microscope. A total of 205 perikaryia was identified in the two brain samples. The distribution of GnRH neurons was 43.3% in the preoptic area, 13.4% in the anterior hypothalamus, 41.2% in the medial hypothalamus, and 2.0% in the posterior hypothalamus. There was a high concentration of GnRH positive neurons in the Arcuate Nucleus and Diagonal Band of Broca. Axons were observed in groups or isolated single nerve fiber throughout the preoptic area and hypothalamus, and were detected in a high density in the median eminence. In conclusion, GnRH neurons in cow were accumulated in the Diagonal Band of Broca in the preoptic area, and the Arcuate Nucleus of medial hypothalamus. This study will allow future research to determine the pathways of neuroendocrine control of GnRH secretion.

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Effect of administration of different doses of estradiol followed by progesterone on gene expression of endometrial estrogen and progesterone receptors in non-cyclic recipient mares

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Keywords: endometrial receptors, gene expression, non-cyclic mares.

The aim of this study was to evaluate the effect of administration of different doses of estradiol benzoate (EB) followed by long-acting progesterone (LA P4) on gene expression of endometrial estrogen and progesterone receptors in non-cyclic embryo recipient mares. Fourteen mares were evaluated during anestrus and distributed into two groups: 10 mg BE+P4 (n=7), which received three decreasing doses (5, 3 and 2 mg on consecutive days) of intramuscular EB (Estrogin® - Farmavet), followed by administration of 1.500 mg of intramuscular LA P4 (Sincrogest® - Ourofino) 24 hours after the last dose of EB; and 5 mg EB+P4 (n=7), which received two decreasing doses (3 and 2 mg on consecutive days) of EB followed by administration of 1.500 mg of LA P4, 24 hours after the last injection of EB. As the control group, seven of these mares were reevaluated and used during the cyclic phase. To measure the gene expression of estrogen and progesterone uterine receptors, biopsies were performed immediately before the initiation of treatment with EB (M1), 24 hours after the last administration of EB (M2) and five days after injection of LA P4 (M3). In the control group, biopsies were performed in estrus, when uterine edema (score 2-3) and the presence of ≥35 mm in diameter follicles were detected (M2); and in diestrus, on day five after ovulation (M3). Gene expression analysis of estrogen receptor alpha (ERα), beta (ERβ) and progesterone (PR) were performed by real time RT-qPCR, using beta-2-microglobulin (B2M) as the reference gene. The Wilcoxon signed-rank test for paired data was used to compare relative gene expression between the studied moments. Expression of ERα tended to be higher in 1.88-fold (P=0.06) after administration of EB and in 1.34-fold (P=0.06) after administration of LA P4 in group 10 mg EB+P4, which was not observed in group 5 mg EB+P4 (P>0.05). When gene expression dynamics were compared between groups, there was a 1.21-fold increase in PR expression when M3 was compared to M2 in 10 mg EB+P4 group, which tended to be different (P=0.06) from the 1.73-fold reduction found in the control group when PR expression between diestrus and estrus were compared. No differences were observed when the dynamics of ERα and ERβ expression in M3 in relation to M2 were compared between groups (P>0.05), which were reduced after administration of LA P4 or ovulation. No differences were detected when the mRNA expression of ERα, ERβ and PR from 5 mg EB+P4 group were compared to the control (P>0.05) or 10 mg EB+P4 groups (P>0.05). In conclusion, the administration of 10 mg of EB followed by 1.500 mg of LA P4 was not able to reduce the endometrial gene expression of PR after LA P4 injection, as observed in cyclic mares in diestrus, and the dose of 5 mg of EB followed by LA P4 promoted the most similar ERα, ERβ and PR gene expression changes to those observed in cyclic mares.

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Effect of melatonin on DNA fragmentation and in vitro maturation of bovine oocytes subjected to heat shock

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Keywords: DNA fragmentation, heat shock, *in vitro* maturation.

The aim of this study was to evaluate the effect of different concentrations of melatonin added to the medium IVM in DNA fragmentation and maturation of oocytes subjected to heat shock. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in factorial experiment design 3x2. Three concentrations of melatonin (0 M, 10-6 M and 10-4 M; M5250 - Sigma, St. Louis, MO , USA) added to the medium and two MIV incubation conditions (conventional: 24 hours at 38.5°C and 5% CO_2; or heat shock: 12 hours at 41ºC followed by 12 hours at 38.5ºC and 5% CO_2) were tested, resulting in treatments: M1 (0 M; 38.5ºC; n = 156), M2 (10-6 M; 38.5ºC; n = 154), M3 (10-4 M; 38.5ºC; n = 161), M4 (0 M; 41ºC; n = 154), M5 (10-6 M; 41ºC; n = 143) and M6 (10-4 M; 41ºC; n = 159). The IVM was performed in Nunc plate containing 400 µL of TCM-199 (Tissue Culture Medium 199 - Invitrogen, California, USA) supplemented with 20 µg/mL of FSH (Pluset®, Calier Laboratories, Spain) and 10% of estrus cow serum. After the maturation period, the cumulus-oocytes complex were denuded in a solution of PBS plus 0.1% hyaluronidase (Sigma, St. Louis, USA) by vortexing for 5 minutes and washed twice in PBS containing 0.1% PVP. The oocytes were fixed in 4% paraformaldehyde in PBS for one hour and evaluated by the TUNEL assay (deadend ™ Fluorometric TUNEL System - Promega, Madison, WI, USA) about the percentage of TUNEL positive oocytes (DNA fragmentation) and percentage of nuclear maturation (percentage of oocytes in metaphase II). Four replicates were performed. Data were analyzed by Proc Genmod of SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA) considering effects of repetition, melatonin concentration, incubation conditions and interaction between the factors. Values shown are the mean ± s.e.m. Addition of melatonin did not affect ($P > 0.05$) the percentage of TUNEL positive oocytes (M1 = 2.1% ± 0.7; M2 = 1.9% ± 1.9; M3 = 1.9% ± 1.3; M4 = 5.1% ± 2.7; M5 = 2.3% ± 1.5; and M6 = 1.9% ± 0.7) and there was no interaction between concentration and incubation conditions. Melatonin did not affect the percentage of nuclear maturation in the temperature of 38ºC ($P > 0.05$), however, in the heat shock, the percentage of maturation was higher in M6 treatment when compared to M4 ($P < 0.05$) (M1 = 85.8% ± 2.9^{a}; M2 = 84.0% ± 2.8^{a}; M3 = 79.5% ± 2.9^{ab}; M4 = 61.6% ± 6.9^{c}; M5 = 62.8% ± 8.5^{cd}; M6 = 72.6% ± 5.3^{bd}). The DNA fragmentation was not influenced by melatonin supplementation to the medium MIV. However, there was an increase in the percentage of maturation of oocytes subjected to heat shock in maturation medium with a concentration of 10-4 M in comparison with 0M concentration.

Acknowledgments: Fapemig, CNPq and Embrapa.

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Cortisol effect and its receptor on bovine embryo production

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Keywords: bovine embryo, glucocorticoid, RNAi.

Cortisol, the main glucocorticoid (GC) found in cattle, acts in several physiological processes, playing a key role at the beginning of pregnancy, because it regulates mechanisms involved in embryo implantation process in the endometrium (Michael; Papageorghiou, Human reproduction update, v 14, p. 497-517, 2008). The main mechanism of GC action is via its interaction with glucocorticoid receptor (GR), which has been identified in bovine embryos. According to this the objective of this study was to evaluate the GC mechanism of action in bovine IVP embryos. In experiment 1 we check if GR is important for early embryonic development. For this we silenced the translation of mRNA for GR, using the RNAi technique. IVP Bovine zygotes were injected with siRNA for GR 16 hours after IVF and were IVC for 8 days until blastocyst stage to analyze embryonic development and quantification of mRNA and protein for GR. Cleavage, blastocyst rates, relative quantitation of mRNA for GR and fluorescence were subjected to ANOVA ($P < 5\%$). The relative quantification of mRNA for GR decreased in 2-4 cell embryos and blastocysts, as in immunofluorescence blastocyst ($P < 0.05$), revealing that there was the translation block. Regarding cleavage and blastocyst rates, they were reduced in the injected group (59.7% ± 5.0, 9.7 ± 1.5%, respectively) compared to control (81.2% ± 9.7; 27 0% ± 8.5, respectively) ($P < 0.05$), furthermore the embryos injected with siRNA for GR were of inferior quality. From the observation that the GR is important for early embryonic development the effect of adding different cortisol concentrations in in vitro embryo culture medium was evaluated in experiment 2. The embryonic development and gene expression (NRF1, COX, TFAM, HSP70, FASN, GLUT1) was assessed. For this CCO were IVM, IVF and subsequently IVC in SOF medium containing 0 (control); 0.01 ug/mL; 0.1 ug/mL or 1 ug/mL cortisol. There was no significant difference in the embryos treated with GC (66.6 ± 6 and 30.5 ± 8.9 to 0.01µg/ mL; 70.0 ± 5 and 35.6 ±10.1 to 0.1 ug/mL; 70.1 ± 11 and 27.7 ± 4.5 to 1 ug/mL, $P > 0.05$) compared to control (67.1 ± 11 and 34.8 ± 9.8) according to cleavage and blastocyst rates, respectively. Because of greater morphological similarities between group of 0.1 µg/mL and the control, this concentration was chosen for analysis of the relative quantification of mRNA. Thus, in vitro produced embryos were incubated from the 1st day with or without 0.1 µg/mL of Cortisol, and on the 8th day were analyzed for gene expression, however there was no difference between groups for any of the transcripts analyzed. Therefore, we conclude that GR is important for the early embryonic development in cattle, however its action is not directly related to interaction with GC.

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Effect of fetal bovine serum in FASN expression in bovine embryos cultivated *in vitro*

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Keywords: embryo, FASN, fetal serum.

Supplementation of the medium with fetal bovine serum (FBS) is widely used to increase the embryo rate, however presents a drawback because it is related to increased intracellular lipid accumulation in embryos (DODE, RBRA, v. 37, 145-150 , 2013). The action of FASN enzyme can be related to this process, since it is responsible for the synthesis of palmitate fatty acid from the precursors malonyl-CoA, acetyl CoA and NADPH (URSTAD-JENSEN, BBA- Mol. Cell Biol. Lipids, v. 1821, 747-753, 2011). Thus, the aim of this study is to evaluate the FASN expression in bovine embryos produced supplemented with different concentrations of FBS. Bovine ovaries were collected at a local slaughterhouse and the cumulus-oocyte complexes (COCs) were matured in vitro in TCM-199 supplemented with 10% FBS, FSH, LH and antibiotic for 22 hours at 38.5°C in 5% CO_2. For IVF, COCs and sptz were co-incubated in TALP medium supplemented with FERT-penicillamine, hipotaurina, epinephrine, heparin and BSA under the same conditions mentioned for IVM. After 24 hours of co-incubation presumptive zygotes were then distributed in droplets cultivation in SOF medium supplemented with BSA and antibiotics according to the experimental groups (0, 2.5, 5 and 10% FBS). For counting the number of total cell, embryos were stained with fluorochrome Hoechst 33342. For the analysis of gene expression, the mRNA was extracted by Trizol® method (CA, USA), subjected to reverse transcription with the help of High- kit Capacity cDNA Reverse Transciption (CA, USA) and then analyzed using an assay relative quantification PCR (kit Power SYBR Green® PCR Master Mix) (CA, USA). Statistical significance was estimated by ANOVA with Tukey's post-test, adopting the significance level of 5%. Regarding the blastocyst rate, a significant difference ($p < 0.05$) was observed between the group without adding FBS (15% ± 7.6) compared to the groups with the addition of SFB - 2.5; 5 and 10% (39% ± 5.9; 37% ± 44% ± 3.9 and 10.2, respectively). With respect to counting the number of embryonic cells, the group supplemented with 2.5% FBS performed better than the non supplemented group with SFB (136 ± 8.5 and 108 ± 12.4, respectively) ($P < 0.05$), however this group did not perform significantly better than the other groups. Furthermore, in the analysis of gene expression, FASN gene regulation was not altered in *in vitro* cultured embryos regardless of the addition of FBS ($P <0.05$). Thus, it is concluded that the culture of bovine embryos with different concentrations of fetal calf serum does not affect FASN gene expression. However, supplementation contributes to embryo development in a quantitative and qualitative way, corroborating previous studies.

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Genetic paternal effects on ovary characteristics and ovarian structures of canchim (*Bos indicus* vs *Bos taurus*) heifers: preliminary data

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Keywords: heifers, physiology, ovary.

The aim of this study was to identify the influence of the paternal genotype on ovarian characteristics of Canchim heifers. Heifers (n=140) were evaluated by transrectal ultrasonography (US; Mindray, DP 2200VET, Shenzhen Mindray Bio-Medical Electronics Co., Shenzhen, China) twice with a 14 d interval to detect the presence of a CL. The presence of a CL was not detected in 45 heifers following evaluations, and 32 heifers were randomly selected (16.0 months; 211.0 ± 3.3 kg) daughters of 6 bulls (A, B, C, D, E and F). Heifers were allocated in a grazing intensive pasture system at an experimental station of the Brazilian Agricultural Research Corporation (EMBRAPA), located in São Carlos, state of São Paulo, Southeast of Brazil. US evaluations were performed every 14 days, from January to April 2015. The ovaries were classified according to their diameter (OC) as: I (< 1.5 cm); II (1.6 cm to 2.5 cm); III (2.6 to 3.5 cm); IV (3.6 to 4.5cm). The largest follicle (LF) and the second largest follicle (SLF) present on the ovaries were also recorded. Statistical analysis were performed using the GLIMMIX procedure of SAS® considering the effects of sire and repetition and the results were presented as least squares mean ± SE. Results were significant when $P < 0.05$. Heifers daughters of bulls A, C and F had greater CO (2.0 ± 0.07, 2.1 ± 0.08 and 2.1 ± 0.05, respectively) than heifers daughters of bulls B, D and E (1.8 ± 0.06, 1.8 ± 0.05 and 1.9 ± 0.06, respectively). Furthermore, heifers daughters of bulls A, C, E and F had greater MF (10.3 ± 0.39; 10.1 ± 0.44; 10.4 ± 0.34 and 10.4 ± 0.29mm, respectively) than the daughters of bulls B and D (0.34mm ± 8.9, 9.1 ± 0.27, respectively). In addition, the SMF of heifers daughters of sires A, C, E and F (5.9 ± 0.31, 6.2 ± 0.36, 6.2 ± 0.28 and 6.1 ± 0.24mm respectively) was also greater than heifers daughters of bulls B and D (5.3 ± 0.28 and 5.4 ± 0.22mm, respectively). This study corroborated data from the literature that showed genotype (sire) effects in reproductive tract characteristics of females and also in the development of ovarian structures.

