001 Reproductive Endocrinology

Effects of follicular ablation on follicular growth and codominance in beef cattle

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The process of follicular selection results in one or two dominant follicles that reach a size of > 9 mm of diameter. Selection of two dominant follicles with ovulatory capacity from a follicular wave is called co-dominance. The mechanisms by which two follicles reach dominance and acquire ovulatory capacity remains unclear. To increase our understanding about ovarian physiology, we evaluated the patterns of follicular growth in beef cows during the periovulatory period. Twenty multiparous cows weighing > 350 kg were selected, estrus was synchronized with intravaginal implant of progesterone (0.5 g), estradiol benzoate (2 mg i.m.) and prostaglandin F2α (Cloprostenol 500 µg i.m.). The cows were randomly divided into two groups: Group 1 (G1) ablated group: All follicles larger than 5 mm were ablated using a 20-gauge needle and transvaginal guided ultrasonography 52 h after removal of intravaginal progesterone and injection of prostaglandin F2α in ten cows; Group 2 (G2) control group: Ten cows without follicular ablation were scanned daily to examine follicular growth, appearance of co-dominance and corpus luteum development by transrectal ultrasonography. The largest follicle reached a diameter of > 9 mm between the second and third day of the follicular ablation. The cows of the control group (G2) ovulated between 3 and 4 days after prostaglandin F2α treatment. Ovulation in the ablated group occurred between 5 and 8 days after ablation. Five cows from the ablated group developed co-dominance and double ovulation, as confirmed by the presence of two corpora lutea. However, co-dominance and double ovulation was not observed in the control group. In conclusion, follicular aspiration resulted in an increase in co-dominance and double ovulation in beef cattle.
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Supplementation of 17ß-estradiol and progesterone in the co-culture medium of bovine oviductal epithelial cells and ovine spermatozoa reduces sperm kinematics and capacitation

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In mammals, spermatozoa attach to oviductal epithelial cell and this interaction is able to maintain fertilizing ability until ovulation occurs. During the estrous cycle, sperm-oviduct interactions and oviduct-secreted proteins are influenced by the dynamics of the hormonal environment. Thus, this study assessed the effect of hormonally supplemented bovine oviductal epithelial cell (BOEC) co-culture on ram sperm function throughout 24 h of incubation. Ram cooled-stored spermatozoa were selected by swim-up and then co-cultured separately for 24 h at 38.5ºC under 5% CO_2 with either: (1) Fert-TALP medium (positive control – POSControl); (2) Fert-TALP medium without any capacitating substance (modified Fert-TALP) supplemented with 17beta-estradiol (E2) and progesterone (P4) at concentrations similar to follicular phase (Follicular NEGControl); (3) modified Fert-TALP medium supplemented with E2 and P4 concentrations similar to luteal phase (Luteal NEGControl); (4) BOEC cultured in the same medium of Follicular NEGControl group (Follicular BOEC group); (5) BOEC cultured in the same medium of Luteal NEGControl group (Luteal BOEC group). Sperm kinematics (analyzed by computer-assisted semen analysis - CASA), capacitation status and sperm plasma membrane integrity were evaluated in different intervals (0, 2, 4, 6, 18 and 24 h). The variables were subjected to a repeated measure two- way ANOVA (mixed model) and Bonferroni *post hoc* test ($P < 0.05$). Sperm plasma membrane integrity was not affected ($P > 0.05$) by BOEC co-culture, regardless the phase of the estrous cycle. At 2 and 4 h, the Luteal BOEC group presented lower ($P < 0.05$) progressive motility (PM) and total motility than the Luteal NEGControl group. At 4 h, the Follicular BOEC group showed lower ($P < 0.05$) velocimetric parameters (straight-line velocity, average path velocity, curvilinear velocity and beat/cross frequency) and PM than the Follicular NEGControl group. Throughout incubation, both BOEC co-culture groups showed a decrease ($P < 0.05$) in their capacitation rate compared with the POSControl group. On the other hand, the Luteal BOEC group had a greater ($P < 0.05$) noncapacitated rate than the POSControl group and Luteal NEGControl group. In conclusion, co-culture between ram cooled-stored spermatozoa and BOEC submitted to a hormonal environment similar to the follicular and luteal phases is able to suppress motility and also has a role on delaying sperm *in vitro* capacitation and consequently, prolonging the life span of spermatozoa. This study was supported by FINEP and FAPERJ.

