Workshop 2: "Embryo *in vitro* production" - Coordinator: Yeda Watanabe

In vitro embryo culture models "mimicking" physiological conditions

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Keywords: bovine, development, embryo quality, *in vitro*.

It is widely accepted that the quality of the embryos produced *in vitro* is inferior to those derived *in vivo.* However, culture of *in vitro*-produced bovine zygotes *in vivo* in the ewe or cow oviduct improves the quality of the resulting blastocysts similar to that of totally *in vivo* produced embryos. Conversely, *in vivo*-produced bovine zygotes cultured *in vitro* develop into blastocysts of low quality. Current systems of *in vitro* culture support up to 30-40% blastocyst yield although they provide a suboptimal environment to the early embryo development that has both short- and long-term consequences. One of the most common media for the culture of bovine embryos is synthetic oviductal fluid (SOF) that is frequently supplemented with serum and/or albumin. The use of serum during culture has a negative effect on embryo quality, which has been evidenced in terms of changes in crytotolerance, gene expression, pregnancy rate after embryo transfer, and alterations in the phenotype of newborn calves (i.e. large offspring syndrome). In an attempt to improve the quality of the embryos obtained *in vitro*, different systems of embryo culture have been developed. Culture in the presence of oviductal epithelial cells, oviductal fluid (OF) and extracellular vesicles (EV) may represent appropriate *in vitro* models "mimicking" the physiological conditions the pertain *in vivo*. For example, co-culture of embryos with bovine oviductal epithelial cells (BOEC) has been considered a suitable model to produce embryos of better quality and also to study oviduct-embryo interactions. These cells can be cultured as monolayers on conventional culture plates or on hanging inserts in a polarized cell culture system and cell suspensions. However, the drawback of monolayers is that during their *in vitro* culture they dedifferentiate losing important morphological characteristics. An important component of the oviductal environment is the OF containing simple and complex carbohydrates, ions, lipids, phospholipids and proteins. We showed in cattle that low concentrations of OF in embryo culture media in the absence of serum had a positive effect on development and quality in terms of cryotolerance, cell number and expression of quality-related genes. EV is a general term encompassing several different vesicle types, released by somatic cells that are present in body fluids, and contain bioactive molecules (i.e. proteins, RNAs, mRNAs, miRNAs) and lipids. Recently, we demonstrated that embryo culture in the presence of EV derived either from BOEC conditioned media or OF improved blastocyst quality. In conclusion, the goals of *in vitro* embryo production are to simulate as close as possible the conditions *in vivo* in order to obtain high quality embryos capable of continuing development, implantation after transfer and resulting in viable births, as well as to provide information and new insights on early embryo-maternal communication.

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The role of extracellular vesicles in bovine ovarian follicular development and embryo quality

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Keywords: bovine, extracellular vesicles, miRNAs, ovarian follicle.

The use of assisted reproduction techniques presents an alternative to solve reproductive problems in domestic species and humans. However, there is concern that epigenetic changes occurring during *in vitro* culture adversely affect tissue programing during fetal development and could lead to a higher pre-disposition to development of diseases and reproductive problems, in adulthood. Epigenetic changes are regulated by DNA methylation/hydroxymethylation, posttranscriptional histone modifications or microRNAs. MicroRNAs are non-coding RNA molecules that regulates gene expression during different tissue developing processes. Recently, cell-secreted vesicles called exosomes and microvesicles carrying bioactive materials including miRNAs were identified in different body fluids. Cell-secreted vesicles are considered a new class of intercellular communication with possible implications in different physiological processes. However, its role during oocyte maturation and embryo development is not well understood. Our main goal is to determine if cell-secreted vesicles from follicular fluid can modulate epigenetic changes during *in vitro* culture of bovine oocytes and embryos. In a series of experiments in our laboratory we evaluated the effects of exosome treatment during oocyte maturation and early embryo development. Initially, we performed RNA-seq and miRNA analysis in order to identify RNA and miRNA molecules present within exosomes, and the data demonstrated the presence of different coding and non-coding RNA. Bioinformatics analysis demonstrated that messenger RNAs identified within exosomes were involved in controlling DNA-protein interaction and translation machinery. Additionally, we exposed granulosa, cumulus-oocyte-complexes and embryos to labelled exosomes and we verified that granulosa, cumulus, transzonal projections and even the embryo incorporated exosomes. Treatment with exosomes induced changes in genes known as epigenetic modifiers and in genes associated with embryo quality. Additionally, exosomes from 3-6 mm ovarian follicles changed global DNA methylation and hydroxymethylation, thus suggesting that normally used *in vitro* production system is missing some of the contents carried by these extracellular vesicles present in follicular fluid. Our results demonstrated that extracellular vesicles present in follicular fluid can modulate mRNA and miRNA, global DNA methylation and hydroxymethylation levels as well as blastocyst quality in *in vitro* produced bovine embryos. Results obtained in our research will establish the role of extracellular vesicles from follicular fluid modulating the regulation of epigenetic changes acquired during *in vitro* culture of oocytes and embryos, thus generating new therapeutic tools for domestic agriculture species and humans.

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Workshop 3: "*In vivo* conversations with the embryo: the oviductal and uterine dialects"
(IV Workshop of the Thematic Project Signatures of Receptivity – Coordinator: Mario Binelli)

Oviductal transcriptomics

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Keywords: cattle, gene expression, oviduct.

The oviduct provides the environment for the transport of sperm and oocyte, fertilization and early embryo development. In the cow, between days 3.5 and 4 after fertilization, at the 8- to 16-cell stage, the embryo moves from the oviduct to the uterus where development continues through blastocyst formation, conceptus elongation and implantation. Clear evidence indicates a two-way interaction between the uterus and developing conceptus. However, the evidence for reciprocal crosstalk during the transit of the early embryo through the oviduct is less clear. Indeed, the importance of the oviduct during early embryo development could be underestimated because embryos can be produced *in vitro*. Nevertheless, it has been clearly demonstrated that culture of *in vitro* produced bovine zygotes in the oviducts of cattle, sheep or even mice improves embryo quality measured in terms of morphology, gene expression, cryotolerance and pregnancy rate after transfer. Internally, the oviductal epithelium is made up of ciliary and secretory cells; the secretory cells are responsible for secreting and actively transporting the proteins, amino acids and ions that are present in the oviductal fluid. During the oestrous cycle, modifications in the proportion of secretory and ciliary cells, in the transcriptome of these cells and also in the composition of the oviductal fluid have been reported. Moreover, transcriptomic differences have also been described between the ipsilateral and contralateral oviduct as well as within the epithelium of the ampulla and isthmus of cyclic heifers in the follicular or luteal phase. Regarding the effect of the embryo(s) on the oviduct, studies in mice and pigs have reported an alteration on expression of a number of genes in response to the presence of embryos. In a recent study from our group it was necessary to transfer multiple embryos into the oviduct of heifers to detect differences in the transcriptome of the oviduct epithelium, while when a single embryo was present in the oviduct no differences were found, suggesting a local effect of the embryo. In addition, a local influence of the embryo on the transcriptome of the equine oviduct epithelium has been reported. In these studies in cattle, horses and pigs, the presence of an embryo induced subtle changes in the oviductal expression of genes related to immune function. Identifying and understanding the mechanisms involved in embryo-oviduct cross-talk during the critical period of early reproductive events would lead to an improvement in the success of reproductive assisted technologies in domestic mammals and humans.