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Efficacy of half dose of Lutalyse® for reducing progesterone plasma concentrations in bovines

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Keywords: lutalyse, luteolysis, progesterone.

The use of Dinoprost in lactating cows is efficient in inducing luteolysis after 72 hours (P4≤1.0mg/ml) in 91.3% of the animals (Steverson et al., 2010). Based on this data, the aim of this study was to evaluate the efficacy of half a dose of Lutalyse® (Dinoprost Trometamina – Zoetis, São Paulo, Brazil). The study was conducted in 3 commercial farms: the first farm with 54 Holstein heifers (Senador Firmino/MG); the second farm with 108 crossbreed animals (Fama/MG, 54 heifers and 54 cows) and the third farm with 108 Nelore animals (Governador Valadares/MG, 54 heifers and 54 cows). The experiment was carried out the same way for all three farms: after day 6 of the estrous cycle, the animals were randomly divided into 3 groups: 1) Control Group; 2) Lutalyse® full dose group (25mg) and 3) Lutalyse® half dose group (12.5mg). Each group was divided into 2 subgroups (from day 7 to 11 and from day 12 to 17 of the estrous cycle). Behavioral signs of estrus was considered Day 0 of estrous cycle. Two blood samples were collected, the first one immediately before the administration of Lutalyse® and the second one 36 hours after. Based on other studies (Steverson et al., 2010), luteolysis occurred if P4 concentration was higher than 1.0 ng/mL before the administration of Lutalyse and lower than 1.0 ng/mL 36 hours after. Animals with low P4 concentrations (n=9; <0.1 ng/mL) before Lutalyse administration were characterized as non-ovulated and removed from the analysis. Radioimmunoassay was used to measure P4 concentrations and data was analyzed using ANOVA (GLM procedure, SAS Inst. Inc, USA). There was no interaction between treatments and subgroups (P>0.1). No interaction between calving order, breed and treatments on P4 concentration reduction (P>0.1). Average P4 concentration before treatment and 36 hours after was 4.83±0.20 ng/ml and 2.15±0.17 ng/ml (n=261), respectively. P4 concentration before administration did not differ (4.17±0.20 [89]; 4.79±0.24 [87]; 4.99±0.24 [85]; P>0.1) between full dose, half dose and control group, respectively. However, P4 concentration 36 hours after Lutalyse® administration was similar for full dose groups (0.46±0.07 [89]) and half dose groups (0.78±0.12 [85]), and both groups showed lower P4 concentrations than the control group (5.23±0.28 [87]). Overall, a full dose treatment had the same efficacy (90.0% [81/89]) as a half dose (83.7% [81/89]) and both treatments were better in decreasing P4 concentrations than the control group (8.0% [7/87]). Efficacy results were similar among the farms, considering full and half dose groups (Farm 1= 94.4% [17/18] and 88.2% [15/7]; Farm 2= 91.7% [33/36] and 85.7% [30/35] and Farm 3= 88.6% [31/35] and 78.8% [26/33], respectively) and had higher efficacy than the Control group (Farm 1= 11.8% [2/17]; Farm 2= 11.1% [4/36] and Farm 3= 2.9% [1/34]). Half dose of Lutalyse® (12.5mg) had efficiently reduced P4 concentrations 36 hours after the administration.

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The impact of neonatal treatment with a GnRH agonist on reproductive and metabolic endpoints in prenatally androgenised sheep

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Keywords: androgenised, ewes, GnRH agonist.

Aims – The minipuberty (MP) is a transitory period after labor, characterized for the abrupt reduction in gonadotropic axis (for few weeks) which persists until the beginning of the puberty. For its features it may be considered a possible window for therapeutic interventions (Jansen HT et al., Endocrinology 152:4288, 2011). The present study evaluated whether a GnRH agonist (leuprolide acetate i.m.) could change the development of typical features of PCOS such as anovulation and insulin resistance at adult age (19 months). Materials and Methods: Animals – Overall, 49 adult sheep from Corriedale breed were obtained from a farmer from the state of RS after the approval of Animal Ethics Committee (CEUA –UFSM). The androgenisation protocol consisted in the administration of testosterone propionate in the mothers (Androgenol®, Juatuba, Brasil) 100 mg i.m. biweekly from the day 30 to 90 of gestation. No treatments were performed in the control group. Part of androgenized sheep offspring (n=4) received up to 48h after birth 5m i.m. of leuprolide acetate (LA). Along the time, 18 ewes were evaluated: 7 (4 controls and 3 androgenised) were euthanized at 13 months of age, and 11 (5 controls, 3 androgenised and 3 androgenised and treated with LA) euthanized at 19 months of age. Results: Reproductive abnormalities – As expected, the ano-genital distance was higher in androgenized females at birth (mean + SD) of 8.0 ± 0.78 cm in comparison to controls 0.58 ± 0.18 cm ($P < 0.0001$, T Student). Control sheep exhibited a marked ovulatory production of Progesterone along 6 consecutive weeks while androgenized animals shown a clear disruption of ciclicity (66%). No significant changes in the pattern of ovulation could be demonstrated in prenatally androgenized sheep treated with LA. Moreover, evidence of insulin resistance was observed in both androgenized groups through the intravenous glucose test (10 mg/kg) ($P < 0.01$, T Student). Conclusions: According to our preliminar results, the neonatal treatment with LA was not able to avoid the development of metabolic (insulin resistance) and reproductive (at least ovulation) in an animal model of PCOS.

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Roles of cell death in sexual dimorphism during preimplantation development

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Keywords: apoptosis, female embryo, sex dimorphism.

Female bovine embryos progress at lower rates and originate smaller blastocysts than male counterparts. However, when and how sex dimorphism starts to occur is not clear. The knowledge of singularities among female and male embryos can be useful for human assisted reproductive medicine, when X-linked disorders risk is detected, and for livestock sex-specific breeding programs. The aim of this study was to characterize the roles of cell death in development of female and male embryos. Using sex-sorted semen from three different bulls for fertilization, we compared bovine sex-specific embryos at 96, 120 and 144 hpi, assessing quality parameters. For that, embryos with more than 4 cells at 96 and 120 hpi; or more than 8 cells at 144hpi were fixed in 4% PFA and stained for caspase 3 (apoptosis marker) by immunofluorescence. Nuclei were counterstained using HOECHST. Cell fragmentation was estimated by number of enucleated cytoplasm fragments inside zona pellucida. Results were grouped as Female and Male, since consistency among bulls 1, 2 and 3 data was detected. The analysis was performed as follows: I. Total cell number; II. Apoptosis (rate of apoptotic cells in embryos); III. Fragmentation (rate of fragmented cells in embryos). The effect of time over each embryo sex (Kruskall-Wallis/ Dunn, F96xF120xF144; M96xM120xM144) and the effect of sex over each moment (Mann Whitney, F96xM96; F120xM120; F144xM144) were analysed using GraphPad InStat (p=0.05). In this study, 379 embryos (65-93 per group) were evaluated, obtained in three replicates. As expected, mean cell numbers increased from 96 to 144 hpi (F: 11.88 ± 0.53^{a}, 15.42 ± 1.04^{a}, 28.1 ± 2.44^{b}; M: 11.33 ± 0.64^{A}, 16.62 ± 1.12^{B}, 40.19 ± 2.86^{C}). Comparing Female vs Male, decreased cell numbers was detected at 144hpi (F: 28.1±2.44, M: 40.19±2.86*). Regarding apoptosis, in female groups the higher rate was detected at 96hpi (23.08 ± 2.54^{a}, 14.62 ± 2.0^{b}, 14.46 ± 1.94^{b}). For male embryos, at 144 hpi the lowest rate was detected (21.40 ± 2.68^{A}, 15.23 ± 1.63^{A}, 9.71 ± 1.43^{B}). Female embryos presented higher apoptosis rates at 144 hpi (F: 14.46±1.94, M: 9.71±1.43*), in reflex to a cell number decrease and to a tendency (p=0.07) of increase in number of apoptotic cells (F: 2.91±1.50, M: 2.38±1.52). Cell fragmentation remained unaltered for female embryos (17.19±1.67, 15.55±1.55, 14.97±1.34), and for male embryos decreased at 144 hpi (15.76 ± 1.36^{A}, 13.11 ± 1.01^{A}, 10.98 ± 1.19^{B}). Female embryos presented higher fragmentation rates comparing to male group at 144 hpi (F: 14.97±1.34, M: 10.98±1.19*), and this increase was also due to a numeric increase in fragmented cell numbers (3.47±0.22, 2.73±0.17*). These new results lead us to propose that sex dimorphism is established at 144hpi in bovine, during morula-blastocyst transition, and cell death is involved in this process.

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Influence of follicle diameter and time of cleavage on embryo production and profile of histone H3K4 methylation in bovine blastocysts

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Keywords: early cleavage, histone modifications, late cleavage.

The diameter of follicles from which oocytes are retrieved and the time of the first cleavage potentially influence competence and epigenetic reprogramming in early embryonic development. This study aimed to investigate the developmental potential and the occurrence of monomethylation at lysine 4 of histone H3 (H3K4me) in bovine blastocysts of early, intermediate and late cleavage after fertilizing oocytes from small and large follicles. Small (≤ 2 mm) and large follicles (4-8 mm) from slaughterhouse ovaries (n = 1982) were punched. Among the collected oocytes, 699 from ≤ 2 mm diameter follicles and 639 from 4-8 mm diameter follicles were subjected to *in vitro* maturation and fertilization. The presumptive zygotes were cultured, and cleavage rates were evaluated by separating the embryos into early (≤ 28 h post-IVF), intermediate (> 28 h and ≤ 34 h post-IVF) and late (> 34 h and ≤ 54 h post-IVF) groups. The blastocyst rates were further evaluated after 7 and 8 days of culture. The blastocyst and cleavage rates were compared by logistic regression and differences were considered statistically significant at a confidence level of 95% ($P < 0.05$). Among blastocysts, we randomly selected 5 embryos per group and investigated H3K4me by immunofluorescence. The percentage of late-cleaved embryos was higher ($P < 0.05$) than that of early-cleaved embryos for 4-8 mm follicles (late: 30% vs. early: 19%) and ≤ 2 mm follicles (late: 33.8% vs. early: 16.6%), indicating that most embryos start the first cell division cycle later. The blastocyst rate for the 4-8 mm group (36.3%) was higher than that for the ≤ 2 mm group (22.9%, $P < 0.05$). In addition, the blastocyst rates for the early and intermediate cleavage groups (45.3% and 33.8%, respectively) were higher than that for the late cleavage group (13.5%, $P < 0.05$). The blastocysts from all the groups displayed H3K4me staining by immunofluoresence; the staining was particularly intense in the trophectoderm region and was weak or absent in the inner cell mass region. Data from this study demonstrate that higher blastocyst embryo rates are obtained from embryos that cleave within 34 hours after fertilization and from those produced from follicles of 4 to 8 millimeters in diameter, indicating a greater ability of these embryos to develop to the stage of embryonic preimplantation. Furthermore, the presence of monomethylation at H3K4 in all the evaluated blastocysts suggests that this histone modification plays a key role in maintaining embryo viability at this important developmental stage.

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Follicular fluid influence on oocyte competence: identification of factors involved in oocyte quality and embryonic development

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Keywords: follicular fluid, *in vitro* production, oocyte competence.

In the process of *in vitro* production of embryos (IVP), one of the most important stages for embryo development is *in vitro* maturation (IVM), since essential events that influence the quality of the future embryo occur during this phase. The oocyte removal from the follicular environment results in the spontaneous resumption of meiosis, interfering in the oocyte capacitation period *in vitro*. Thus, follicular conditions can result in differences that can later impact the embryo phenotype. The objective of this study was the investigation of fundamental molecules present in follicular fluid (FF) that are possibly involved in oocyte capacitation, embryo viability and competence. For this analysis, bovine ovaries were obtained in commercial slaughterhouse. Follicles of 7-8mm were individually aspirated and Cumulus-oocyte complexes (COCs) with their respective FF (5 follicles/ovary) were separated. *In vitro* maturation of COCs from the same ovary were made in 50µl drops of culture medium using Well of the Well system for 22-23 hours in an incubator at 38.5°C and 5% CO2 and high humidity. Oocyte fertilization was made at the same atmospheric conditions of the IVM for 18 hours, followed by in vitro culture (IVC) until D7 in SOFaa medium containing 5% of FCS, 20 L / mL of essential amino acids and 10 uL / mL of nonessential amino acids. Cleavage and blastocyst rates were evaluated at 40hpi and 168hpi respectively. Glucose, cholesterol and pyruvate molecules present in the FF were quantified by fluorimetric assays using commercial kits and analyzed according to the cleavage and blastocyst rates. The data obtained was then analyzed using the Wilcoxon-Mann-Whitney test (n = 6 replicates) on GraphPad Prism 5.0 software. The results showed a higher glucose level in the FF of cleaved embryos than of non-cleaved ones (CI = 0.234 ± 1.327µM; NCl = 0.554 ± 0.108 µM). Likewise, oocytes that were able to develop into blastocysts were obtained from FF with higher pyruvate and cholesterol concentration (cholesterol - Bl = 33.14µM ±1.98; NBl= 28.86µM ± 1.32), (pyruvate - Bl= 35.83µM ± 2.67; NBl= 28.42µM ± 2.30). These results indicate that glucose can be an important substrate for embryo cleavage and that the presence of cholesterol and pyruvate in the FF is essential for the development to blastocyst stage, thus resulting in higher oocyte quality, which is an essential factor for a better embryo development.

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The effect of insulin-like growth factor-I (IGF-I) on mitochondrial gene expression of bovine oocytes subjected to heat shock

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Keywords: heat shock, IGF-I, mitochondria.