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Effect of zinc supplementation on the area of corpus luteum and progesterone serum concentration

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Zinc (Zn) is a relevant trace element in the body. The function of Zn involves a wide range of biological processes including cell proliferation, immune function, and defense against free radicals. Zinc is an essential component of Cu/Zn-SOD that plays a key role in the maintenance of a functional corpus luteum (CL), in its morphology and progesterone (P_4) production (Kawaguchi *et al*., 2013). The aim of this study was to evaluate the effect of parenteral Zn supplementation at the beginning of fixed-time artificial insemination (FTAI) on corpus luteum size, P_4 and Zn serum concentration. Multiparous Aberdeen Angus cows (n =27) were randomly assigned to two groups: Control (n=16) and Zinc (n=11) group supplemented with 400 mg $ZnSO_4$ injected at the beginning of FTAI protocol (day 0). Follicular waves were synchronized by intravaginal insertion of a CIDR for seven days and an intramuscular (i.m.) injection of estradiol (E_2) benzoate (day 0). At day 7, CIDR was removed and a i.m. injection of PGF2α and E_2 cypionate was applied. All cows were inseminated on day 9. Blood samples were collected on day 0, 7, 9 and 16. The variables assessed were Zn serum concentration (day 0, 7, 9 and 16), area of preovulatory follicle (**APF**), and E_2 serum concentration (**E_2SC**) at insemination time (day 9), area of corpus luteum (**ACL**) and P_4 serum concentration (**P_4SC**) at day 16 and pregnancy rate (day 40). The statistical analysis was carried out with SAS. Continuous response variables were analyzed with linear models and pregnancy rate (percentage) was analyzed by logistic regression. Serum zinc concentrations (Mean ± SEM) were not affected by Zn supplementation for Control= 92,8 ± 8,3; 130,4 ± 8,3; 99 ± 8,3; 89,3± 8,3 µg/dL; and Zinc= 89,6 ± 10; 121,6 ± 10; 100,8 ± 10; 90,6 ± 10 µg/dL at days 0, 7, 9 and 16 respectively ($P > 0.05$). These results showed that 66.6 % cows (18/27) had serum Zn deficiency (< 90 µg/dL) at the beginning of FTAI protocol (day 0). Zinc supplementation did not modify APF (Control= 10.1 ± 1.0; Zn= 12.9 ± 1.2 mm^2), E_2SC (Control= 17.8 ± 1.0; Zn= 16.3 ± 1.2 pg/mL) and ACL (Control= 34.8 ± 2.7; Zn= 38.6 ± 3.7 mm^2) when all cows were considered. However, Zn supplementation increased ACL (Control= 32.6 ± 2.9; Zn= 43.5 ± 3.9 mm^2; $P < 0.05$) in Zn deficient cow and tended to increase APF (Control= 9.7 ± 1.2; Zn= 13.6 ± 1.5 mm^2; $P = 0.097$). The P_4SC were increased by Zn supplementation when all cows were considered (Control= 4.2 ± 0.4; Zn= 5.7 ± 0.5 ng/mL; $P < 0.05$). The P_4SC of deficient cow were similar between treatments (Control= 4.1 ± 0.6; Zn= 5.4 ± 0.8 ng/mL; $P > 0.05$). Pregnancy rates at day 40 was higher but not significantly different for cows injected with Zn respect to Control group (Control= 46.1%, 50%; Zn= 80%, 100%, considering all and deficient cows respectively $P > 0.05$). In conclusion, Zn supplementation at the beginning of the FTAI protocol in deficient cows increased corpus luteum area, and increase serum progesterone concentrations when all cows were considered in the analyses. This study provide evidence that parenteral Zn supplementation may enhance pregnancy rates, even in those cows that present adequate Zn serum concentrations.