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Workshop 3: "*In vivo* conversations with the embryo: the oviductal and uterine dialects"
(IV Workshop of the Thematic Project Signatures of Receptivity – Coordinator: Mario Binelli)

Oviductal transcriptome and morphology between high and low fertility beef cows

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Keywords: ampulla; isthmus; sex-steroids.

In cattle, animals ovulating larger follicles have higher concentrations of estradiol (E2) during the follicular phase and a larger corpus luteum (CL), which produces greater amounts of progesterone (P4) during the luteal phase. This sex-steroid profile favors the formation of a more receptive oviductal and uterine environment for embryo development and implantation. The objective was to compare the transcriptome and ovidutal morphology of Nelore cows treated to ovulate large follicles (LF-LCL group, associated with high receptivity to the embryo; n = 21) with cows treated to ovulate small follicles (SF-SCL group; n = 20). On day 4 of the estrous cycle, ampulla and isthmus samples were collected and stored at -80°C for molecular tests or fixed in formalin to be embedded in paraffin. After RNA extraction, transcriptome (n = 3 / group / region) was evaluated by RNA-sequencing. Fixed tissue samples were cut and stained with hematoxylin-eosin or periodic acid Schiff for the evaluation of morphology and counting of cell populations (First count: ciliated and secretory cells; second count: cells with or without cytoplasmic granules), respectively. The transcripts profile shows a series of differentially expressed genes (DEG) between the groups associated with functional characteristics of the oviduct (ampulla: 692 DEG; isthmus: 590 DEG). Genes involved in extracellular matrix remodeling, cellular proliferation and secretion were more expressed in cows on the FG-CLG group. Additionally, these cows also had a greater number of primary (P 0.04) and secondary (P = 0.07) folds in the mucosa layer and a greater number of secretory cells (P = 0.004) and of cells containing cytoplasmic granules (P = 0.03). Extracellular matrix remodeling and morphogenic branching process, with the subsequent release of growth factors and increased cellular secretory activity could lead to a better embryonic development. In conclusion, the higher fertility of LF-LCL cows is related to molecular and morphological changes in the oviduct favoring embryo transport and development.

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Workshop 3: "*In vivo* conversations with the embryo: the oviductal and uterine dialects"
(IV Workshop of the Thematic Project Signatures of Receptivity – Coordinator: Mario Binelli)

Oviductal microRNA expression profile between high and low fertility beef cows

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Keywords: ampulla, cattle, isthmus.

MicroRNAs (miRNAs) are small endogenous RNA molecules (21 to 25 nucleotides) that act in the post-transcriptional gene silencing. miRNAs are involved in fertilization, embryo development and implantation, however, there is not yet known which miRNAs are in bovine oviduct or if their abundance varies between high and low fertility cows. The main objective was to compare miRNAs expression profile in ampulla and isthmus of Nelore cows treated to ovulate large follicles (LF-LCL group, associated with high receptivity to the embryo; n = 21) with cows treated to ovulate small follicles (SF-SCL group, n = 20). On day 4 of the estrous cycle, ampulla and isthmus samples were collected and stored at -80°C for subsequent total RNA extraction. cDNA synthesis was performed using the miScript II RT kit (Qiagen). RT-qPCR was performed with miScript SYBR® Green PCR kit (Qiagen) and specific primers to evaluate the abundance of 348 target miRNAs and 3 endogenous miRNAs (RNT43, T1 and TR-miR-99b). Using a pool of ampulla and isthmus samples, the correct miRNAs amplification was asses and 88 ampulla microRNAs and 88 isthmus microRNAs were selected to perform region and groups comparisons. Of these, 24 were common to both regions. Thirty four miRNAs were differentially expressed (DE) between groups ($P < 0.05$) in the ampulla, being 20 up-regulated in LF-LCL group and 14 up-regulated in the SF-SCL group. While in the isthmus, 48 miRNAs were DE between groups ($P < 0.05$) being 17 up-regulated in LF-LCL group and 31 up-regulated the SF-SCL group. The prediction of targets genes and biological pathways regulated by DE miRNAs was performed using the miRTarBase and TargetScan platforms. The results show that in the LF-LCL group, the up-regulated miRNAs were involved in pathways such as cellular proliferation, secretion, protein folding, homeostasis and extracellular matrix remodeling. In comparison, in the SF-SCL group, pathways involved in apoptosis, antigen presentation and processing, and nucleotide biosynthesis were predicted. This study is the first to identify and define the expression of miRNAs in bovine oviduct. Present data provide a basis for future studies on the predictive role of miRNAs in bovine fertility and the potential biological processes that they could regulate.

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(IV Workshop of the Thematic Project Signatures of Receptivity – Coordinator: Mario Binelli)

The peri-ovulatory endocrine profile modifies the expression of DICER and AGO4 but not AGO1, AGO2 or XPO5 in bovine oviduct

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Keywords: microRNAs, RISC, sex-steroids.