Exposure of bovine oocytes to elevated temperature causes many cellular changes such as increased production of reactive oxygen species and reduced mitochondrial activity. Mitochondrial activity has been shown to be associated with expression of nuclear DNA (nDNA) and mitochondrial (mtDNA) encoded genes. There is evidence that the negative effect of heat shock on the oocyte mitochondrial activity was attenuated by insulin-like growth factor-I (IGF-I) supplementation during in vitro maturation (IVM). Therefore, the objective of this study was to determine the effect of IGF-I on mRNA expression of nDNA (TFAM: mitochondrial transcription factor A and ATP5S: ATP synthase subunit s) and mtDNA (ATP6: ATP synthase subunit 6 and COX1: cytochrome C oxidase subunit 1) encoded genes in bovine oocytes subjected to heat shock during IVM. Cumulus-oocyte complexes (COCs) recovered from slaughterhouse ovaries were distributed in control (22h at 38.5°C) and heat shock (14h at 41°C and 8 hours at 38.5°C) groups in the presence of 0 or 25 ng/mL IGF-I during IVM. After IVM, COCs were mechanically denuded by repeated pipetting for complete removal of cumulus cells. Denuded oocytes were stored at -80°C until RT-PCR. Groups of 30 oocytes per replicate were collected from each experimental group (n = 5 replicates) and submitted to total RNA extraction (RNeasy Mini kit, Qiagen). Reverse transcription (RT) reaction was performed using the Superscript III Kit (Invitrogen). Amplification of target genes was carried out using power SybrGreen® PCR Master Mix. The expression of genes ATP6, COX1, and TFAM ATP5S was determined by real time RT-PCR. Cyclophilin A expression was used as reference gene according to the RefFinder program. Relative gene expression values were obtained by ΔΔCt method corrected by the amplification efficiency for each gene (Pfaffl equation). Data were submitted to least squares analysis of variance using the SAS statistical software. There was no effect of temperature and IGF-I on ATP6, TFAM and ATP5S mRNA expression. Exposure of bovine oocytes to heat shock during IVM increased (Temperature: $P < 0.0005$; Temperature x IGF: $P < 0.01$) COX1 mRNA expression as compared to control. However, supplementation of heat shocked oocytes with 25 ng/mL IGF-I during IVM recovered COX1 gene expression to levels similar to the control group. In conclusion, IGF-I has a regulatory action in COX1 gene expression, possibly acting indirectly on the respiratory chain activity under heat shock.

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Follicular fluid thermoprotective role during *in vitro* maturation of bovine oocytes subjected to heat shock

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Keywords: follicular fluid, heat shock, maturation.

Follicular fluid (FF) is a component of oocyte microenvironment containing plasma factors and specialized molecules secreted by follicular cells and oocyte. Follicular fluid products play an important role in follicular growth and oocyte maturation. Heat stress has been shown to compromise the follicular microenvironment and oocyte maturation. Exposure of bovine oocytes to elevated temperature compromise oocyte maturation and developmental competence. Therefore, the objective of this study was to determine the role of FF on oocyte nuclear maturation and cumulus cells (CCs) expansion in cumulus-oocyte complexes (COCs) subjected to heat shocked. Follicular fluid was collected by aspiration from slaughterhouse ovaries during the winter months, processed and stored at -80ºC. Slaughterhouse COCs were matured in Maturation Medium (MM) (TCM199-Bicarbonate with 50 µg/mL gentamicin, 0.2 mM sodium pyruvate, 10 µg/mL FSH, 10 µg/mL LH and 1 µg/mL estradiol 17-b). COCs were distributed in positive control (MM + 10% fetal bovine serum at 38.5ºC for 22 h), 0% FF control (MM + 0% FF at 38.5ºC for 22 h) and heat shock (MM at 41ºC for 14 h followed by 38.5°C for 8 h) in the presence of 0, 10, 15 e 20% FF during in vitro maturation (IVM). After 22h IVM, CCs expansion was evaluated by image analysis (software ImageJ) of each COC before and after IVM (N = 5 replicates using 139-154 COCs/treatment). COCs were vortexed in 100 mg/mL hyaluronidase for 5 minutes to remove cumulus cells. Denuded oocytes were fixed in 3.7% formaldehyde for 30 minutes and stained with 1 mM Hoechst 33342 for 15 minutes to determine meiotic progression (N = 6 replicates using 126–136 COCs/treatment). Data were analyzed by ANOVA (SAS). Heat shock reduced CCs expansion ($P < 0.001$) from 3.38 ± 0.14 (positive control) and 3.00 ± 0.14 (0%FF control) to 1.91 ± 0.14 fold (0% FF heat shock). The proportion of metaphase II (MII) oocytes was reduced ($P < 0.001$) by heat shock from 84.0 ± 4.0% in positive control and 74.9 ± 4.0% in 0% FF control to 46.5 ± 4.0% in 0% FF heat shock. However, addition of 10.15 and 20% FF rescued the deleterious effect of heat shock in CCs expansion (2.62 ± 0.14, 2.63 ± 0.14 and 2.63 ± 0.14 fold for 10.15 and 20% FF, respectively) while the doses of 10 and 15% FF rescued nuclear maturation of heat shocked oocytes (64.5 ± 4.0% and 64.0 ± 4% for 10 and 15% FF, respectively) which was similar to 0% FF control at 38.5°C. In conclusion, addition of 10 and 15% FF to MM rescued oocyte expansion and nuclear maturation of heat shocked bovine oocytes, suggesting that FF factors prevent the deleterious effect of heat shock.

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Profile of mRNA expression of (pro)renin receptor and prorenin during luteinization and luteolysis in cattle

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Keywords: corpus luteum, PGF2α, progesterone.

The effect of renin independent-prorenin on the P4 synthesis in response to LH peak was proposed in the 80s (Sealey et al., Proc. Natl. Acad. Sci. USA, 82, 8705-9, 1986). Recently the (pro)renin receptor [(P)RR], which binds to prorenin and renin, was identified in the bovine corpus luteum (CL). Moreover, our research group demonstrated that intrafollicular blockade of (P)RR decreases P4 plasmatic levels during the initial process of luteinization. The aim of this study was to evaluate the profile of mRNA expression of prorenin and (P)RR during bovine luteinization and luteolysis. Thirty cyclic cows of European breed were synchronized with an injection of 500µg of sodic cloprostenol (PGF2α-IM). The estrus was observed and the ovulation was monitored by ultrasonography. The animals were randomly ovariectomized on days 5 (n=4) and 10 (n=5) after ovulation to characterize prorenin/(P)RR during CL formation. To study the profile of mRNA expression of prorenin/(P)RR during luteolysis, cows with CL of 10 days (0h) of estrus cycle received an injection of 500µg of sodic cloprostenol (PGF-IM) and were ovariectomized at 2 (n=3), 12 (n=3), 24 (n=4) e 48h (n=4) after PGF2α injection. The in vivo model was adapted from Shirasuna et al. (Domest. Anim. Endocrin., 43, 227-238, 2012) and confirmed by serum P4. The CL tissue was submitted to Trizol® (Invitrogen, Carlsbad, CA) protocol to extract total RNA, which were quantified by spectrophotometer (NanoDrop, Thermo Scientific, USA). The total RNA was treated with DNase (Promega, Madison, WI) and transcriptase reverse reaction was performed with iScript (Bio-Rad, Hercules, CA), according to the fabricant instructions. The genic expression was evaluated by qPCR and the variability in the quantification of mRNA was evaluated in relation to GAPDH. The results of mRNA expression of prorenin and (P)RR were evaluated by multi-comparison of means test least squares means (LSMEANS). All continuous variables were tested to normality using Shapiro-Wilk test and when necessary normalized. On the 10 day after ovulation (0h after PGF2α), the expression of prorenin mRNA (5.17±2.73) and (P)RR (1.99± 0.57) was significantly increase compared to 5 day of the estrous cycle (0.57±0.21 e 0.53± 0.12 respectively; $P<0.05$). After PGF2α treatment, results of mRNA expression of prorenin and (P)RR suggested a decrease in all hours tested (3.72±2.83; 3.43±0.84; 2.18±0.52; 1.02±0.12 e 1.56±0.56; 1.48±0.17; 1.06±0.10; 1.49±0.44, respectively) compared to 0h, except 12h after $PGF_{2\alpha}$. In conclusion, our results evidence the presence of prorenin/(P)RR in the bovine CL and suggest a major involvement on luteinization than in the luteolysis.

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Plasma progesterone profile and luteal characteristics in pregnant and non pregnant Saanen goats

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Keywords: cavity corpus luteum, estrous cycle, ovary.

The aim of this study was to associate the plasma progesterone profile (P4) in pregnant and non-pregnant Saanen does with the number of corpora lutea (CL) and presence of luteal cavity in an entire estrous cycle. A total of 23 does (64.1 ± 5.4 kg / 3.3 ± 0.4 BCS / 2.8 ± 1.4 years old) had their estrous synchronized, during the breeding season, using two doses of 37.5 ug d-cloprostenol (Prolise®, Tecnopec LTDA, São Paulo, Brazil) at seven days interval. After estrus detection, all does were mated. From the first day of the estrous cycle (day after ovulation), daily, blood was sampled to P4 measurement and ultrasound monitoring (Sonoscape®, Shenzhen, China) of the luteal dynamics was performed until luteolysis and subsequent ovulation or pregnancy at 21 days. Plasma P4 was assessment with a commercial solid phase radioimmunoassay (RIA) kit (Beckman Coulter®– Immunotech, Marseille, France). Data were analyzed by ANOVA, followed by Bonferroni test ($P< 0.05$). A total of 50 CLs from 10 pregnant does and 13 non-pregnant were assessed. Non-pregnant females showed greater frequency of solitary CLs (83.3%) than pregnant ones (16.7%). No difference between the presence of two (54.5% vs. 45.5%) or three (33.3% vs. 66.7%) CLs, as well as the presence or absence of luteal cavity (54.0% vs. 46.0%) on the pregnancy rate was observed. There was no effect of the number of CLs and the presence of luteal cavity on the plasma P4 in does that became pregnant or not. An effect in the day of estrous cycle and interaction between day x pregnancy on P4 values was found. Pregnant does had different plasma profile from 16th day of the cycle (16.7 ± 4.8 vs 10.2 ± 5.8 ng/mL) compared to non-pregnant does. Within the pregnant does group, a stabilization and maximum P4 values from the 8th day of the cycle (12.8 ± 2.2 ng/mL) to the 21st day was observed, with concentrations averaging 13.6 ± 2.9 ng/mL throughout time. In the non-pregnant group, a stabilization and maximum P4 values from the 6th day of the cycle (9.8 ± 2.8 ng/mL) to the 16th day was found, with concentrations averaging 12.3 ± 3.8 ng/mL throughout time. Furthermore, there was an intense hormone drop from the 17th day (5.9 ± 5.3 ng/mL) to achieve baseline in the 21st day (1.1 ± 0.8 ng/mL). In conclusion, non-pregnant does showed greater frequency of solitary CL. Although no difference in P4 values on the number of CL and presence or absence of luteal cavity in the physiological state of the doe were found, pregnant does demonstrated stabilization and maximum P4 production later in comparison with non-pregnant females.

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Main causes of abortion in beef herds in southern Brazil and Uruguay

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Keywords: *Leptospira sp*, reproductive management, *Ureaplasma sp*.

The pregnancy losses represent a significant in genetic and economy in the industry, that often leads to involuntary culling of females. Especially, if the cause comes from a contagious disease to other animals and/or humans (Grooms, Vet Clin North Am Food Anim Pract 20:5-19, 2004). The aim of this study was to determine the main causes of abortion in beef herds from two farms with an extensive management system. The herd with 10.832 *Bos taurus taurus* and mixed animals (A) was located in Uruguay, Melo/Cerro Largo. The other farm located in Cachoeira do Sul, RS/Brazil had 3.280 animals *Bos taurus taurus* (B) and neither of the farms used vaccines for diseases in their herds. The animals were separated by categories (nulliparous, primiparous, multiparous) and according to their reproductive management: artificial insemination (AI) and natural mating (NM). Blood samples from the farm A (152 females and 16 bulls) and B (90 females and 14 bulls) were collected. The serum was used for diagnosis of *Leptospira* sp with microagglutination test (MAT), and vaginal swab or preputial wash for *Ureaplasma* sp identification with nested-PCR. The statistical analysis were performed using MEANS e PROC GLIMMIX from SAS (SAS 9.3, USA, 2003). On the farm A: 57.2% (87/152) of females and 87.5% of bulls (14/16); and in the farm B: 78.9% (71/90) of females and 57.1% (8/14) of bulls were MAT-positive serum for *Leptospira* sp. *Ureaplasma* sp was identified on 28.3% (43/152) of females and 62.5% (10/16) of the bulls from farm A, and 40.0% (36/90) of the females and 57.1% (8/14) of bulls from farm B. The serotypes identified most frequently were *L. pomona* 87.2% (157/180) and *L. hardjo* 37.7% (68/180). Other serotypes with concomitant and a lower serology percentage were: *L. icterohaemorrhagiae*, *L. butembo*, *L. bratislava* and *L. canicola*. The abortion rate in MAT-positive serum females for *Leptospira* sp was 64% (101/158) and 25% (21/84) for MAT-negative serum females. *Ureaplasma* sp was responsible for 70.9% (56/79) of abortion (P = 0.0169). The abortion rate in seronegative females to Leptospirosis and negative for Ureaplasma was 6.6% (3/45). Higher abortion rate occurred in nulliparous, 67.5% (27/40), while on primiparous was 49.2% (31/63), and 46% (64/139) on multiparous. The management practice type did not interfere in the rate (P = 0.8242), because natural mating was responsible for 54.9% (84/153) of the abortions and artificial insemination for 42.7% (38/89). The advantage of using the AI was shown here by the lowest percentage of positive females for Ureaplasma, 4.5% (4/89), while females submitted to the NM had 49.0% (75/153) (P = 0.0001). Regarding the number of calvings, 84.6% (11/13) of nulliparous aborted. Furthermore, *Ureaplasma* sp and *Leptospira* sp led to significant gestation losses in both herds.

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Prorenin blocks forskolin effect and resumes meiosis in bovine oocytes

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Keywords: cAMP, cGMP, *cumulus* and oocyte cell.

Prorenin levels are increased in follicular fluid after LH surge. Recently, our group identified the presence of (pro)renin receptor [(P)RR] in bovine cumulus-oocyte complex (COC). Our aim was to evaluate the effect of prorenin in resumption of meiosis and in the levels of cAMP and cGMP in cumulus cells (CCs) and oocytes. For this, bovine ovaries were obtained in a local abattoir and approximately 20 COCs/ treatment were cultured in 200µl of TCM-199 for 15 hours at 39°C. The treatments were: positive control, negative control (FSK; 200µM), FSK and prorenin (10-10M), and FSK, prorenin plus aliskiren (ALK; direct renin inhibitor, 10-7M). Nuclear maturation was considered when oocytes reached the stage of metaphase I (MI) using10mg/mL of bisbezimide (Hoechst 33342) in fluorescence microscope. Statistical analysis was performed using SAS with significance of 5%. The rate of oocytes that reached MI was greater in the group treated with FSK plus prorenin (38.39%) compared with negative control (18.92%), and FSK, prorenin and ALK groups (8.68%, $P<0.05$). To determine the effect of prorenin on cAMP and cGMP concentrations in oocytes and CCs, COCs were cultured for 6 and 15 hours under the same conditions previously described and distributed as follows: positive control, negative control (FSK), prorenin and FSK plus prorenin. The concentrations of cAMP and cGMP were measured on CC (n=60 COCs) and oocytes (n=50) after 6h of culture using cAMP EIA kit (No. 581 001; Cayman Chemical) and cGMP EIA kit (No. 581 021; Cayman Chemical) according to manufacturer's instructions. Data were tested for normal distribution using the Shapiro-Wilk test and normalized when necessary. Variables from different treatments were compared by ANOVA. Nuclear maturation from COCs cultured by 15 hours were considered as controls. Oocytes treated with FSK plus prorenin reached higher MI percentage (49.95%) than negative control (25.59%; $P<0.05$), however lower percentage than positive control (83.12% MI) and prorenin (78.34%) groups. Intra-oocyte cAMP concentrations were slightly reduced in COCs treated with prorenin plus FSK (8.66 ± 1.20) compared to negative control (10.33 ± 0.88). The positive control (4.00 ± 1.52) and prorenin (3.00 ± 0.57) groups showed lower concentration of cAMP compared to the negative control. Furthermore, concentration of cAMP (15.08±6.7, 9.91±3.68, 2.02±1.43 and 5.05±4.66) and cGMP (0.42±0.19, 0.31±0.07, 0.78±0.4 e 3.61±2.96) in cumulus cells and cGMP in oocytes (0.57±0.09, 0.71±0.09, 0.78±0.12 e 0.60±0.01) did not differ following the treatments (FSK, FSK plus prorenin, positive control and prorenin, respectively). In conclusion, our results indicate that prorenin acts on resumption of meiosis in bovine and suggest a regulation in the concentration of intra-oocyte cAMP.