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Delayed time of luteolysis using ovulatory doses of GnRH on days 8 and 15 after insemination in dairy cows

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Avoidance of corpus luteum (CL) regression during the critical period after fertilization is a main goal for reproductive success. Synthesis of uterine prostaglandin F2-alpha is stimulated by the increased responsiveness of the endometrial epithelium to oxytocin pulses following a coordinated action of progesterone (P4) and estradiol (E2) on their receptors (Silvia *et al*., 1991. Biol Reprod, 45:655-663). Experiments using follicular ablation between days 9-15 after ovulation managed to decrease E2 concentrations and delay the time of luteolysis (Araujo *et al*., 2009. Biol Reprod, 81:426-437). This study aimed to test the following hypotheses: 1) Cows receiving Gonadotropin Releasing Hormone (GnRH) on day 8 after artificial insemination (AI) will generate accessory CL and start a new follicular wave. 2) Cows receiving GnRH 15 days after AI will ovulate the dominant follicle and have delayed luteolysis. 3) The combination of treatments would improve the number of pregnant cows (P/AI). Four treatments were randomly assigned to 200 lactating Holstein cows submitted to fixed time AI 72 days after calving using double-ovsynch (Souza *et al*., 2008. Theriogenology, 70:208-215) synchronization protocol: 1) Control: No further treatment received (n=51); 2) G8: Cows received an intramuscular injection of 200µl of GnRH (Gonadorelin Acetate, Gonabreed, Parnell Pharmaceuticals, Overland Park, KS, USA) on day 8 after AI (n=46); 3) G15: Cows received 200µl of GnRH on day 15 after AI (n=51); 4) G8-15: Cows received 200µl of GnRH on day 8 and 200µl of GnRH on day 15 after AI (n=52). Blood samples were taken on days -3, -1, 8, 15, 18, 20, 22, 25, and 27 relative to AI to determine P4 concentrations, and ultrasound of ovarian structures was performed in order to determine CL regression in the non-pregnant cows. Undetermined CL regressions or ovulations were excluded from the analysis. Ovulatory response to GnRH on day 8 (80.6%, 79/98) was higher (P< 0.0001) than on day 15 (48.5%, 50/103), with no differences between G8 and G8-15 (P=0.44) nor G15 and G8-15 (P=1.0). Timing of luteolysis in non-pregnant cows was delayed in the G15 treatment (22.07±0.82d, n=14) compared to control (19.71±0.43d, n=17; P= 0.01) and G8 (19.62±0.67d, n=21; P= 0.03). For cows receiving GnRH on day 15, timing of luteolysis was longer for ovulating (24.71±0.80d, n=7) compared to not-ovulating cows (21.18±0.75, n=11; P= 0.007). However, not-ovulating cows to day 15 GnRH tended (P=0.09) to have longer time of luteolysis than cows not receiving GnRH (19.66±0.42, n=38). Overall conception rate (P/AI) was 45% (90/200) for pregnancy diagnose at day 32, with no difference detected between groups (control 41.2% (21/51); G8 37.0% (17/46); G15 52.9% (27/51); and G8-15 48.1% (25/52); P=0.40). Injection of GnRH on day 15 after AI causes ovulation of dominant follicle present in about half of the cows treated and extends the life of the CL increasing the risk of pregnancy success. Experiments with larger number of animals are required to determine whether injecting GnRH at day 15 after AI will improve P/AI, and also to determine whether steroidogenesis of E2 is affected after treatment regardless ovulation occurs or not.