In cattle, it is known that plasmatic concentrations of estradiol (E2) during pro-estrus/ estrus and progesterone (P4) during the initial diestrus modifies the oviductal transcriptome by the activation of their specific receptors. Steroid receptors interact with other molecules within the target cell to initiate a signaling cascade. Several studies suggest that estradiol receptor affects the biosynthesis of microRNAs (miRNAs) by its interaction with proteins involved in this process. Thus, the main objective was to study if the variations in peri-ovulatory E2 and P4 concentrations could modify the abundance of transcripts encoding XPO5, DICER and Argonaut proteins (AGO1, AGO2 and AGO4) in the ampulla and isthmus of cows with different peri-ovultory hormonal profiles. Nelore cows were synchronized with an E2 and P4-based protocol to ovulate small (SF-SCL, n = 20) or large (LF-LCL, n = 21) follicles and consequently result in different luteal development and E2 and P4 plasmatic concentrations between groups. On day 4 of the estrous cycle, the animals were slaughtered and ampulla and isthmus samples were collected. After RNA extraction and cDNA synthesis, the abundance of transcripts was assessed using qRT-PCR. The results were analyzed using ANOVA and considering region and group effects, and their interaction. The XPO 5 transport the pre-miRNAs from the nucleus to the cytoplasm and its abundance was similar between groups and regions. The DICER is a type III ribonuclease that is responsible for pre-miRNAs cleavage producing double-stranded miRNAs and its abundance was 18.71 and 3.0 times greater in the ampulla and the isthmus of SF-SCL group compared to LF-LCL group, respectively (interaction group * region P = 0.08). The AGO family plays catalytic role in the RNA-induced silencing complex (RISC). The abundance of AGO1 and AGO2 was 8.3 (P = 0.02) and 4.2 (P = 0.02) times higher in the ampulla and the isthmus, respectively, but they did were not affected by group nor by the group * region interaction. The abundance of AGO4 expression was transcript was 2.1 times higher in ampulla than isthmus of the LF-LCL group and 1.2 times higher in isthmus than ampulla of the SF-SCL group (region * group interaction; P = 0.03). We conclude that the peri-ovulatory endocrine profile modify the expression of DICER and AGO4 but not that of XPO5, AGO1 and AGO2. Further studies, in which other components of the miRNAs biosynthetic process are evaluated, and, immature and mature microRNAs forms are identified, are needed to determine the role of steroid hormones controlling this pathway.

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Conceptus-maternal crosstalk and pregnancy establishment in cattle

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Keywords: conceptus, cow, pregnancy establishment.

Successful establishment of pregnancy during the pre-implantation period is dependent on timely interactions between the developing embryo/conceptus and the uterine environment. During the first week of development, the embryo is somewhat autonomous and does not require contact with the maternal reproductive tract as evidenced by the ability to produce blastocysts *in vitro* and to successfully transfer embryos from donors to recipient animals. However, conceptus elongation, a feature of ruminant embryos, does not occur outside the uterine environment or in the absence of uterine glands *in vivo,* indicating that uterine-derived factors are responsible for this elongation. In cattle, progesterone (P4) from the corpus luteum induces both temporal and spatial (cell-specific) changes in the endometrial transcriptome necessary to establish uterine receptivity. These changes include down-regulation of the progesterone receptor in the luminal and then glandular epithelium, which allows expression of genes and secretion of their protein products, as well as active transport of other molecules, required for conceptus elongation. The timing of conceptus elongation is clearly associated with concentrations of P4 in circulation, which act via the uterus to alter the timing of PGR down-regulation and thus onset of expression of key genes. Consequently, P4 has an indirect effect on the secretion of interferon tau (IFNT) by the conceptus, the pregnancy recognition signal in cattle. Interestingly, studies aimed at elevating P4 in the week post mating have resulted in no effect or a modest and variable response in terms of pregnancy rate. Conceptus-derived factors other than IFNT, including prostaglandins and cortisol, may also modify the endometrium prior to pregnancy recognition. By cross-referencing the protein content of uterine lumen fluid (ULF) from pregnant and cyclic heifers on Day 16 post-estrus to RNA sequencing gene expression data from similarly staged tissues as well as proteins produced by Day 16 conceptuses following short term culture *in vitro,* we identifed a putative tissue source of these proteins (i.e., endometrium, conceptus or both). We have subsequently characterized differences in the composition of micro-vesicles from the ULF of pregnant and cyclic heifers at Day 16. This generated a list of 299 and 254 proteins detected in exosomes recovered from pregnant and cyclic heifers, respectively, of which 232 were common to both, 67 were only detected in pregnant ULF exosomes and 22 only detected in cyclic ULF exosomes. Of the 67 proteins only identified in exosomes recovered from the ULF of pregnant heifers, 35 matched those previously reported in media following short-term culture of Day 16 conceptuses and include Actinin alpha 1, Creatine kinase U-type, mitochondrial, Transketolase, Elongation factor 2, Protein disulfide-isomerase, and Thioredoxin. These and other data will be discussed in the context of conceptus maternal interaction and uterine receptivity to conceptus elongation and implantation.

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Long-acting progesterone supplementation at early diestrus in beef cattle: fertility responses in TAI and TET programs

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Keywords: embryo, bovine, luteolysis.

In dairy and beef cattle, the circulating progesterone (P4) concentrations at early diestrus are positively associated with pregnancy outcome. Recently, many studies using different hormonal strategies were performed to increase the P4 concentrations during early and mid diestrus with the purpose of enhancing fertility in timed-AI (TAI) and timed-ET (TET) programs. Supplemental P4 using intravaginal P4 devices during early diestrus stimulates conceptus growth, but may not be practical for use in large cattle operations. Therefore, we have performed sequential studies aiming to evaluate the use of an injectable, single-dose long-acting P4 treatment as a novel strategy to supplement P4 during diestrus and potentially improve fertility in TAI and TET in beef programs. In our first trial, doses of 150 or 300 mg of long-acting P4 were given on Day 2 or 3 post-ovulation and efficiently increased the plasma P4 concentrations for at least 3 days in non-lactating beef cattle. This strategy did not affect initial luteal development nor vascularization, but accelerated the onset of functional and structural luteolysis on about 3 days in non-inseminated cows (Reprod.Dom.Anim.49,85-91, 2014). Despite this paradoxical effect, in a subsequent study (Theriogenology.85,1239-1248, 2016) we tested and confirmed the hypothesis that the supplementation with 150-mg long-acting P4 at Day 4 post-TAI improves fertility in lactating beef cows submitted to TAI. Pregnancy rate (P/TAI) was similar ($P > 0.1$) between the control and P4-treated cows (53.2%; 209/393 vs. 56.2%; 219/390), but when the P/TAI was evaluated according the size of the CL, cows with a CL < 0.9 cm^2 benefited from the P4 treatment and showed P/TAI 35% greater than non-supplemented cows. This result indicated a positive effect of P4 supplementation in those cows with impaired corpus luteum (CL) function. We also observed that the P4-stimulated embryotrophic effects increased in 20% the pregnancy/TAI in lactating anestrous beef cows submitted to the same TAI protocol. In high producing dairy cattle, greater increases in P/TAI were obtained using 900 mg long-acting P4. However, additional trials from our group using 150 mg long-acting P4 at Day 4 post-TAI did not result in improvements on fertility of crossbreed and Nelore beef cows at early postpartum, indicating that further understanding of potential interactions between the reproductive status of the cow and the supplemental P4 dose are needed. In TET programs, our preliminary data is indicating a potential increase of 7 to 12% on P/TET in crossbreed beef recipients for supplementation with 150 mg P4, on Day 4 or Day 7 (day of embryo transfer). In summary, the use of long-acting P4 at early diestrus is a potential strategy to favor embryo development and maintenance. Although fertility responses varied among TAI studies, the expressive increase on P/TAI in lactating anestrous cows and in cows with a smaller CL indicate a potential use of long-acting P4 supplementation for fertility improvement in TAI and TET programs. For additional advances or understanding of the inconsistency in the fertility's trails, the causes of early luteolysis and the new combinations of timing and dose of supplemental P4 for cows under different environmental and physiological conditions need to be explored in further studies.