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Natriuretic peptide receptor 3 (NPR-3) is negatively regulated by LH + FSH in bovine cumulus cells

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Keywords: cumulus oophorus, expansion, natriuretic peptides.

The natriuretic peptides (NP) system consists of three distinct endogenous peptides: A-type NP (ANP), B-Type (BNP) and C-type (CNP), and three receptors: NP-1 receptor (NPR-1), NPR-2 and NPR-3. It has been demonstrated in mice (Zhang et al., 2010, Science, 330, 366-369) and pigs (Zhang et al., 2015, J Cell Physiol, 230, 71–81) that CNP produced by mural granulosa cells, binds to NPR-2 in cumulus cells and maintains the oocyte arrested at germinal vesicle stage. After the LH surge, expression of CNP in granulosa and NPR-2 in cumulus cells declines (Kawamura et al., Hum. Reprod. 26, 3094–3101). However, the role of NP receptors on meiotic regulation has not been systematically investigated in monovular species. Previous studies from our laboratory, using cattle as an experimental model, revealed an increase in CNP expression in granulosa cells after the LH surge. Moreover, we found that the three NPs stimulate meiosis resumption and cumulus cell expansion in cumulus-oocyte complex (COC) cultured with forskolin. The aim of this study was to evaluate the expression kinetics of NP receptors during in vitro maturation of bovine COCs cultured with or without gonadotropins. COCs were collected from ovaries obtained from a slaughterhouse, selected (quality 1 and 2) and cultured in TCM 199 for 3, 6, 9 and 12h, with or without FSH (0.5mg/mL) and LH (5.0µg/mL). In each time point, an image was captured from 10 COCs of each treatment and the total area was measured (µm2/COC; LeicaApplication) to evaluate cumulus expansion. To evaluate meiotic progression and expression profile of NP receptors in cumulus cells, 30 oocytes from each treatment and time point were used. The experiments were performed in quadruplicate. Transcript levels were quantified by qPCR and differences between groups determined using LSMean and Student's t test with 5% significance. We observed that up to 6 h of culture, over 80% of the oocytes remained in germinal vesicle in both groups. However, at 9 h and 12 h of maturation, meiotic resumption was observed in 86.9% and 99.0% of oocytes in the gonadotropin group, and 56.3% and 70.4% in the control group, respectively. In addition, we observed that COCs of the control group had similar cumulus area at 0 h during 12 h of culture. Contrarily, a three-fold increase in the COCs area was observed from 6 h to 12 h of maturation in the gonadotropin group. There was no difference in the transcript levels of NPR-1 and NPR-2 between treatments or time in culture. However, NPR-3 mRNA levels in cumulus cells decreased from 0 h to 9 h in the gonadotropin group. In the control group the highest levels of NPR-3 mRNA were detected at 12 h of culture. These findings indicate that NPR-3 expression is negatively regulated by FSH+LH during in vitro maturation of bovine COCs.

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Dynamic remodeling of endometrial extracellular matrix regulates embryo receptivity in cattle

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Keywords: cattle, endometrium, receptivity.

We aimed to evaluate in the bovine endometrium whether (1) key genes involved in endometrial extracellular matrix (ECM) remodeling are regulated by the endocrine peri-ovulatory milieu; and (2) specific endometrial ECM-related transcriptome can be linked to pregnancy outcome. In Experiment 1, pre-ovulatory follicle growth of cows was manipulated to obtain two groups with specific endocrine peri-ovulatory profiles: the Large Follicle Large CL group (LF-LCL) served as a paradigm for greater receptivity and fertility and showed greater plasma pre-ovulatory estradiol and post-ovulatory progesterone concentrations when compared to the Small Follicle-Small CL group (SF-SCL cows). Endometrium was collected on days 4 and 7 of the estrous cycle. Histology revealed a greater abundance of total collagen fibers in SF-SCL on days 4 and 7 endometrium. In Experiment 2, cows were artificially inseminated and, six days later, endometrial biopsies were collected. Cows were retrospectively divided into pregnant and non-pregnant (P vs. NP) groups after diagnosis on day 30. In both Experiments, expression of genes related to ECM remodeling in the endometrium was studied by RNAseq and qPCR. Gene ontology analysis showed an inhibition in the expression of ECM-related genes in the high receptivity groups (LF-LCL and P). Specifically, there was downregulation of TGFB2, ADAMTS2, 5 and 14, TIMP3 and COL1A2, COL3A1, COL7A1 and COL3A3 in the LF-LCL and P groups, and this was confirmed by qPCR. Results suggest receptivity is associated with a tight control of the abundance of ECM components and that disregulation could perturb the initial embryonic contact with maternal endometrial tissue leading to failure of pregnancy. In summary, the overlapping set of genes differently expressed in both fertility models: (1) suggests that disregulation of ECM remodeling can impair receptivity and (2) can be used as markers to predict pregnancy outcome in cattle.

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Transfer of two demi embryos increases pregnancy rate but not the birth rate

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Keywords: embryo, gestation, micromanipulation.

This study aimed to compare the viability of bovine demi-embryos inovulated alone or in pars versus intact embryos. Twenty five Simmental and Aberdeen Angus cows were used as embryo donors, and 153 crossbred heifers were used as recipients. Donors were superovulated by conventional protocol with eight decresasing doses of FSH; and the embryos were collected by nonsurgical technique. Only excellent embryos morphologically (G1) classified as morulae, early blastocyst and blastocyst stage, were split. Seventy eight embryos were submitted to bisection by using a micro surgical blade, and 52 were kept intact. Embryos were transferred into the recipients in three treatments: T1 (intact embryos; n = 52 recipients); T2 (1 demi-embryo/receptora; n = 54 recipients); T3 (2 demi-embryos; n = 51 recipients). Recipients were synchronized by a single injection of sodic cloprostenol. Embryos and demi-embryos were non-surgically inovulated 6 to 8 days after estrus in the uterine horn ipsilateral to the corpus luteum. Pregnancy diagnosis were done at 30 and 60 days of gestation. The means of gestation rate were compared by $\chi 2$. An economic analysis was performed considering the costs of the: recipients, the embryo production and transfer (i.e., hormones, disposables materials, handling media, semen, and the hand-to-work). The inovulated cows pregnancy rate did not differ among treatments (P>0.05) at 30 (55.8; 47.1 and 62.0%) and 60 days (51.9; 37.3 and 54.0% for T1; T2 and T3, respectively). Pregnancy rate per original embryo was greater in T2 (88.9%) than T1 (55.8%) and T3 cows (60.8%) (P < 0.05) at 30 days, however, there were no differences (P > 0.05) among treatments at 60 days (51.9; 70.4 and 52.9 for T1; T2 and T3 cows, respectively). The percentage range of twin pregnancies was 0 – 0%; 1 – 5.3% and 10 – 37.0% for T1; T2 and T3, respectively. The means percentage of live born calf by inovulated recipient did not differ (48.1; 31.4 and 34.0% for T1; T2 and T3, respectively). The percentage of live born calf using one original embryo was better (P > 0.05) in T2 (59.2%) than T3 cows (33.3%). The abortion rate was higher in T3 cows (P < 0.05). The mean cost of calf born alive was U$287.3; 262.5 and 385.4 for T1, T2 and T3 cows, respectively. It is concluded that bisection and transfer of one demi-embryo do not reduce costs of calf born alive. Transfer of two demi-embryos into the same recipient did not improve pregnancy rate.

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Intrauterine treatment in repeat breeder dairy cows: preliminary data

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Keywords: endometritis, PMN, uterus.

Postpartum uterine infections have a high prevalence and a negative effect on reproductive performance (RP) in dairy cattle. The meta-analysis of the efficacy of treatments for endometritis with PGF2α (Heimerl & Heuwieser J Dairy Sci 96, 2973-2987, 2013) revealed large discrepancies between results. In the present study the impact of endometritis on the RP of repeater breeder dairy cows (n = 30) with chronic postpartum endometritis was evaluated after: uterine infusion of saline solution (500ml) + sodium cloprostenol IM (25mg, Sincrocio®, Ouro Fino , Brazil) (n = 10; SFPG), uterine infusion of ceftiofur (500mg, Ceftiomax®, Biogenesis Bago, Brazil) (n = 11; CPG) or sodium cloprostenol IM (25mg, Sincrocio®, Ouro Fino, Brazil) (n = 9; PG). Gynecological examination and ultrasonography were performed in all females. To establish the presence of endometritis, a endometrial cytology (Cytobrush, Minitube, Brazil) was done according to Barlund et al. (Theryogenology 69, 714-723, 2008) (PMN1) in the uterine body, and the cutoff point was the presence of ≥7% of polymorphonuclear neutrophils (PMN) (Gilbert et al. Theriogenology 64, 1879-1888, 2005). The slides were stained with fast panoptic. Cytological evaluation determined the percentage of neutrophils (%PMN), counting at least 200 cells under optical microscopy (400X) for the quantitative evaluation of endometrial inflammation. Repeat breeder cows with more than three AI above 100d of postpartum, and ≥7% of PMN were included in the study. Ten days after treatment new cytology was obtained as the first examination (PMN2). The AI was conducted after estrous detecting and RP observed during the three subsequent cycles. Ultrasonography examination was done 28d after AI in order to detect the early pregnancy (P/AI). Statistical analysis was performed using PROC GLM and PROC GLIMMIX from SAS (SAS 9.3, USA, 2003). The average DIM was 209.6 ± 13.9 days (SFPG = 227.4 ± 31.8; CPG = 197.1 ± 0.4; PG = 219.4 ± 28.4; P = 0.7213), the number of AI was 4.4 ± 0.31 AI/cow (SFPG = 4 ± 0.5; CPG = 4.7 ± 0.6; PG = 4 ± 0.53; P = 0.716) and the average production was 26.5 ± 1.4 kg milk/cow/day (SFPG = 27 ± 3; CPG = 27.4 ± 2.1; PG = 25 ± 1.3; P = 0.5902). In PMN1 the average was 7.07 ± 0.7 and 0.99 ± 0.25 after, and showed significant effect (P = 0.002) in reducing PMN after treatment [(SFPG: 8.6 ± 0.8% vs 0.6 ± 0.4%), (CPG: 8.5 ± 0.6% vs 0.5 ± 0.2%) and (PG: 8.75 ± 0.9% vs 2.5 ± 1.45%)]. The results showed a tendency (P = 0.067) of greater reductions in PMNs in SFPG and CPG treatments, and smaller in PG. The P/AI in the first, second and third cycle after treatment was to SFPG: 45.4%, 33% and 33%, CPG: 30%, 14.3%, and 33.3% and for PG: 25%, 14.3%, and 33.3%, respectively. After three cycles the accumulated P/IA was 72.7%, 60%, and 55.5% (P = 0.769). The treatments reduced PMN with satisfactory rates of P/AI. The continuity of the study with a larger number of animals is required to confirm the indications of these findings.

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Post puerperal endometritis treatment in dairy cows

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UNIFENAS.

Keywords: cefapirin, endometritis, uterine infusion.

This study was aimed to evaluate and compare the efficacy of Cefapirin (Metricure®; MSD; São Paulo-SP; Brazil) with other conventional antimicrobial applied in local route, for the treatment of post puerperal infections. The experiment was accomplished for a sample of 90 cows between the 3rd and the 5th week postpartum, divided into three groups according to birth order. This experiment was approved by the ethics committee of the University of José do Rosário Vellano with the protocol number 0024/2013. The first group (n = 30) received a single uterine infusion with 500 mg Cefapirin, the second (n = 30) was treated with infusion of 2 g of oxytetracycline hydrochloride diluted in 50 mL of saline (single dose) and third (n = 30) received only uterine infusion with 20 mL of saline (single dose). The total rate of clinical cure was 73.3% for Group 1, 46.7% for group 2 and 10% for Group 3 ($P < 0.05$). In relation to uterine cytology results of groups 1 and 2 were compared, and it is considered negative cytology animals with less than 10% of polymorphonucleate cells. Then, the first group was 81.8% and the second group with 57.1% negative cytology ($P < 0.05$). Regarding the reproductive performance after treatments the service period (SP) of the group 1 was 142.3 days and Group 2 164.5 days ($P < 0.05$). In addition, the Group 1 also obtained the highest number of pregnant animals to 1st insemination and 60, 90, 120 and 180 days postpartum. Therefore, it was concluded that the uterine infusion with Cephapirin 500 mg in single dose for the treatment of endometritis showed higher efficiency than conventional treatment with Oxytetracycline.