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(IV Workshop of the Thematic Project Signatures of Receptivity – Coordinator: Mario Binelli)

Effect of AI on the incidence of short luteal lifespan in cows supplemented with P4 at early diestrus

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Keywords: embryo, luteolysis, bovine.

In cattle, progesterone supplementation (P4-suppl) during early diestrus stimulates conceptus growth and the proportion of pregnant cows (P/AI). Despite such beneficial effects, a proportion (~40%) of non-inseminated and P4-suppl animals undergo reduction on luteal lifespan (early luteolysis) (Reprod. Dom. Anim. 49, 85-91, 2014) which may reduces the potential of further improvements of this strategy in inseminated cows. Accordingly, the presence of an embryo or conceptus between days 13 and 16 of diestrus alters gene expression in the endometrium (Biolreprod. 85,144-156, 2011). We hypothesized that presence of the conceptus in P4-suppl cows reduces the incidence of early luteolysis. Non-lactating multiparous cows (n = 94) were synchronized using an estradiol/P4-based protocol followed by estrus detection twice a day for 4 days. Cows detected in estrus (n = 66), were split to receive 12h after estrus an AI with semen from a single high fertility bull (n = 23; AI subgroup) or semen extender only (n = 43; non-AI subgroup). At AI, the pre-ovulatory follicle diameter was measured and ovulation (d0) was monitored every 12h by transrectal ultrasonography. On d3, an IM injection with 150 mg of long acting P4 (Sincrogest; Ourofino Saúde Animal, Cravinhos, SP) was given to non-inseminated (P4-suppl group; n = 21) and inseminated cows (AI + P4-suppl group; n = 23). Non-inseminated and non-treated cows (Control; n = 22) received at the same moment (d3) a sham injection (NaCL 0.9%). From d3 to d23 CL development and function was evaluated by B-mode and Color Doppler ultrassonography. Criteria for classifying cows that underwent luteolysis was CL area <2.0 cm^2 and colored blood flow signals that covered ≤ 25% of total CL area. Additionally, blood samples were collected every 48 h from d9 to d21 for measurement of P4 concentrations by RIA. On d30, pregnancy was checked by ultrasonography. Data were analysed with SAS v.9.3. Frequency of luteolysis was analyzed using Chi-square test and continuous variables using Kruskal-Wallis test. Treatment with P4 ($P = 0.03$) reduced luteal lifespan (Control: $17.6^{a} \pm 0.37$; P4-suppl: $16.4^{b} \pm 0.37$ and AI + P4-suppl non-pregnant cows: $15.9^{b} \pm 0.71$ days). The proportion of cows in luteolysis on d15 was not affected by AI (Control: $4.5\%^{b}$ [1/22]; P4-suppl: $33.3\%^{a}$ [7/21] and AI + P4-suppl: $26.1\%^{a}$ [6/23]; $P \leq 0.05$) but on d16, the proportion was similar between AI + P4-suppl and the control group and tended to differ between AI + P4-suppl and the P4-suppl (Control: $31.8\%^{b}$ [7/22]; P4-suppl: $61.9\%^{a,X}$ [13/21] and AI + P4-suppl: $34.8\%^{a,b,Y}$ [8/23]; $^{a,b}P = 0.05$; $^{X,Y}P = 0.07$). On d30 the P/AI was 30.4% (7/23). Removing the D30 confirmed pregnant cows, the proportion of D30 non-pregnant cows that showed luteolysis until d16 was 50.0% (8/16). In conclusion, AI affected incidence of short luteal lifespan, but this effect is limited by the conceptus developmental stage.

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Molecular control of early luteolysis in Nelore (*Bos indicus*) cows supplemented with injectable long-acting progesterone during the early luteal phase

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Keywords: cattle, corpus luteum, cytobrush.

Long-acting injectable (i) progesterone (P4) supplementation at the onset of diestrus increases conception rate in beef cattle. However, earlier onset of functional and structural regression of the corpus luteum (CL) has been observed, resulting in pregnancy loss in 30 to 50% of animals subjected to this treatment. Therefore, we tested the hypothesis that iP4-induced premature luteolysis is associated with earlier decrease in the abundance of endometrial P4 receptor (PGR) and an earlier rise in the abundance of oxytocin receptors (OXTR). Cycling, multiparous, non-lactating Nelore cows (n = 24) were selected after detection a CL in at least one of two consecutive exams, conducted 12 days apart. Cows received a P4-releasing device (Sincrogest®, OuroFino Saude Animal), 2 mg of estradiol benzoate i.m. (Sincrodiol®, Ouro Fino Saude Animal) and $PGF_2\alpha$ treatment (500 µg sodium cloprostenol; Sincrocio®, Ouro Fino Saude Animal). After 8 days an additional $PGF_2\alpha$ treatment was performed. The day of ovulation was defined as Day 0. According to pre-ovulatory follicle size, cows that ovulated were assigned to receive no treatment (Control group; n = 12) or 300 mg of iP4 on Day 3 post-ovulation (Sincrogest®, Ouro Fino Saude Animal, Brazil; iP4 group; n = 12). Endometrial tissue was collected transcervically from every cow using a cytological brush on days 3, 5, 7, 9, 11, 13 and 16, immediately submerged in 1 mL Trizol reagent and stored at -80°C. Color Doppler ovarian ultrasonography was conducted every 12 h from device removal to verify ovulation and daily from Day 3 to 25 for evaluation of development and regression of the CL. The day of structural luteolysis was defined as the day when CL area (cm^2) decreased by 25% of the average CL area measured on Days 7 and 8. Data were examined for normality using Shapiro-Wilk test. Data that were not normally distributed were transformed to natural logarithms, square or ranks. The SAS PROC MIXED procedure (version 9.2; SAS Institute, Cary, NC, USA) was used for analysis. Fisher`s exact test distribution was used for comparisons of frequency data. Data are presented as the mean ± SEM, unless otherwise indicated. Structural luteolysis occurred earlier ($P = 0.03$) in the iP4 group (16.4 ± 0.9 d) compared to the control group (18.8 ± 0.7 d). The frequency of cows that began functional luteolysis before Day 17 was greater ($P = 0.03$) in the iP4 group (50.0%) than in the control group (8.3%). There was a group by time interaction ($P < 0.05$) for abundance of the OXTR gene. Cows in the iP4 group that presented earlier onset of luteolysis had a greater abundance of OXTR on Day 16 then the previous days while cows of P4 group that did not have early luteolysis showed similar abundance of OXTR across the experiment. There was a group by time interaction ($P < 0.10$) for abundance of the PGR transcript. Cows in the iP4 group that presented earlier onset of luteolysis had greater abundance of PGR on Day 16 compared to Day 13, while cows of iP4 group that did not have early luteolysis showed greatest abundance of PGR on Day 5 that decreased on Day 7 and then remained low across time. In conclusion, iP4-induced early luteolysis is associated with an early rise in the abundance of endometrial OXTR and PGR. We speculate that this molecular event is associated with earlier release of endometrial $PGF_2\alpha$ pulses, involved in CL regression.

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(IV Workshop of the Thematic Project Signatures of Receptivity – Coordinator: Mario Binelli)

Cytobrush: a tool for molecular evaluation of the bovine endometrium

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Keywords: cattle, cytobrush, uterus.

In cattle, molecular understanding of uterine biology *in vivo* requires probing of the uterus using invasive techniques, such as biopsies. Repeated sampling may cause inflammation, physical damage and impact fertility. Therefore, validation of less traumatic sampling techniques that are repeatable and allow the collection of representative and reliable samples are necessary. In this context, this study aimed to: (1) compare the structural and functional aspect of endometrial samples collected by transcervical biopsy and cytobrush and (2) characterize the expression profile of genes involved in the luteolytic mechanism in bovine endometrium using the cytobrush technique. In Experiment 1, estrus of five Nelore cows was synchronized using a protocol based on progesterone and estradiol injections. Ovulations were detected by ultrasonography. Ten days after ovulation, endometrial samples were taken transcervically from the uterus, both by a cervical brush adapted to the tip of an artificial insemination gun and, subsequently, using a biopsy apparatus. Samples were submerged in Trizol reagent and stored at -80C until RNA extraction. The abundance of transcripts characteristic of epithelium (KRT18), stroma (VIM), immune cells (CD3D) and endothelial cells (FLT1) and transcripts involved in uterine function during the estrous cycle (PGR, PTGES, AKR1C4) was measured by RT-qPCR and compared between probing techniques. The fixed effect of technique was analyzed by one-way ANOVA using the PROC MIXED procedure (SAS version 9.2). Abundance of VIM and FLT1 was 9.2 and 275 fold greater in biopsy samples, respectively ($P < 0.05$). In contrast, abundance of KRT18 and CD3D was 5.2 and 2.2 greater in cytobrush samples, respectively ($P < 0.05$). Although there was a greater abundance PTGES mRNA in cytobrush samples ($P < 0.05$), abundance of AKR1C4 and PGR genes was not altered by the collection method ($P > 0.05$). In Experiment 2, estrus of five Nelore cows was synchronized and the day of ovulation (D0) estimated by ultrasonography. On days 10, 13, 16 and 19 endometrial samples were collected using the cytobrush method. OXTR, ESR1 and PGR2 transcript abundance was measured by qPCR and analyzed by repeated-measures ANOVA using the PROC MIXED procedure (SAS version 9.2). The least significant difference test was used for mean comparisons among days. There was an effect of day on the abundance of all transcripts (PGR2 and OXTR, $P < 0.05$; ESR1, $P < 0.1$). Abundance of ESR1 decreased gradually from D10 to D16 then increased again on D19. There was a gradual increase in the abundance of OXTR over time, and its greatest abundance was noted on day 19. In contrast, abundance of PGR2 mRNA was maximum on day 10 and then gradually decreased ($P < 0.05$). In summary, tissues obtained using the cytobrush technique provide representative samples, enriched in epithelial and immune cells, compared with biopsies. Temporal dynamics of expression of select transcripts were consistent with uterine function at the end of the estrous cycle. In conclusion, cytobrush sampling can conveniently substitute biopsy sampling, providing a safe, repeatable and less traumatic method to study bovine uterine biology *in vivo*.

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The maternal immune system and pregnancy in cattle

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Keywords: embryo, endometrium, maternal recognition.

Mammalian embryo implantation requires the priming of the maternal immune system, but, not the provocation. There are many examples of conditions where a disturbed or aberrant immune profile during embryo implantation leads to pregnancy loss. However, these studies are primarily associated with human and mouse species; data is generally limited for cattle and livestock. Most available information centres on the endometrial response to interferon tau (IFNT), a type I antiviral cytokine, which is the maternal recognition factor for cattle and sheep. Interferon tau secretion by the embryo and detection by the dam is critical to corpus luteum (CL) maintenance and pregnancy retention. However, the large volume of bovine endometrial and conceptual transcriptomic data highlights a broader more integral role of the maternal immune system in the establishment of pregnancy in cattle. Which when taken together with available immunohistochemistry and flow cytometry data from livestock, mouse, and human, presents a profile of immune cell involvement from ovulation to conception and placentation. The key events of pregnancy establishment in cattle and the involvement of the maternal immune system will be discussed.

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Spatio-specific functional transcriptomic signature of bovine endometrium 7 days after insemination

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Keywords: embryo development, gene expression, uterus.