A250 Embryology, Developmental Biology and Physiology of Reproduction

Use of different intravaginal devices and doses of progesterone to induce synchronous estrus in Santa Inês sheep

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Keywords: embryo transfer, induction of estrus, sheep

The embryo transfer (ET) routine in sheep implies in successive and increased use of gonadotropin stimuli to ewes. Because these conditions can decrease the fertility of the females, it has been recommended to interval ET procedures with gestations. The objective of this study was to test the efficiency of protocols to induce synchronous estrus in Santa Inês donor ewes. The study was conducted from January to March in Coronel Pacheco-MG. Twenty four ewes previously subjected to six successive non-surgical embryo recovery were equally allocated according body weight (kg) and condition score (BCS; variation 1 to 5) in three experimental groups. Ewes of G1 (n=8; 57.6±12.0 kg; 3.3±0.8), G2 (n=8; 58.5±13.8 kg; 3.4±0.5) and G3 (n=8; 56.6±13.5 kg; 3.3±0.6) received intravaginal devices containing P4 for six days plus 37.5µg d-cloprostenol (Prolise®; ARSA S.R.L., Buenos Aires, Argentina) latero-vulvar and 300 IU eCG (Novormon 5000®; Coopers, São Paulo, Brazil) i.m. 24 h before device removal. It was used CIDR (G1; CIDR®; 330mg P4, Pfizer Saúde Animal, São Paulo, Brasil), human absorbent (G2 and G3; O.B®; Johnson & Johnson, São José dos Campos, Brazil) imbibed with 200 (G2) or 400mg (G3) P4 (Evocanil®; Zodiac Produtos Farmacêuticos, Diadema, Brazil). After device removal, ewes were twice daily (morning/afternoon) checked for estrus and natural mated not exceeding four mating per ram per day.Statistical data are presented in descriptive form. Estrous response and interval to estrus were respectively 100.0% and 41.3±12.2h for G1, 62.5% and 30.0±7.0h for G2 and 100.0% and 28.5±14.2h for G3. Pregnancy rate was 50% (4/8), 25% (2/8) and 50% (4/8) to ewes from G1, G2 and G3, respectively. Overall pregnancy rate considering only ewes mated was 47.6% (10/21). Results of this study suggest that the use of alternative device and source of P4 can be considered to induction of synchronous estrus in Santa Inês ewes after successive non-surgical embryo collections.

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A251 Embryology, Developmental Biology and Physiology of Reproduction

Progesterone concentrations as marker of monitoring equine placental changes and fetal viability

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Keywords: mares, pregnancy, progesterone.

Placentitis is the most common cause of abortion and stillbirth in the horse, compromising fetal-maternal unit by hypoxemia or infection (McKinnon; AAEP; 11; 87; 2009). The aim of this study was to evaluate the progesterone (P4) as a potential marker of monitoring placental changes and equine fetal viability. For this study were used 10 mares with induced ascending placentitis, according to the method already described (Feijó; Arq. Bra. Med. Vet. Zootec; 66; 1663; 2014). Five induced mares were untreated and five induced mares were treated with Trimethoprim- Sulfamethoxazole (30mg/Kg, 12 hours, intravenous) and Flunixin Meglumine (1.1mg/Kg, 24 hours, intravenous). The treatment begging 48 hours post induced to foaling or abortion. Serum sample were collected previous induction (15 days), at induction day, post-induction (2 to 6 days) and at parturition to quantification of progesterone by Immunotech® (Beckman Coulter Company, Marseille, France) comercial diagnosis radioimmunooassay technique. All foaling was assisted and foal viability were evaluated and classified as: A) viable and B) stillbirths or debilitated do not survive. All viable foals were monitored every day by 30 days of age. It was performed grossly and histopathologic evaluation of placentas. The mares were divided into two groups, according histopathological changes in placenta: 1) subacute and acute placentitis; 2) chronic placentitis or unchanged. Groups of placental histology and placentitis treatment were compared by t test. To compare the viability of foals was used kruskal-wallis test, significance was assigned to all values $P<0.05$. No difference was observed in the P4 concentrations at pre-induction, inducton day, post-induction and at parturition in relation to placental injury and treatment. In the post-induction evaluation, mares that delivered viable foals showed higher P4 concentration ($P=0.034$) in relation to the stillbirth foals or debilitated do not survive, the results of mean and standard deviation are respectively: 23.48±20.10 and 8.90±3.47 ng/mL. At birth, P4 did not differ regarding the viability of the foal. All dead or unviable foals (group B) came from mares with subacute or acute placentitis (group1). Progesterone not proved to be a good marker of placental alterations. The progesterone values are lower in mares foaling stillbirth foals or debilitated that do not survive, suggesting that this may be used as an indicator of fetal/neonatal viability.

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A252E Embryology, Developmental Biology and Physiology of Reproduction

A novel 3-D culture system to study bovine oviduct physiology, gamete interaction and early embryo development

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Keywords: 3-D culture system, cattle, embryo development, gamete interaction, oviduct physiology.

Successful fertilization depends on processes that take place in the oviduct. Due to its intra-abdominal location, it is difficult to study intra-oviductal processes *in vivo* in mammals. Instead, *in vitro* models that retain essential cell morphological and functional characteristics are being developed. In culture, bovine oviduct epithelial cells (BOECs) rapidly lose differentiated cell properties (e.g. secretory activity and cilia), while suspended cells have a limited lifespan. Progress with insert culture models and 3-D printing technologies prompted us to develop two independent BOEC culture systems, in which *in vivo*-like differentiation and function is re-established, to study oviduct physiology: (i) 3-D printed U-shaped inserts mounted with PET membranes with 0.4 µm pores (3D U-shaped culture) and (ii) hanging inserts (polycarbonate with 0.4 µm pores) containing 150 µL of Matrigel (3D culture). BOECs were harvested by scraping, and cultured for 24h to agglomerate into floating vesicles with outwardly oriented cilia. The vesicles were plated and, 7 days later, the resulting monolayers were scraped, washed and seeded onto the 2 systems described above and cultured at an air-liquid interface. For comparison, BOECs were also seeded onto coverslips as monolayers (2D culture). After 28 days, the apical side of all BOEC monolayers was washed to harvest secreted proteins, and the inserts were fixed for immunocytochemistry. Proteins (20 µg) were separated by SDS-PAGE and visualized by silver staining, or blotted onto nitrocellulose and immunostained for oviduct specific glycoprotein (OVGP1). Epithelial cell differentiation was indicated by immunodetection of laminin and the presence of primary cilia. Ciliated cell presence (acetylated α-tubulin) and secretory activity (OVGP1) characteristics of BOECs in 3D cultures were comparable to freshly harvested BOECs. The 3D culture yielded 46 silver-stainable protein bands versus 30 in 2D cultures (n=3 per system). In 3D U-shaped cultures, the polarized state (laminin and primary cilia) and their amenability to direct fluorescence microscopy (allowing live cell imaging) are currently determined. In conclusion, 3D culture methods promote polarization and differentiation of BOECs. The extent to which physiological function is maintained is under investigation. Studies in progress to assess the BOEC differentiation using the 3D U-shaped cultures include basolateral co-culture of stromal cells. Ultimately, we aim to develop an oviduct-like environment to study gamete activation, fertilization and early embryo development *in situ*.

A253E Embryology, Developmental Biology and Physiology of Reproduction

Detection of pregnancy-associated glycoproteins (PAGs) in prolific and non prolific ewes from early to late gestation and postpartum

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Keywords: ELISA, PAGs, non prolific, prolific, RIA, sheep.

Pregnancy-associated glycoproteins (PAGs) are placental antigens that were initially characterized as pregnancy markers in the maternal circulation of bovine species (Zoli et al., 1992, Biol. Reprod. 46: 83-92). After that, the measurement of such molecules in maternal blood as a method for pregnancy diagnosis in other ruminants has been demonstrated in several species. It can give useful information to develop appropriate feeding strategies for pregnant females and to assure requirements of the mother and the growing of fetuses and to avoid metabolic disorder associated to pregnancy. The aim of the present study was to investigate the use of a PAG ELISA-Sandwich kit (Ref. code E.G.7. CER. Marloie, Belgium) *vs* two homologous radio-immunoassay described in El Amiri et al. (2007; Reprod. Domest. Anim. 42:257-62) to detect PAGs in blood samples collected from Boujaâd (non prolific, n=8) and Boujaâd x D'man (prolific, n=20) sheep from early to late gestation and postpartum. Ewes were assumed to be pregnant when PAG concentrations were higher than 0.8 ng/ml in ELISA and 0.3 in RIAs. In addition the samples were also explored by the double immunodiffusion radial (El Amiri et al., 2003, Theriogenology. 59:1291-301) after PAG extractions. The results show that in both systems (ELISA *vs* RIAs), the PAG concentrations were significantly lower in Boujaâd a non prolific sheep than in Boujaâd x D'man a prolific sheep. Furtemore, the concentrations in RIAs were 3 folds higher than those in ELISA. In all systems, the concentrations decreased rapidly after lambing (21 weeks) reaching basal values at fourth week postpartum in ELISA *vs* RIAs. In ELISA all pregnant females showed PAGs level above 1.4 ng/ml from day 24. The double radial immunodiffusion showed positive reactions in ewes carrying more dead fetus. In conclusion, the plasma PAG investigated in the present study showed that the ELISA technique is proved to be a convenient and reliable means for early pregnancy diagnosis as well as for pregnancy fellow up in sheep. From 24 days of gestation, its reliability achieved 100% and, therefore, matches conventional approaches of pregnancy detection. The PAGs could also be detected after extraction from plasma of pregnant ewes using the double radial immunodiffusion. However, for using this technique in routine, further studies are necessary.

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A254E Embryology, Developmental Biology and Physiology of Reproduction

Effect of asynchronous embryo transfer on glucose transporter expression in equine endometrium

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Keywords: asynchronous embryo transfer, endometrium, expression of the glucose transporter, horse.

Equine pregnancy is characterized by an unusually long pre-implantation period (40 days) during which the conceptus is entirely dependent on uterine secretions for nutrient provision. Moreover, horse embryos tolerate a wide range of uterine asynchrony following embryo transfer (ET); however negative asynchrony (recipient behind the donor) of more than 5 days markedly retards conceptus growth and development, and thereby offers a unique tool for studying the effect of the uterine environment on early development. Glucose is an important nutrient during pre-implantation development, however little is known about its transport from the endometrium into the uterine lumen. The aim of the current study was to evaluate the effect of uterine asynchrony on glucose transporter expression in the equine endometrium. Day 8 horse embryos were transferred to recipient mares that ovulated on the same day (synchronous; n=10), or 5 days after (asynchronous; n=10) the donor mare. The resulting conceptuses and matched endometrial biopsies were collected 6 or 11 days after ET (14 or 19 days of embryo development: n=5 per group). Endometrial expression of mRNA for glucose transporters was evaluated by qRT-PCR, and the effects of asynchronous ET and stage of pregnancy were analyzed by two-way ANOVA followed by independent-samples t-tests. Gene expression for SLC2A3, 4, 5, 8, 10 and SLC5A1 was stable over time and treatment, whereas endometrial SLC2A1 mRNA expression was down-regulated in the asynchronous group at day 14 of embryonic development ($p<0.05$), but did not show differences between the two treatment groups at day 19. In summary, the expression of SLC2A1, one of the main glucose transporters in the endometrium, is negatively affected by asynchronous ET and, although its expression seems to be restored by day 19 of conceptus development, this might be a contributor to the delayed development observed in asynchronous pregnancies.

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A255E Embryology, Developmental Biology and Physiology of Reproduction

The effects of hypo- and hyperglycemia during lipolysis-like conditions on bovine oocyte maturation, subsequent embryo developmental and glucose metabolism

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Keywords: glucose, metabolic disorders, NEFA, oocyte.

Elevated follicular NEFA concentrations, commonly present in cattle in NEB or women suffering obesity or type 2 diabetes, are known to disrupt oocyte and embryo development and alter subsequent embryo metabolism. However, NEB cows exhibit systemic hypoglycemia whereas humans suffering metabolic disorders have hyperglycemic insults. Both metabolic features may affect oocyte development. Little is known about whether elevated NEFA concentrations in combination with hyper- or hypoglycemic conditions influences oocyte viability. In this study, we hypothesized that glucose interacts with high NEFA levels during *in vitro* oocyte maturation to affect developmental capacity and metabolism of the resulting blastocysts. Thus, 647 bovine grade I COCs were matured (3 repeats) under 4 conditions: 1) physiological NEFA (72µM; palmitic, stearic and oleic acid) and routine IVM glucose (GLUC) concentrations (5.50mM) (CNTRL), 2) pathophysiological NEFA (420µM) and routine GLUC (HI NEFA), 3) HI NEFA and high GLUC (10mM) (HI NEFA+HI GLUC) and 4) HI NEFA and low GLUC (2.75mM) (HI NEFA+LO GLUC). Subsequently, matured oocytes were routinely fertilized and cultured for 7 days. At day (D) 7 post insemination (pi) all blastocysts were individually cultured for 24 hours in 4µl drops of modified SOF medium under oil after which droplets were analyzed on GLUC concentrations as described by Guerif *et al.* (PLOSone, 8, e67834, 2013). Cleavage (48h pi), blastocyst rates (D8 pi) and the rates of D8 blastocysts from cleaved zygotes were recorded. Developmental competence and GLUC consumption data were compared between 4 treatments using a binary logistic regression model and mixed model ANOVA, respectively. Replicate, treatment and the interaction of both factors were taken into account (IBM SPSS Statistics 20). Significant lower cleavage rates were observed for HI NEFA+LO GLUC (56%) compared with CNTRL (73%; P=0.006) and HI NEFA+HI GLUC conditions (70%; P=0.048). At D8 pi, blastocyst rates of HI NEFA+LO GLUC exposed oocytes (18%) were significantly lower compared with CNTRL (38%, P<0.001), whereas development of HI NEFA+HI GLUC D8 blastocysts (25%) tended to be reduced compared with CNTRL (P=0.066). The capacity of cleaved zygotes to develop to blastocyst stage by D8 showed a similar profile: HI NEFA+LO GLUC (32%) significantly reduced and HI NEFA+HI GLUC (35%) tended to reduce development compared with CNTRL (53%; P=0.024 and P=0.066, respectively). Interestingly, with no significant difference in developmental stage at D7, these HI NEFA+LO GLUC blastocysts consumed significantly less GLUC from D7 to D8 (12.14 ± 4.10 pmol/embryo/h) compared with CNTRL (25.53 ± 2.96 pmol/embryo/h; P=0.020). In conclusion, low GLUC concentrations seem to be more deleterious than high GLUC concentrations in the presence of elevated NEFAs in terms of embryo development and the lower ability of the surviving D7 embryo to consume GLUC as an energy source for its further development.

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A256E Embryology, Developmental Biology and Physiology of Reproduction

Integreated andrological evaluation in Angora goat

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Keywords: integrated andrological evaluation, sperm parameters, testicular parameters.

Integrated andrological evaluation (IAE) is a practice to identify Satisfactory (S), Questionable (Q) and Unsatisfactory (US) males. IAE procedure mainly involves classical and modern methods. The routine evaluation system contains physical and reproductive examination, while the innovative approach is more based on ultrasound detection of testicular and accessory glands, scrotal surface thermography, GnRH challenge test, CASA semen analysis (Computer Assisted Semen Analyzer), fluorescent staining, seminal plasma biochemistry, testicular fine needle aspiration cytology (TFNAC). The aim of this trial was to conduct a pilot study with an IAE based evaluation system (except of the TFNAC) in four healthy adult (4/6 years) Angora goat in Kazan-Turkey. Physical traits such as age, BSC, hereditable defects on: eyes, mouth, legs/feet, prepuce, penis, scrotum and its components were recorded and scrotal circumference, testicular ultrasound (ESAOTE MyLab5, Genoa, Italy with convex probe 2.2 – 6.6 MHz) and scrotal thermography (Flir, E60 during GnRH challenge test with 8.4 μg Busereline IV) were performed. Semen parameters such as color, volume, concentration, sperm motility (SCA Microptics), viability and morphology (Eosin-Nigrosin stain), acrosome integrity (FITC-PNA) were measured in fresh and frozen-thawed semen samples. Correlation indices and mathematical tendencies were calculated using Sigma Stat Software 2.05 and Microsoft Excel version 14.4.9. Three males were evaluated as Q, and one as US because of the presence of feet and mouth defects. One buck has not been evaluated by reasons of higher delta Testosteronemia during GnRH Challenge Test and echotexture testicular classification (Lower Mineralization Index). All mature bucks showed similar scrotal thermal pattern. Seminal plasma mean values of cholesterol, glucose, LDH, triglycerides, total protein, GGT and magnesium were 30.5 mg/dl, 77.8 mg/dl, 470.1 u/L, 8.8 mg/dl, 82.5 g/l, 46.8 u/L and 2.03 mg/dl, respectively. Bucks with higher testicular functionality, according to the physical examination, had the best freezability (Delta Viability and Intact Acrosome) and higher levels of cholesterol (34.5 mg/dl) glucose (87.4 mg/dl), LDH (585.4 u/L), triglycerides (11.15 mg/dl), total protein (89.0 g/l), GGT (53.4 u/L) as well as the lower levels of magnesium (1.88 mg/dl) in the seminal plasma. A correlation between testicular functionality and frozen-thawed semen parameters was also confirmed by sperm kinetic parameters, viability and morphology results. Application of IAE in Angora goat may indicate the buck selection for specific purposes such as breeding, cryopreservation or exclusion from any application.

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A257E Embryology, Developmental Biology and Physiology of Reproduction

Effect of epidermal growth factor on nuclear and cytoplasmic *in vitro* maturation of guinea pig oocytes

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Keywords: apoptosis, cortical granule, EGF, guinea pig, mitochondria, oocyte maturation.

The guinea pig may represent an animal model for research on ovarian infertility and improvement of the in vitro maturation (IVM) conditions is needed in this species. The aim of the present work was to immunolocalize the Epidermal Growth Factor (EGF)-Receptor in the guinea pig ovaries and to study the effect of EGF on meiotic and cytoplasmic maturation, and apoptotic rate in cumulus-oocyte-complexes (COCs). Immunohistochemistry was performed in paraffined ovaries using a rabbit polyclonal antibody EGF-R (1:100; Santa Cruz Biotechnology) and the ABC Vector Elite kit (Vector Laboratories). For the IVM, COCs were collected by aspiration of follicles >700µm under a stereoscopic microscope. They were cultured at 37°C in 5% CO2 during 17 h with TCM-199 supplemented with glutamine, pyruvate, BSA, and different concentrations of EGF (Sigma) [0 (control), 10, 50 or 100 ng/mL] or 10% Fetal Calf Serum (FCS). After IVM, 564 oocytes were fixed and stained with 10 µg/mL Hoechst to assess nuclear configuration in terms of Metaphase II (MII) rate. A total of 143 oocytes were treated progressively with 0.5% pronase, 4% paraformaldehyde, 0.02% Triton X-100, 7.5% BSA and 100 µg/mL FITC-LCA for cortical granule (CG) staining. Also, 78 oocytes were stained with 180 nm MitoTracker RedCMXRos (Molecular Probes Inc) for active mitochondria visualization. CG and mitochondria patterns were analyzed with laser scanning confocal microscopy (Leica TCS SP2). Apoptosis rate in cumulus cells (n=58 COCs) were visualized with TUNEL (In Situ Cell Death Detection Kit, Roche) and analyzed with Image J software. Chi-square test was used to compare nuclear maturation, CG and mitochondria migration rates. The apoptotic index was analyzed by a one-way ANOVA using Duncan post-hoc test. Positive immunostaining for EGF-R was found in granulosa and theca cells and oocytes in all follicular stages. MII were significantly higher in oocytes supplemented with 50 ng/mL EGF group (75.9%) compared to other experimental groups (43.5, 51.8, 53.7 and 59.5% for 0, 10, 100 ng/mL EGF and 10% FCS, respectively, $P<0.05$). Group matured with 50 ng/mL EGF showed higher rate of oocytes with peripheral migration pattern of CG (compatible with cytoplasmic maturation) compared to control group (71.9 vs. 32.4%; $P<0.05$) and migrated mitochondrial pattern compared to the control group and the group supplemented with 100 ng/mL EGF (80.0% vs. 27.8% and 31.3%, respectively; $P<0.05$). Apoptotic rate was lower in 50 ng/mL EGF (17.2±0.9%) and 10% FCS (16.0±1.2%) groups related to the control one (28.7±1.4%) ($P<0.05$). In conclusion, the presence of EGF-R in guinea pig ovaries, suggests that EGF may exert a direct effect on ovarian function. A dose of 50 ng/mL EGF seems to be the most appropriate concentration for IVM of guinea pig oocytes, since it improves nuclear and cytoplasmic oocyte maturation and reduces apoptosis in the cumulus cells.

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A258E Embryology, Developmental Biology and Physiology of Reproduction

Immunoradiometric assay (IRMA) of Pregnancy-Associated Glycoproteins (PAG) in bovine milk: determination of profiles in ongoing and failed pregnancies

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Keywords: cattle, milk, pregnancy, pregnancy-associated glycoproteins,

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Pregnancy-Associated Glycoproteins (PAGs) are used since early eighties as pregnancy markers in cattle and other ruminant species. Until now, they are mainly assayed in plasma or serum samples by using radioimmunoassay or ELISA systems. In cattle, concentrations of PAG are detectable in maternal blood from Day 28 to Day 30 after fertilization. Milk concentrations are 20-30 times lower than in blood samples and cannot be quantified by existing immunoassay systems. Recently, a new sensitive and robust immunoradiometric assay (IRMA) was developed allowing PAG quantification in bovine milk. Purified bovine PAG 67kDa was used as standard at concentrations ranging from 100 to 50,000 pg/mL. Highly purified immunoglobulins (hp-Ig) were obtained from two distinct rabbit polyclonal antisera by using a specific affinity chromatography (anti-PAG 4B-Sepharose gel). The hp-Ig708 (purified from polyclonal antiserum raised against caprine PAG 55kDa+59kDa) was used as capture antibody (0.01 µg/tube). The hp-Ig727 (purified from polyclonal antiserum raised against purified boPAG67kDa) was used as detection antibody (1:8,000). Radiolabeled streptavidin (125I-Strep; 50,000 cpm/100 µL) was used to reveal the Ab-Ag-Ab-Biot complexes. The aim of this study was to quantify PAG concentrations in bovine milk for pregnancy follow-up in cattle. Milk was collected from pregnant cows (n=20) during the whole duration of lactation until dry-off. Samples were frozen until assay. Before analysis, milk samples were thawed at 37°C, centrifuged (2,500 x g) and fat was removed. Samples giving high PAG concentrations were serially diluted in order to fit with standard curve range. In non-pregnant cows, concentrations remain lower than 40-50 pg/mL at all time points. In pregnant cows, milk PAG concentrations increased from Week 10 (56.9 ± 13.1 pg/mL) to Week 11 (93.5 ± 20.4 pg/mL) and Week 12 (135.2 ± 27.7 pg/mL). Thereafter, PAG concentrations increased regularly until Week 32 (2,177.6 ± 496.2 pg/mL) and slightly decreased until dry-off at Week 35 (1,615.9 ± 663.9 pg/mL). Immediately after parturition, PAG concentrations reached 5,615.3 ± 615.7 pg/mL and decreased continuously until Week 11 postpartum (36.6 ± 2.1 pg/mL). In this experiment, we could also follow three cows with pregnancy failure (2330, 7722 and 7725). Two of these cows (7722 and 7725) showed very low levels of PAG before pregnancy failure. In Cow 2330, PAG concentrations clearly decreased around the time of pregnancy failure. In conclusion, in the present study we describe the use of a sensitive and quantitative IRMA allowing pregnancy follow-up in dairy cows. This approach offers the possibility (in time or in retrospective studies) of an individual follow-up without any additional manipulation of female neither any stress induced by the investigator.

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A259E Embryology, Developmental Biology and Physiology of Reproduction

Cumulus cells protect the oocyte against free fatty acids

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Keyword: bovine cumulus cell, Carnitine-PalmitoylTransferase-1A, DiGlyceride-AcylTransferase, free fatty acids, Stearoyl-CoA-Desaturase.

Cumulus cells have an intimate contact with, and provide metabolites to, the oocyte. The importance of cumulus cells for the oocyte extends into potential protection of the oocyte against free fatty acids (FFA)[1]. Exposure of cumulus-oocyte-complexes (COCs) to elevated FFA levels results in massive lipid accumulation in cumulus cells and normal developmental competence of oocytes (Aardema et al., BoR, 2013; 88, 164). Two potential mechanistic routes by which cells are protected against saturated FFA are lipid storage and β-oxidation (Henique et al., JBC, 2010; 285, 36818-827). To further unravel the presumed protection against FFA by cumulus cells, oocytes with and without cumulus cells were exposed to FFA. To investigate the potential mechanism by which cumulus cells may protect the oocyte, gene expression of cumulus cells from COCs matured in the presence or absence of FFA was analysed for DiGlyceride-AcylTransferase (*DGAT*; lipid storage), Carnitine-PalmitoylTransferase-1A (*CPT-1A*; β-oxidation) and Stearoyl-CoA-Desaturase *(SCD)*, the enzyme that converts saturated FFA into unsaturated. COCs were collected from bovine slaughterhouse ovaries and during 23h matured with or without 250μM saturated stearic acid followed by standard fertilization and culture. After 8h of maturation, cumulus cells were removed from part of the COCs and oocytes were placed back in maturation medium. Gene expression of cumulus cells from COCs was analysed by QPCR for *DGAT, CPT-1A* and *SCD* before and after 23h culture with or without FFA, and from cumulus cells without an oocyte for *CPT-1A* and *SCD*. Statistical analysis was performed by a paired sample t-test (gene expression) and general linear model (culture data). Materials and methods according to Aardema et al. (BoR, 2013; 88, 164) Removal of cumulus cells after 8h maturation resulted in oocytes with normal developmental competence (27±2.8%; 24±1.1% for COCs). Exposure to stearic acid resulted in strongly reduced developmental competence of oocytes cultured without cumulus during the last 15h (1±1.0%; $P<0.01$) compared to oocytes matured as COC (18±4.2%). Expression of *CPT-1A* ($P<0.01$) and *SCD* ($P<0.01$) in cumulus cells increased during maturation of COCs, independent of the presence of FFA. *DGAT* expression was not different among groups. The presence of an oocyte during culture resulted in higher *SCD* expression levels in cumulus cells after 23h of culture ($P<0.05$). These data indicate that cumulus cells are essential to protect the oocyte against saturated stearic acid. The increase in *CPT-1A* expression was independent of the condition and is in line with the necessity of β-oxidation during COC maturation. *SCD* expression has to our knowledge, not been investigated before and showed a marked, oocyte dependent, increase during maturation. We suggest that conversion of saturated FFA into harmless unsaturated FFA by cumulus cells protects the developmental competence of the oocyte.

A260E Embryology, Developmental Biology and Physiology of Reproduction

Mobilization of intracellular lipids by supplementation of IVM and IVC media with L-carnitine improves bovine embryo quality

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Keywords: gene expression, *in vitro*, mitochondria.

Mobilization of embryo lipid by supplementing culture media with metabolic activator is one of the promising tools to improve quality of in vitro produced bovine embryos. Therefore, the present study investigated the effect of L-carnitine supplementation during in vitro maturation (2.5 mM) and embryo culture (1.5 mM) on embryo developmental rates, quality and gene expression profiles. Cumulus-oocyte complexes recovered from slaughter house ovaries were morphologically evaluated and only grades 1and 2 were used in this study. Treatment groups were: T1=IVM+LC, T2=IVC+LC, T3=(IVM and IVC)+LC and control. In vivo produced embryos were included in all analyses. Development rate was calculated based on the number of embryos reached blastocyst stage at day 8 of culture. Total cell count as well as number of apoptotic cells was evaluated using Tunnel-Hoechst assay. The activity of mitochondria and intensity of lipid was measured using fluorescent probes. Expression of embryo selected candidate genes was profiled using quantitative real-time PCR. Our results showed no differences ($P < 0.05$) in cleavage rate between L-carnitine treated groups and control. Although there was an increase in blastocyst rate in T2 (44.4%) and T3 (42.1%) groups compared to T1 (39.2%) and control (38.2%), it was not statistically significant. Embryos cultured with L-carnitine and in vivo group had greater total cell number (T1: n=140.2, T2: n=164.8, T3: n=155.9 and in vivo: n=160) than the control (n=129.4). On the other hand, the percentage of apoptotic cells from total number of cells was greater ($P < 0.05$) in control (11.2%) than L-carnitine treated (T1: 4.2, T2: 3.8 and T3: 2.9%) and in vivo derived blastocysts (0.3%). Cytoplasmic lipid content was reduced by 1.8, 2.7, 2.4 and 5.1 times in T1, T2, T3 and in vivo produced blastocysts compared to their control counterparts. Whereas, intracellular mitochondria density was increased by 2.0, 4.8, 4.5 and 6.3 folds in embryos cultured with L-carnitine and in vivo group. Genes regulating lipid oxidation (CPT2 and CPT1B), fatty acid transport (SLC27A1) and mitochondria transcription (TFAM) were up-regulated while a lipid storage marker transcript (PLIN2) was down-regulated in embryos cultured in presence of L-carnitine and in vivo ones compared to control. Collectively, the lipolytic effect of L-carnitine was linked with increased mitochondrial activity, reducing apoptotic cells and modulating gene expression of in vitro produced embryos which will most likely enhance their survival after cryopreservation and transfer to recipients.

A261E Embryology, Developmental Biology and Physiology of Reproduction

Intrauterine expression of insulin-like-growth factor family members during early equine pregnancy

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Keywords: conceptus, horse, IGF-family members, pre-implantation period.