Establishment of pregnancy depends on a well-balanced interplay between sex-steroid profiles, the maternal reproductive tract and the developing conceptus. The bovine endometrium is a dynamic tissue that undergoes spatio-temporal functional changes directed by the ovarian hormones, estradiol (E2) and progesterone (P4). Due to local vascular arrangements, sex steroid input to the uterus varies both along its wide and the broad axis. A long standing unresolved question is how early can the embryo signal to the reproductive tract and change its function. The present study tested two hypotheses simultaneously: (1) there is a spatio-specific transcriptomic signature in the endometrium and, (2) presence of an embryo influences such signature 7 days after AI (D7). It was expected that abundance of sex-steroid-responsive, receptivity related genes would decrease from anterior to posterior thirds of the uterine horn and from mesometrial to antimesometrial side. Moreover, due to its limited number of cells, presence of a D7 embryo would not affect endometrial gene expression. Estrous cycles of multiparous, non-lactating Nelore cows were synchronized and animals were allocated to one of two experimental groups at estrus (D0): Control (Con; n = 8), cows were sham-inseminated and received semen diluent; or Pregnant (Preg; n = 16), cows were inseminated with semen from the same batch of a commercial bull, 12h post estrus. Size of the pre-ovulatory follicle, subsequent CL area and plasmatic P4 concentrations on D7 were similar between groups ($P > 0.1$). Cows were slaughtered on D7 and the uterine horn ipsilateral to the CL was isolated and divided in anterior, middle and posterior thirds, starting from the uterotubaric junction. Each uterine third was washed individually with DMPBS and presence of an embryo in the flushing was confirmed in the Preg group. All embryos found (n = 10) were in the anterior third flushings. Subsequently, intercaruncular endometrial samples were collected from each uterine third in the mesometrial and antimesometrial region. Relative abundance of transcripts for 89 key genes was measured by PCR using Fluidigm platform (Biomark HD). Data were analyzed by split-split-plot ANOVA and included the effects of group (Con vs. Preg.), third (anterior, middle and posterior) and region (mesometrial and antimesometrial) and interactions. Spatial regulation of gene expression across the uterine horn was verified with upregulation for expression of genes associated to secretory activity, transporters, prostaglandin cascade and redox environment pathways on the anterior and decreasing to the posterior third. In contrast, for transcripts associated with extracellular matrix remodeling and growth factors, there was an upregulation of transcript abundance from the anterior towards the posterior third. In conclusion, the expression pattern of specific genes varied among regions of the female reproductive tract.

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Histotroph and uterine receptivity

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Keywords: bovine, pregnancy, serpin.

In cattle, before implantation, conceptus development depends on the uterine secretome (i.e., histotroph). Despite such pivotal role, little is known about the dynamics of histotroph synthesis and changes in composition throughout the early diestrus and the relevance of this entirely dynamic process for pregnancy establishment. Objective was to alter histotroph composition at early diestrus and verify the effect on (1) embryo survival and (2) timing to recover composition. Follicle growth of cycling, non-suckled, multiparous Nelore cows was synchronized with an estradiol/progesterone based protocol followed by estrus detection (d0) twice a day for 4 days. In Exp1, each uterine horn was flushed with 30 mL of D-PBS on d1 (n = 15), d4 (n = 17) or d7 (n = 16), or were not flushed (control, n = 16). On d7.5, 3 IVP embryos were transferred to the uterine horn ipsilateral to the CL. Pregnancy was checked on d25 by transrectal ultrasonography. In Exp2, each uterine horn was flushed thrice with 30 mL of D-PBS on d1 (n = 9), d4 (n = 9), d7 (n = 9), d1 + 4 + 7 (n = 9) or was not flushed (control, n = 9). A sham flushing (all procedures except delivering D-PBS to the uterus) was performed in each cow, in each experimental day when no flushing was scheduled. On d7.5, the uterine horns of all cows were flushed with 30 mL D-PBS and flushings were used to quantify total protein (BCA protein assay kit), abundance of albumin, a common serum protein (ALB; SDS-PAGE followed by Coomassie staining and densitometry) and abundance of SERPINA14, a pregnancy-related protein (SERP; Western Blotting). Preliminary data were analysed by SAS using Chi-square test for Exp1 and PROC MIXED for Exp2. In Exp1, pregnancy (P)/ET was similar between the control group, (60%; 9/15) and the flushing on d1 group (60%; 9/15) but compared to the control, the P/ET from flushing on d4 group tended to be lower (29.4%; 5/17; p = 0.06) and in the flushing on d7 group, it was numerically lower (37.5%; 6/16; p = 0.16). In Exp2, flushing increased total protein content. Compared to the control, flushing on d4 or d7 increased total protein 4.1 and 3.8 fold ($P < 0.05$), while flushing on d1 or d1 + 4 + 7 resulted in no changes or only a moderate increase (1.6 fold, $P = 0.07$), respectively. Similarly, flushings on d4 or d7 increased ($P < 0.05$) ALB abundance 5.8 and 5.2 fold, while flushing on d1 or d1 + 4 + 7 resulted in increases of 2.1 and 2.4-fold, respectively. For SERP, flushing on d4 or d7 resulted in increases of 9 and 14.5-folds ($P < 0.01$), respectively, and flushing on d1 or d1 + 4 + 7 did not change it. In conclusion, embryo retention was decreased by flushings conducted at time points approaching ET, highlighting the importance of an appropriate intrauterine milieu for the success of pregnancy. Uterine flushing at early diestrus successfully altered histotroph composition, sharply increasing the abundance of total, serum and pregnancy-related proteins.

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Pre-ovulatory endocrine profiles influence endometrial aminoacids transport, metabolism and availability in the uterine lumen during early diestrus in beef cows

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Keywords: cattle, sex steroids, uterine receptivity.

In beef cattle, a large size of the pre-ovulatory follicle (POF) and resulting elevated proestrus/estrus estradiol (E2) and diestrus progesterone (P4) concentrations positively affect conceptus growth and fertility. However, sex-steroid-mediated mechanisms that influence uterine receptivity to the embryo need to be elucidated. Amino acids are important components of maternally-derived secretions that are crucial for embryo survival before implantation. The hypothesis is that the size of the POF, E2 and P4 concentrations modulate endometrial abundance of solute carrier proteins (SLC) transcripts related to AA transport and metabolism and subsequently affect lumenal amino acids concentrations. Therefore, follicle growth of Nelore cows was manipulated to produce two experimental groups: large POF and CL (LF-LCL group) and small POF and CL (SF-SCL group). On Day 4 (D4; Experiment 1) and Day 7 (D7; Experiment 2) post GnRH injection to induce ovulation, endometrial tissue and uterine washings were collected post-mortem. Transcript abundance was evaluated by qRT-PCR and amino acid concentrations were quantified in washings by HPLC. On Experiment 1, POF size, plasma E2 concentration on D-1, and plasma concentration of P4 on D4 were 15.70mm ± 0.43 vs. 11.31mm ± 0.23 ($P < 0.01$), 2.44pg/ml ± 0.19 vs. 0.65pg/ml ($P < 0.01$) and 1.40ng/ml ± 0.23 vs. 0.80ng/ml ± 0.10 ($P < 0.01$) for the LF-LCL vs. SF-SCL groups, respectively. For Experiment 2, POF size, plasma E2 concentration on D-1 and plasma P4 concentration on D7 were 13.18mm ± 0.44 vs. 10.63mm ± 0.30 ($P < 0.01$), 2.30pg/ml ± 0.57 vs. 0.50pg/ml ± 0.13 ($P < 0.01$) and 3.68ng/ml ± 0.38 vs. 2.49ng/ml ± 0.43 ($P = 0.04$) for the LF-LCL vs. SF-SCL groups, respectively. On D4, abundance of *SLC1A4, SLC38A1, SLC6A6, SLC7A4, SLCY* and *PRMT6* and on D7, *SLC1A4, SLC6A1, SLC6A14, SLC7A4, SLC7A8, SLC38A1, SLC38A7, SLC43A2, PRMT6* and *DDO* was greater in the endometrium of cows from the LF-LCL group ($P < 0.05$). On D4, higher concentrations of taurine, alanine and α-aminobutiric acid were observed in SF-SCL ($P < 0.05$). In contrast, lower concentrations of valine and cystathionine were quantified in D7 uterine washings from SF-SCL cows ($P < 0.05$). On D4, animals from LF-LCL group, associated with greater fertility, presented less aminoacid content in uterine secretion but abundance of transporters was compatible to greater transport in comparison to animals from SF-SCL group. This suggests that before embryo moves from oviduct to uterus, amino acids transport and metabolism pathways prioritizes endometrium cells preparation for receiving the embryo but not accumulation in uterine secretions. However, on D7, when the embryo is in direct contact with uterine secretions, genes related to amino acids transport in endometrium and amino acids concentration in histotroph are up-regulated in LF-LCL cows. The latter insights indicate that amino acids metabolism and transport, towards endometrial cells or uterine secretions, might be mechanisms contributing to maternal receptivity.