Insulin-like growth factor (IGF) family members are known to regulate fetal and placental growth and development. Insulin (INS), IGF1 and IGF2 stimulate cell proliferation and differentiation via their receptors INSR, IGF1R and IGF2R. The actions of IGF are further regulated by the IGF-binding proteins. The horse is unique with regard to an unusually long pre-implantation period (40 days) offering a unique tool to study the dialogue between conceptus and endometrium. We evaluated the expression of IGF system components in equine conceptus membranes, and endometrium during the cycle and early pregnancy. Endometrial biopsies were harvested on days 7, 14, 21 and 28 from pregnant mares, following conceptus collection, and days 7, 14 and 21 from cycling mares (n=4 per group). Bilaminar trophoblast was isolated from day 14 and 21 conceptuses, and the yolk-sac and allantochorion from 28 day conceptuses were separated. Expression of mRNA for IGF system components (INS, INSR, IGF1, IGF1R, IGF2, IGF2R) were investigated by qRT-PCR. The effect of conceptus developmental stage was analyzed by one-way ANOVA, and the effects of pregnancy and days after ovulation on endometrium by two-way ANOVA followed by independent-sample T-tests. INS mRNA was not detected in endometrium or conceptus membranes. IGF1 and IGF2R mRNA levels were uniform in cycling and pregnant mare endometrium. INSR gene expression increased in the endometrium of pregnant mares only from day 7 to 14 ($p<0.05$) and showed a higher expression than in cyclic mares on day 21 of pregnancy ($p<0.05$). IGF2 mRNA increased sequentially from day 7 to 14 to 21 of pregnancy ($p<0.05$). IGF1R expression was elevated on day 14 in both cyclic and pregnant mares ($p<0.05$). In the conceptus membranes, mRNA expression for INSR, IGF1, IGF1R, IGF2 and IGF2R was low on days 7 and 14 but showed up-regulation from day 21 ($p<0.05$). In summary, IGF family members are expressed uniformly in endometrium from cycling mares whereas endometrial expression increases during early pregnancy. Conceptus membrane expressions of IGF family genes increases from day 21, when the blastocyst capsule would start to disintegrate. We propose that the INS/IGF system plays an important role in early equine embryonic growth and the preparation for placentation.

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A262E Embryology, Developmental Biology and Physiology of Reproduction

Bovine oviduct epithelial cells: an *in vitro* model to study early embryo-maternal communication

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Keywords: bovine oviduct epithelial cells, embryo-maternal communication, IVP.

We aimed in this study to: (1) assess the expression of oviduct epithelial cells markers on bovine oviduct epithelial cells (BOECs) cultured *in vitro* under two different systems (suspension or monolayer) and (2) determine the BOECs response to the presence of early bovine embryos. BOECs were mechanically extracted by squeezing the isthmus regions of oviducts collected from slaughtered heifers during the early luteal phase, determined by the appearance of the corpus luteum. Part of the oviduct extract was frozen in liquid nitrogen and stored at -80^{o}C for gene expression analysis (fresh BOEC), while the rest was cultured in SOF+10% FCS for either 24 h (suspension) or 7 days (monolayer). Suspension or monolayer BOECs were co-incubated for 24 h with Day 2 (2- to 4-cell) or 3 (8- to 16-cell) bovine embryos produced *in vitro* to determine the embryonic effect on BOECs. A control group without embryos was included for each BOEC culture. RNA extraction from BOECs was carried out by Trisure™ (Bioline, Madrid, Spain) and Dynabeads (Dynal Biotech, Oslo, Norway) and gene expression was analyzed by qPCR, using *ACTG1* as housekeeping gene. Statistical differences were assessed by ANOVA. *OVGP1*, *GPX4* and *FOXJ1* were chosen as markers for oviductal epithelial cells and based on their function to support early embryo development, protect gametes against oxidative stress, and cilia formation, respectively. *KERA* and *PRELP* are genes implicated in extracellular matrix and *ROCK2* and *SOCS3* are genes involved in cytokinesis, all of which were found to display a response to the early embryo *in vivo* (Maillo et al., Biol Reprod 2015, DOI:10.1095/biolreprod.115.127969). Among BOECs markers, *OVGP1* and *FOXJ1* were significantly downregulated in suspension cells compared with fresh BOECs, losing their expression in a monolayer; however, *GPX4* was significantly higher in monolayer than fresh and suspension BOECs, suggesting that although monolayer BOECs lost some of their functional characteristics, they still conserved others like protection against oxidative stress. Regarding the effect of the embryos on *in vitro* cultured BOECs, only suspension BOECs showed an embryonic effect on gene expression: *ROCK2* and *SOCS3* were significantly upregulated in cells co-cultured with Day 2 compared with Day 3 embryos. In conclusion, based on the markers studied, BOECs cultured *in vitro* lost some of their functional characteristics, with suspension cells being closer to *in vivo* controls than monolayer. In addition, under our experimental conditions, suspension cells were more adequate to detect possible embryo signals than monolayer.

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A263E Embryology, Developmental Biology and Physiology of Reproduction

Effect of high hydrostatic pressure (HHP) stress on intercellular ATP content in pig embryo

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Keyword: embryo development, hydrostatic pressure, pig.

Embryos exposed to high hydrostatic pressure (HHP) have a greater resistance to further stress and a higher survival rate in cryopreservation or nuclear transfer processes (Pribenszky C., Biology of Reproduction 83; 690-697, 2010). It is known that efficient metabolism is one of the main factors response for a proper development of pig embryos (Romek M., Reproduction in Domestic Animals 46; 471-80, 2011). In addition, preliminary measurements of the inner mitochondrial membrane potential (ΔΨm) has shown lower ΔΨm in HHP embryos compared to untreated embryos. If the HHP directly affects on metabolism of embryos reducing the ΔΨm, perhaps the total amount of adenosine triphosphate (ATP) content is changing. The aim of this study was to examine the effect of HHP treatment of porcine zygote, on ATP level in embryos at various stages of development. Pig zygotes (number of 217 embryos) used in the experiment were collected surgically from superovulated gilts breed polskiej zwisłouchej. Gilts were superovulated by an intramuscular injection of 1500 IU of PMSG (pregnant mare serum gonadotrophin, Serogonadotropin, Biowet) followed 72 h later by 1000 IU of hCG (human chorionic gonadotropin, Chorulon, Biowet). Embryos were treated by HHP in HHP device (Cryo-Innovation Ltd, Hungary) for 1 h in 39°C at a pressure of 20 MPa. Afterwards cultured in vitro in medium NCSU-23 in 39°C and 5% CO2. Before ATP analysis, embryos from experimental and control groups were frozen in 5µl Gibco® HEPES buffer (Thermo Fisher Scientific Inc., MA USA) in 1.5ml eppendorf (4-8 embryos in each tube). Analysis of ATP content was performed using Adenosine 5'-triphosphate (ATP) bioluminescent somatic cell assay kit (Sigma Chemical Company, USA) and the luminometer Lumat^3 LB 9508 (Berthold Technologies, USA). In order to examine the statistical differences a one-way ANOVA were used. The intracellular ATP content in HHP treated group (A) and control group (B) at zygote stage (a), 8-16 cells (b), morula (c) and blastocyst (d), looks like this: Aa 1.63 ± 0.26 pmol/embryo, Ab 1.55 ± 0.25 pmol/embryo, Ac 0.97 ± 0.09 pmol/embryo, Ad 0.88 ± 0.23 pmol/embryo, Ba 1.51 ± 0.40 pmol/embryo, Bb 1.40 ± 0.12 pmol/embryo, Bc 1.01 ± 0.35 pmol/embryo, Bd 0.62 ± 0.15 pmol/embryo. Pig embryos treated by HHP at zygote stage show not significant differences in intercellular ATP content compared to control group. Significant differences in ATP content between zygote, 8-16 cells and morula, blastocyst stages in both groups of HHP treated and untreated embryos were observed. It means that ATP content in pig embryo is changing during development.

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A264E Embryology, Developmental Biology and Physiology of Reproduction

Lipid profile analysis of bovine *in vitro* blastocysts deriving from insulin treated oocytes by desorption electrospray ionization – mass spectrometry (DESI-MS)

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Keywords: blastocyst, cattle, insulin, IVP, lipid profile analysis.

The aim of this study was to characterize the lipid profile of bovine blastocysts produced from oocytes exposed to different insulin concentrations during maturation by DESI-MS. Insulin is a key metabolic hormone and its concentration in blood and follicular fluid changes in situations of metabolic imbalance as obesity, diabetes or negative energy balance (NEB). The impact of insulin on the lipid profile of blastocysts can provide important insights on the metabolic changes induced by this hormone on early development. Blastocysts were produced from abattoir derived oocytes according to standardized IVP-protocols in our laboratory. Insulin treatment was performed during 22 h of maturation using 0 (INS0); 0.1 (INS0.1) or 10 (INS10) µg/ml bovine insulin. After maturation, all treatment groups were submitted to equal conditions during fertilization and culture. On day 8, blastocysts were separately frozen at -80ºC in PBS with 0.1% PVA and individually transferred to glass sides in randomized order. A total of 63 blastocysts were used for DESI-MS lipid profile analysis. Lipids such as diacylglycerols (DAG), triacylglycerols (TAG) cholesteryl esters (CE), squalene and ubiquinone were detected in positive ion mode as silver adducts. Average full scan mass spectra of the three different treatment groups indicated few changes in the lipid profiles. Multivariate statistics by PCA (Principal Component Analysis) was used to comprehensively explore the chemical information of the full mass spectral dataset and visualize the grouping of samples resulting from chemical similarity. PCA showed some extent of discrimination between INS0 and INS10 whereas the discrimination between INS0 and INS0.1 was less evident. Data suggests down-regulated mitochondrial metabolism (indicated by ubiquinone abundance) in INS10 as well as few changes in TAG- and cholesterol metabolism comparing the treated groups (INS10 and INS0.1) with the control (INS0). Overall, the low extension of changes observed in the DESI-MS lipid profiles indicates minimal impact of insulin exposure during oocyte maturation on lipid content during preimplantation embryo development. The results of the lipid profile analysis shows that the lipid profile was not significantly different in the day 8 blastocyst after exposure of insulin during maturation. Possible explanations could be that the insulin exposure during the IVM period is not sufficient to promote extensive end-point metabolism changes in the lipids detected during preimplantation development, or that the early embryo strongly compensates for the impact of a metabolic stressor as insulin during oocyte maturation by a subsequent change in gene expression, leading to compensating mechanisms to obtain balance in the chemical profile and permitting a viable phenotype.

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A265E Embryology, Developmental Biology and Physiology of Reproduction

Laparoendoscopic single site surgery (LESS) approach to the porcine oviduct for *in vivo* evaluation of physicochemical parameter

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Keywords: approach, laparoscopy, monoport, oviduct, pig, single-site surgery.

We aimed to define a surgical approach to the porcine oviduct capable of combining minimally invasive techniques with a time effective and accurate insertion of biosensors of physico-chemical parameters. Gilts (n= 14) and sows (n=6), of a range weight of 85 to 280 Kg were used. Animals were anaesthetized and placed in lateral right recumbent position. Then, a 5-6 cm incision in the skin followed by layer-by-layer surgical approach to the abdominal cavity was done so as to place the single-site monoport device GelPOINT Advanced (Applied Medical®, Rancho Santa Margarita, California, USA). Under laparoscopy conditions -CO2 pneumoperitoneum (8-10 mmHg)- the left uterine horn was grasped with non-traumatic forceps. Pneumoperitoneum and the single port cap were then removed, and the reproductive organs pulled up towards the incision so as to allow a direct manipulation of the oviduct. The rapid identification of the abdominal opening allowed a rapid and effective insertion of biosensors within the lumen, thus allowing the evaluation of the oviduct microambient, i.e. pH, O2 or temperature. After settling and stabilizing the probes within the oviduct lumen, the organs were put back into the abdominal cavity and in vivo recording of physiological parameters started. The laparoendoscopic single-site surgery (LESS) approach was successful in all the animals, independently of weight and reproductive maturity. Manipulation of reproductive organs was always minimal, although in 3 cases (2 gilts and 1 sow), small and slight hyperaemic areas caused by the forceps were observed in the uterine horn. During the approach no damage to the ovary, oviduct or any other abdominal organ such as intestine was produced. The average duration of the whole procedure since the beginning of the incision in the skin till the insertion of the biosensor within the oviduct was approximately 19.5 min (12-27 range), with a current duration of pneumoperitoneum conditions of 5.5 min (4-7 range). Independently of the LESS approach occasional bleeding of the mesosalpingeal vessels was observed during the manipulation required for stabilizing the probes within the oviduct. The laparoendoscopic single-site (LESS) approach described here proved very efficient in terms of allowing a rapid, minimally invasive and hardly manipulative approach to the reproductive organs, and particularly to the oviduct lumen. This approach benefits from the advantages of both laparoendoscopy (minimal trauma) and traditional laparotomy (by-hand manipulation of organs). The LESS approach is been successfully used to evaluate pH, CO2, O2 and temperature within the oviduct.

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A266E Embryology, Developmental Biology and Physiology of Reproduction

Periovulatory pH within the porcine oviduct and uterus obtained by laparoendoscopic single-site surgery

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Keywords: laparoscopy, oviduct, pH, pig, uterus.

To determine *in vivo* pH values within the oviduct (ampulla and isthmus) and uterus in the porcine species with a minimally invasive approach. Eight pre-pubertal gilts (G) and 7 sows (S) were used. G were treated with intramuscular 1500 IU of eCG and 750 IU of hCG to induce ovulation. 36-44 hours after hCG injection (G) or the onset of oestrus external signs (S) pigs were anaesthetized and placed in lateral right recumbent position. A left lateral paralumbar laparoendoscopy single-site surgical approach (GelPOINT Advanced, SingleMedical®) was carried out under CO2 pneumoperitoneum (8-10 mmHg). Laparoscopy non-traumatic forceps were used to pull up the ovary towards the incision and upon visual inspection pigs were assorted into preovulatory (PreO) or postovulatory (PostO) stages. A flexible 1.6 mm diameter pH probe (MI508, Microelectrodes®, New Hampshire, USA) was sequentially inserted into the ampulla (Amp), isthmus (Isth) and uterus (Ut) for a time period of 10-12 min after signal stabilization. A reference electrode (MI401, Microelectrodes®, New Hampshire, USA) was also required for measurements. To simulate the physiological ambient registers were obtained after replacing back the organs -with the pH probe inserted- into the abdominal cavity and the surgical port closed. Anova of repeated measures was carried out with SPSS 19 (IBM®) to evaluate for a significance level of $p<0.05$. pH values (mean ± SD) within the Amp and Isth were significantly different (7.41 ± 0.17 and 7.10 ± 0,21 respectively, $p<0.001$). pH within the uterus (7.55 ± 0.16) was within the range of the Amp ($p>0.05$) and significantly higher than in the Isth ($p<0.001$). Regarding the PreO and PostO stages pH differences were found in the oviduct ($p=0.02$) for either the Amp (7.45 ± 0.15 vs 7.34 ± 0.12) or the Isth (7.15 ± 0.24 vs 7.04 ± 0.12), but not for the Ut (7.57 ± 0.15 vs 7.52 ± 0.07). While no differences between G and S were observed (7.35 ± 0.24 vs 7.33 ± 0.25) a significant interaction between sex maturity (G vs S) and the phase of the estrous cycle (PreO vs PostO) was found ($p=0.02$). The recorded pH values in the oviduct were lower than those of Nichol (Can. J. Physiol. Pharmacol. 75:1069, 1997), which could be related with the use of a different pH probe and surgical approach. The pronounced pH contrast between the Amp and Isth, and between the Isth and Ut is a relevant result that should be considered to better understand the microambient experienced by the porcine gametes and early embryos.

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A267E Embryology, Developmental Biology and Physiology of Reproduction

Omega-3 fatty acids enhance developmental competence of bovine oocytes under metabolic stress conditions *in vitro*

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Keywords: blastocysts, bovine oocytes, developmental competence, IVP, Omega-3 fatty acids.

Metabolic stress conditions such as negative energy balance in dairy cows are associated with fat mobilization and elevated saturated (stearic; SA, palmetic; PA) and monounsaturated (oleic; OA) fatty acids (FAs) in serum and follicular fluid. We have shown that these FAs have direct detrimental effects on oocyte quality (Van Hoeck et al., ARS, 149:19-29, 2014). In contrast, we demonstrated that polyunsaturated α-linolenic acid (*n-3* 18:3; ALA) can enhance oocyte competence (Marei et al., BOR, 81:1064-1072, 2009). Here, we examined the effects of ALA supplementation (at physiological follicular fluid concentration; 50 μM) during *in vitro* oocyte maturation on subsequent embryo development in the presence of high follicular fluid concentrations of SA, PA and OA (HNEFA, 425μM). Cumulus cell expansion was scored at the end of oocyte maturation (0-3: 0; not expanded, 3; fully expanded). The proportions of cleaved and fragmented embryos were recorded on day 2 post-fertilization. Blastocyst rates were recorded on day 7 and 8. Day 8 blastocysts were categorized as Normal (not expanded), Expanded, or Hatched, and were fixed and immunostained with anti-cleaved-caspase-3 antibody and Hoechst. Total cell counts and apoptotic cell indices were calculated. Data were obtained from 5 independent repeats using 1529 oocytes derived from slaughter house material. A total of 179 blastocysts were stained. Categorical data were analyzed by binary logistic regression using SPSS, and numerical data were analyzed using ANOVA. Pairwise comparisons were performed using Bonferroni correction. *P* values <0.05 were considered significant. Compared with FA-free solvent controls, supplementation with HNEFA resulted in: inhibition of cumulus cell expansion (score: 1.7±0.2 vs. 2.8±0.04, $P<0.05$); higher fragmentation rates (16.8% vs. 9.5%, $P<0.05$); and lower blastocyst rates on day 7 ($P<0.05$), either expressed as a proportion from the total number of fertilized oocytes (15.6% vs. 22.8%) or from the total number of cleaved embryos (20.4% vs. 30.6%). Hatched and expanded blastocysts produced from HNEFA-exposed oocytes had higher apoptotic cell indices. In contrast, these negative effects were alleviated by ALA supplementation. In the HNEFA+ALA group, cumulus expansion score (2.4±0.16), fragmentation (6.9%), blastocyst rate on day 7 (21.4% from total fertilized oocytes and 28.7% from cleaved embryos), and apoptotic cell index were similar to the controls. In addition, HNEFA+ALA group had significantly higher total cell numbers in expanded and normal blastocysts compared with those from HNEFA group. In conclusion, ALA supplementation enhanced oocyte developmental capacity during maturation under metabolic stress conditions. The underlying mechanisms of action are currently under investigation. These results may have clinical implications to improve fertility through dietary interventions in animals and humans suffering from metabolic disorders associated with lipolysis.

A268E Embryology, Developmental Biology and Physiology of Reproduction

Addition of omega-3 DHA during *in vitro* maturation affected embryo development

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Keyword: bovine oocytes, *in vitro* maturation, omega-3 DHA.

Several studies have suggested a positive effect of n-3 poly-unsaturated fatty acids (PUFA) on bovine reproduction. Indeed, n-3 PUFA reduced prostaglandin secretion in uterine environment, thus providing more favorable conditions for embryo development. Other studies suggested a direct effect of n-3 PUFA on the oocyte that could enhance fertility. In the present study, we aimed at investigating *in vitro* the effect of docosahexaenoic acid (DHA, C22:6 n-3, Sigma, Saint-Quentin Fallavier, France) on bovine oocyte maturation and developmental competence. Oocyte cumulus complexes (OCC) were collected from slaughtered cows. In first experiment, *in vitro* maturation (IVM) with DHA 1, 10 and 100 µM was performed (n=3 replicates, 50-60 OCC per condition). After IVM, oocyte viability was assessed using Live/DEAD staining and then meiotic stages were determined by using Hoechst staining after oocyte fixation. Neither difference in viability nor in maturation rate was observed after IVM between control and treated oocytes whatever the DHA concentration. 83.1% of mature oocytes in control IVM and 78.9%; 84.0%; and 84.0% in presence of DHA at 1, 10, 100 µM, respectively, were observed. In second experiment (n=5 replicates, 50-60 OCC per condition), after 26h IVM with or without DHA 1, 10 and 100 µM, oocytes were subjected to parthenogenetic activation (ionomycin 5 µM, 5 min and 6DMAP 2 mM, 4h). Oocytes were then *in vitro* developed in modified synthetic oviduct fluid supplemented with 1% estrus cow serum for 7 days. Cleavage rate and a number of blastomers were assessed in resulting embryos at day 2 post activation. Cleavage rate significantly increased after IVM with DHA 1µM (84.3%) but significantly decreased with 100µM DHA (66.2%) as compared to control (76.0%) embryos (Chi-square test p=0.02). Moreover, the percentage of embryos that progressed further than 4 cells at day 2 was significantly higher (p=0.02) in the presence of 1 and 10 µM DHA (40.8% and 40.4%, respectively) than in control (31.2%) and with DHA 100 µM (22.2%). At day 7, embryos from DHA 1 µM-treated oocytes encountered more cells than those from control and other DHA groups (10 and 100 µM). Altogether these data suggest that a low dose of DHA (1µM) during IVM might improve oocyte developmental competence through possible effect on cytoplasm but not nuclear maturation. Also, we confirmed that a high dose of DHA (100µM) is deleterious for oocyte developmental potential.

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A269E Embryology, Developmental Biology and Physiology of Reproduction

Periconceptional body condition induces placental adaptations but does not affect foal growth and metabolism in horses

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Keywords: body condition, foal, glycemia, horse, periconception, placenta, pregnancy,

Objectives: It has been shown in several species that the periconceptional environment can affect offspring long-term phenotype. This study aims to investigate the effects of periconceptional body condition on feto-placental biometry, post-natal foal growth and glucose metabolism. Materials and methods: 32 saddlebred mares of similar size were allocated to one of two groups depending on their body condition score (BCS, 1-5 French scale) at the time of artificial insemination (AI). Group High (H, n=18) had a median BCS of 3.9 (range: 3–4.25) whereas group Low (L, n=14) had a significantly lower BCS (median: 2.5, range: 2–3.75, p=0.01). Both groups were kept in pasture until the 7th month of gestation when they were housed indoors and fed forage and concentrate (barley). Food intake was not different between groups. Mares were weighed every 2 weeks and their BCS was monitored monthly. Placentas and foals were weighed and measured at birth. Foals were measured and their fasting glucose assessed regularly until 12 months of age. A frequently sampled intravenous glucose tolerance test (FSIGT) was performed at 3 days and 4 months of age. Results were analyzed using a Mann-Whitney test. Results: H mares maintained a significantly higher BCS (median ≥3.75) than L mares from AI until foaling (median at foaling: 3.75, p<0.0001). L mares reached a peak BCS of 3.75 at the 7th and 8th month and thereafter lost BCS until foaling (median BCS at foaling: 2.75). Mares' body weight was not different between groups at any time. Gestation length did not differ between groups. H placentas tended to be 15% lighter with a 10% reduced surface compared to L placentas (p=0.071). Foals' weight and measurements at birth were not different but the placental efficiency (foal/placental weight) tended to be 12% higher in H mares (p=0.078). There was no difference in foals' growth until 12 months. H foals' fasting glucose tended to be higher at 3 days (p=0.063) but there was no difference in the glucose response to the FSIGT. Plasma insulin concentrations are pending. Conclusion: H mares tended to have a lighter placenta and with a reduced surface area that was more efficient than L mares. Their foals tended to have greater fasting plasma glucose than L foals at 3 days. The fact that the BCS of H and L mares throughout gestation matched their BCS at AI highlight the importance of periconceptional BCS. This study follows a previous one showing that feeding mares in the 2nd part of gestation with two different energy sources does not affect feto-placental biometry and foal development until the age of 6 months (Peugnet et al. 2015, Plos One 10, e0122596). Nevertheless, periconceptional BCS appears to induce placental adaptations that are currently being characterized.

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A270E Embryology, Developmental Biology and Physiology of Reproduction

Involvement of phosphodiesterase 5 (PDE5) on lipid accumulation in bovine oocytes and embryos produced *in vitro*

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Keywords: cryotolerance, IVP, lipid metabolism, melatonin, nucleotide.

The aim of this study was to investigate the involvement of PDE5 on lipid metabolism in bovine oocytes by assessing the effects of PDE5 inhibition during in vitro culture on lipid contents in oocytes and resulting in vitro produced (IVP) embryos, and their cryotolerance. In Experiment 1, cumulus-oocye complexes (COCs) from slaughterhouse ovaries were submitted to IVM in TCM199 supplemented with 0.4% BSA or 10% FCS associated or not with a PDE5 inhibitor (10^{-5}M sildenafil- Sigma-Aldrich) and after 22h oocytes were denuded and stained with Nile Red (1µg/ml, 30 min) to assess cytoplasmic lipid levels measured by fluorescence intensity. In Experiment 2, 10^{-5}M sildenafil (SDF) was included during IVM and/or IVC (SOFaa) during embryo development after IVF (TALP medium using frozen sperm from the same bull prepared by Percoll gradient). Controls were cultured without SDF and all groups were cultured with 10% FCS. After 22h IVM, 20h IVF and seven days IVC, embryos were assessed for cleavage (Day 4) and blastocyst development rates. Day 7 blastocysts (BL) were fixed and stained with Nile Red to evaluate lipids. In Experiment 3, the same groups were assessed plus two others including melatonin (10^{-7}M) as an antioxidant during IVC in SDF treated groups. Cleavage and BL rates were determined and embryos were vitrified. After thawing, BLs were cultured for 24h to assess reexpansion and 48-72 h for hatching. Cultures were at 38.5ºC under 5%CO_2 in air. Statistical analyses were performed by ANOVA followed by Tukey test using SAS and significance level was 5%. In Experiment 1, SDF reduced ($P<0.05$) lipid content in oocytes matured with BSA (13.1) or FCS (16.3) when compared to controls matured only with BSA (17.6). SDF groups were similar ($P>0.05$). Reduction in lipids was only observed in BLs produced with SDF in both IVM and IVC (30.2; $P<0.05$). Oocytes matured only with FCS had highest lipid content (20.1, $P<0.05$). In Experiment 2, there was no effect of SDF or melatonin on cleavage or BL rates (79 and 31%, respectively, $P>0.05$) or reexpansion and hatching (89 and 64%, respectively, $P>0.05$). In conclusion, PDE5 inhibition during IVM reduces lipid content in oocytes, but in embryos, inhibition is necessary during both IVM and IVC. Lipid reduction, however, did not translate into improved cryotolerance, neither did the addition of the antioxidant melatonin. PDE5 appears to be involved in lipolysis in bovine oocytes and embryos possibly related to cGMP levels and PKG activity and may be an interesting target for studies to understand lipid metabolism in oocytes and IVP embryos. To our knowledge, this is the first study to show the possible relationship between this pathway and lipid metabolism in bovine oocytes and embryos.

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A271E Embryology, Developmental Biology and Physiology of Reproduction

In vitro production of bovine embryos as a toxicological model: impact of polychlorinated biphenyl (PCB) 126 during maturation

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Keywords: 3R, blastocysts, environmental pollutants, neutral lipids.

Many of the experimental animals used in toxicological studies are for assays involving reproductive toxicity and the vast majority use the small rodents as models for the human. There are many factors making the human and cow much more similar than humans and rodents. The aim of this study was to explore the bovine IVP system for the impact of PCB 126 during oocyte maturation. All PCB congeners are lipophilic persistent environmental pollutants, of which PCB 126 is the most dioxin-like (activates the aryl hydrocarbon receptor) and therefore considered to be the most toxic congener. For maturation, 254 abattoir derived oocytes were used (in three replicates). The oocytes were randomly divided into two groups for maturation and the treated group contained an addition of 100.6 pg/ml of PCB 126, a concentration previously found to affect cleavage and blastocyst development (Krogenæs et al., Reprod Toxicol 12:575-80 1998). Apart from the addition of PCB 126, the maturation, fertilization and culture were done according to standardized protocols (Abraham et al., Acta Vet Scand 54:36 2012). The embryo development was assessed through cleavage at 44 h after fertilization and blastocyst development (stage and grade) at day 7 and 8 after fertilization. At day 8 after fertilization the blastocysts were stained for number of nuclei (DraQ-5, Bionordica, Stockholm, Sweden) and neutral lipid (HCS LipidTOX, Invitrogen, Paisley, UK). The embryos (n = 63) were examined for number of nuclei and for neutral lipid staining intensity with fluorescent microscopy and ImageJ 1.48v (http://imagej.nih.gov/ij). Statistical analysis of the effect of PCB 126 on cleavage rate and blastocyst rate, stage and grade was done by logistic regression (logistic procedure of SAS, Milltown, USA). Continuous variables were analysed in the GLM procedure. Replicate was considered as an influencing factor and was included in all models. The mean cleavage rate for the control group was 76.3% ±0.12 (mean ±SD) and in the PCB 126 treated group 70.0% ±0.09. Blastocyst rate (calculated from number of oocytes to maturation) on day 7 was higher in the control group (19.5% ±0.1) than the PCB 126 group (10.4% ±0.04). On day 8 the corresponding figures were 28.5% ±0.06 (control) and 21.4% ±0.04 (PCB 126 group). The difference in blastocyst rate between the control and the PCB 126 group was significant (p=0.04) on day 7, but not on day 8. There was no effect of PCB 126 on blastocyst stages, grades or number of nuclei. The mean pixel intensity of the LipidTOX stain was lower in the control group (334 ±139) compared to the PCB 126 group (454 ±212) but this was not statistically significant (p=0.18). In conclusion, addition of PCB 126 during maturation seemed to affect early embryo development in this small study, and could possibly be related to lipid metabolism. Bovine IVP should be further explored as a model for toxicity on oocytes.