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Study of estradiol mechanisms to stimulate endometrial PGF2α synthesis in cows

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Keywords: estradiol, luteolysis, prostaglandin.

In cattle, estradiol (E2) plays an important role in endometrial release of PGF2α which is associated with luteolysis. We hypothesized that estradiol modulates the expression of genes and proteins involved in the cascade of PGF2α synthesis by the endometrium of cows. 52 cyclic and non-lactating cows received 2mg of E2 benzoate (Sincrodiol, Ourofino) and an intravaginal progesterone device (1g; Sincrogest; Ourofino) for 8 days. Cows received 0.5mg of sodium cloprostenol (Sincrocio; Ourofino), intramuscular, 48 hours prior to P4 device removal and a second application at the day of its removal. On day 15 of the estrous cycle (D0; estrus), animals were assigned to receive one of the following treatments: placebo (P; 5ml 50% ethanol; IV), estradiol (E; 5mL 50% ethanol containing 3mg of 17β E2; IV) or control (untreated). Endometrial biopsies were collected at 0h (C; n = 10), 4h (E4 n = 11 and P4 n = 10) or 7h (E7 n = 10 and P7 n = 11). Two endometrial biopsies samples were obtained per cow for analysis of gene expression (qPCR) and localization of target proteins (Immunohistochemistry). Gene expression was analyzed by two-way ANOVA and mean comparison, and immunostaining was analyzed by T test. Expression of PTGS2 gene was lower in endometrium of animals from P7 and E7 groups if compared to P4 and E4 groups, respectively ($P < 0.05$). The gene expression of PLA2G4 and ESR1 were lower for animals from E4 group if compared to the E7 group ($P < 0.05$). The abundance of ESR2 gene was lower for cows from E4 and E7 groups if compared to the other groups ($P < 0.05$). A higher OXTR expression was observed in E4 and E7 groups if compared to the other groups ($P < 0.05$). PRKCα was down-regulated in endometrium from E7 group if compared to the other groups ($P < 0.05$). A lower expression of PRKCβ was observed in P7 and E7 groups if compared to the other groups ($P < 0.05$). The abundance of AKR1B1 and AKR1C4 were lower in E4 and E7 groups if compared to the other groups ($P < 0.05$). PKCγ protein immunostaining was higher ($P < 0.05$) in the luminal epithelium (LE) of animals from E4 and E7 groups compared to P4 and P7h groups ($P < 0.05$), and also higher in the glandular epithelium (GE) of P7 group compared to E7 group. The AKR1B1 protein immunostaining intensity was stronger ($P < 0.05$) in the LE from E4 and E7 groups if compared to P4 and P7 groups and was also stronger in GE of E4 group if compared to the P4 group. ERα Immunostaining was higher ($P < 0.05$) in GE of P4 and P7 groups if compared to E4 and E7 groups and also higher in LE of P4 group if compared to E4 group. In conclusion, estradiol administration reduced abundance of most genes related to proteins involved in PGF2α synthesis, except for OXTR. Moreover, the E2 treatment increased immunostaining of the PKCγ and AKR1B1 proteins, and decreased ERα. We speculate that E2 increases these proteins and that the reduced transcripts abundance was observed due to a negative feedback. Thus, protein quantification becomes necessary to determine whether modulation of gene expression by E2 is associated to an increased abundance of these proteins.

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Exploring the endometrial transcriptome during the preimplantation period

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Keywords: endometrium, transcriptomics, uterus.

The biological functions of the endometrium evolved with the development of mammalian species, i.e., the evolution of pregnancy. Its main function is the support of embryonic growth and development and to form the maternal part of the placenta after conceptus implantation. Particularly interesting is the fact that considerable differences with respect to establishment and maintenance of pregnancy exist, that suggests to study and compare this tissue in various mammalian organisms. With the invention of omics technologies, many global studies of endometrial gene expression changes during the phase of establishment of pregnancy and during the estrous cycle have been performed in livestock species to obtain a holistic view of genes associated with this biological process. Various time points have been analyzed during the preimplantation phase in bovine, porcine, and equine endometrium, e.g., for the stages of maternal recognition of pregnancy and beginning of implantation. After many transcriptome studies using the DNA microarray technology, RNA sequencing (RNA-Seq) has been used in the course of the development of Next-Generation Sequencing (NGS) technologies to get an even more deeper insight into endometrial gene expression. The results obtained from these studies for different mammalian species including humans were compared in order to find out more about common and species-specific endometrial gene expression changes during the preimplantation phase and during the sexual cycle. This rapid development of omics technologies, particularly NGS, entails also a big challenge with respect to data analysis, interpretation, and integration. More and more data sets are generated at the level of RNA and proteins as well as data from genome-wide association studies (GWAS) or QTL studies for the trait fertility. A few attempts to integrate these data have already been performed for cattle. In addition to the protein-coding mRNAs, non-coding RNA molecules such as microRNAs (miRNAs) which are mainly playing a role in regulation of gene expression are in the focus of more recent investigations in the endometrium as well as in the embryo and conceptus. Very recently, new forms of cell-to-cell communication via microvesicles and exosomes have been found in the uterus and oviduct introducing a new layer of investigation and complexity. In conclusion, although many studies have been performed to explore the endometrial transcriptome we are still at the beginning to understand the complex regulatory network involved in establishment and maintenance of pregnancy.

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Luteal function and morpho-functional aspects of the embryo-/feto-maternal crosstalk in the dog

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Keywords: bitches, corpus luteum, pregnancy.

The reproductive function of the domestic dog (*Canis lupus familiaris*) is characterized by several, species-specific regulatory mechanisms. Some of them, like the lack of luteolysis in absence of pregnancy and the concomitant luteal independence of uterine luteolysin, are unique. Consequently, similar circulating steroids levels and similar luteal life span, are observed in pregnant and non-pregnant cyclic bitches. Another important characteristic of the canine luteal function is the apparent transient independence towards gonadotropic support during the early dioestrus, i.e., at the time of luteal formation. During that time prostaglandins, especially PGE2, are among the most important luteotropic factors. Later on, during luteal maintenance, both PRL and LH are luteotropic, with PRL being the most important factor. In view of the lack of an active luteolytic principle in absence of pregnancy, the strongly extended luteal regression observed in non-pregnant dogs appears to be a passive degenerative process, strongly contrasting with the acute prepartum luteolysis. The different endocrine regulatory mechanisms governing canine luteal function in both situations, i.e. during pregnancy and in non-pregnant cycles, are therefore obvious. Additionally, the importance of canine luteal steroids in the establishment and maintenance of canine gestation arises from the fact that dog is the only domestic animal species lacking placental steroidogenesis. In this context, our recent data put a new focus on the placenta as an endocrine organ involved in regulating the duration of canine pregnancy. Based on these data, we hypothesized that the prepartum luteolysis in the dog might be due to attaining a critical lower threshold of progesterone of luteal origin, leading to activation of the placental feto-maternal crosstalk and resulting in the activation of placental prostaglandin biosynthetic pathways. The maternal stroma-derived decidual cells appear to play a critical signalling role in the underlying progesterone-mediated signalling cascade. Devoid of an anti-luteolytical principle known from other domestic animal species, and characterized by similar hormonal milieu during early dioestrus, no early gestation marker has so far been identified for the dog. Only following implantation, placental fetal relaxin can be used as a reliable marker for pregnancy diagnosis. Recently, driven by the assumption that also in the dog some mechanisms for synchronization between blastocyst development and uterine preparation for pregnancy must have evolved in order to support gestation, several target genes potentially involved in the process of early canine decidualization (the so-called 'decidualization markers') have been identified in early pregnant canine uterus during the pre-implantation stage. The process of canine decidualization was further studied in detail *in vitro* using primary cell cultures. In this regard, the dog seems to be a suitable animal model for investigating early evolutionary mechanisms involved in the preparation for implantation and ensuring further embryo survival. Consequently, during the conference selected aspects concerning canine luteal function in pregnant and non-pregnant dogs will be discussed. The comparison will be made between the mechanisms involved in cessation of luteal function in pregnant and non-pregnant bitches, along with the involvement of the feto-maternal placental crosstalk in the luteolytic cascade in this species.

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Insulin and CL function: lessons from studies in cattle and dogs

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Keywords: bovine, glucose, progesterone.

The corpus luteum (CL) is an amazing endocrine gland, which function and its regulation may differ from one species to another. In cows, for example, the cyclic CL produces progesterone (P4) for 14 days and the pregnant CL for nine months. No CL 17b-estradiol (E2) production has been reported in cows and gonadotrophins and prostaglandin F2a act as luteotrophic and luteolytic factors, respectively. In the cyclic canine CL there is also a remarkable P4 production, which in turn lasts for 60 days, as long as P4 production by the CL of pregnant bitches. Moreover, in this species, the CL produces E2 and there is no reported luteolysin that triggers cyclic CL regression. Recently, our group reported that cyclic canine CL may respond to insulin stimulus, taking up glucose. Proteins belonging to the insulin signaling driving glucose uptake were also localized in CL and quantified, showing a temporally dependent expression. On the contrary, the glucose transporter sensitive to insulin (GLUT4) and cascade related proteins were not studied in the bovine CL yet. Nevertheless, usual hormonal handling for superovulation and stimulation of the dominant follicle are associated with increased P4 production and we demonstrated that these treatments are also involved with modulation of cellular components involved in insulin signaling. Feeding cows with propylene glycol, which raises insulin plasma concentrations, seems to increase reproductive capacity. Based on theses studies we may infer that insulin regulates CL function, through glucose availability and hormone production.

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Supplementation with sunflower seed promotes endometrial changes and increases conception rates of female beef cattle

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The strategic supplementation with polyunsaturated fatty acids, especially linoleic and linolenic acid, improves reproductive performance of bovine females. The mechanisms by which such supplementation would promote benefits in conception rates are poorly understood. The sunflower seed is an important source of linoleic acid. The hypothesis is that supplementation with sunflower seed promotes an increase in conception rates of female beef cattle and that this effect is due to changes taking place in the endometrium and corpus luteum. Therefore, the aim was to evaluate: the effect of supplementation with sunflower seed in conception rates of Nelore cows submitted to TAI (Experiment 1); the conception rates of crossbred heifers, recipients of *in vitro* produced embryos, submitted to TET (Experiment 2); the plasma concentrations of total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and progesterone (P4), as well as diameter, perimeter and area of the corpus luteum, and the proportion of large (LLC) and small (SLC) luteal cells with respect to the total steroidogenic cells in the corpus luteum (Experiment 3); endometrial tissue fatty acid profile (Experiment 4); the relative abundance of transcripts of genes involved in the biosynthesis of eicosanoids in the endometrium (Experiment 5) and its effects on the number, diameter, perimeter and area of the endometrial glands (Experiment 6). In Experiment 1, Nelore cows submitted to TAI protocol, supplemented with sunflower seed for 22 days from the TAI had greater conception rates in the control group (66.67 % vs 47.76 %; P < 0 05). In Experiment 2, zebu crossbred heifers submitted to the TET protocol, supplemented with sunflower seed for 22 days from the removal of the progesterone implant showed greater conception rates in the control group (55.66 % vs. 36.94 %, P < 0.01). In Experiment 3, Nelore cows supplemented with sunflower seed showed greater plasma concentrations of total cholesterol and HDL, greater total area of the corpus luteum on D15 (D0 = day expected to estrus) and greater percentage of the corpus luteum LLC on D7 and D15, however, such features were not correlated to increased plasma concentrations of progesterone. In Experiment 4, there were changes in the endometrial tissue lipid profile of Nelore cows supplemented with sunflower seed. In experiment 5, supplementation with sunflower seed did not alter transcription of genes involved in the biosynthesis of eicosanoids in supplemented Nelore cows. In experiment 6, Nelore cows fed had an increased number of endometrial glands. It is concluded that supplementation promotes an increase in conception rate of female beef cattle and that this effect arises from changes in the endometrium.

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