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The anti-equine Chorionic Gonadotrophin (eCG) antibody response after an eCG treatment during the non-breeding season does not affect the semen quality during the following breeding season in bucks

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Equine Chorionic Gonadotrophin (eCG or PMSG) is a glycoprotein with FSH/LH effects that has been widely used to induce follicular growth and ovulation in females. However, it may also be used to improve male reproduction. In a previous study we administered 5 doses of eCG (an initial dose of 800 IU, followed by 4 doses of 500 IU administered every 5 days), to bucks during the non-breeding season, observing an increase of testosterone secretion and an enhancement of fresh and frozen-thawed semen quality during the period in which eCG was administered. However, we also observed an increase in anti-eCG antibodies titer (435.6 ± 31.3 nmol/L vs 29.6 ± 4.0 nmol/L in treated vs untreated bucks). In female goats, repeated treatment with eCG is associated with a decrease in fertility caused by a possible cross-reaction, as endogenous LH or FSH can be blocked by circulating anti-eCG antibodies. Therefore, the aim of the present study was to determine if eCG treatment of bucks during the non- breeding season had negative consequences on testosterone concentration or seminal parameters during the first breeding season following the treatment. The study was performed during the breeding season (February – March, summer in the southern hemisphere) for 30 days, starting 91 days after the last eCG administration (treated group: n=10, untreated group: n=9). Testosterone was measured in serum from samples collected every 3 days by radioimmunoassay, using a solid phase kit. Anti-eCG antibodies were quantified with an ELISA kit in serum from samples collected on Day 91 and 98. Semen was collected weekly by electroejaculation. Statistical analysis was performed using a mixed model of SAS, considering the treatment (treated vs untreated bucks), the time (day of collection), and the interaction between treatment and time. The anti-eCG titer was still greater in treated than in untreated bucks (181.7 ± 44.0 vs 28.1± 7.6 nmol/L; P=0.009). However, there were no treatment differences, nor an interaction between treatment and time in testosterone concentration, sperm concentration, motility, percentages of motile spermatozoa, progressive motility of spermatozoa, or of spermatozoa with a functional membrane. Overall, it was concluded that although the bucks that were previously treated with eCG had greater amount of anti-eCG antibodies, there were no negative consequences on testosterone concentration or seminal parameters during the first breeding season following the treatment. We acknowledge Milton Pintos, María Noel Viera, Gerardo Less and Matias Fiorelli for their help during the collection of the samples. Also to Syntex Uruguay for providing the hormone used in this study. This study was supported by CSIC (Universidad de la República, Uruguay). FB received a scholarship from Agencia Nacional de Investigación e Innovación (ANII, Uruguay).

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Timing of regression of contralateral accessory corpora lutea in pregnant lactating dairy cows

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Preliminary data have suggested that in cows with accessory corpus luteum (ACL) pregnancy loss is greater when this ACL is contralateral to the pregnancy. The aim of the study was to investigate an experimental model related to regression of ACL that were ipsilateral or contralateral to the pregnancy (until day 70 of gestation) in dairy cows. Sixteen lactating Holstein cows bred by fixed-time artificial insemination (FTAI; D0) were treated with GnRH 5 d after AI (D5) to form an ACL. Ovarian and uterine ultrasound evaluations were performed and blood samples were collected daily until D70 of pregnancy to evaluate ovulation, pregnancy, CL size and maintenance, and to analyze circulating progesterone (P4) concentrations. On D28, 13 cows were confirmed pregnant and all had ACL. On D70, cows were submitted to an oxytocin challenge with 100 IU oxytocin i.v. and concentrations of PGFM (pg/mL) were analyzed 0, 30, 60, 90, and 120 min after the oxytocin infusion. Statistical analyzes were performed using PROC GLIMMIX and MIXED of SAS 9.4 (LSM ± SEM; $P \leq 0.05$). In 9 pregnant cows, the ACL was contralateral to the original CL and in 5 pregnant cows, the CL was ipsilateral (1 cow had ACL in both ovaries). There were 2 periods in which luteolysis of the contralateral ACL occurred: between D22-24 (early luteolysis) or D48-53 (late luteolysis) after AI. Luteolysis occurred more frequently in cows with contralateral ACL [88.9% (8/9)] than in cows with ipsilateral ACL [0% (0/5)]. Interestingly, most [75% (6/8)] of contralateral ACL regressed late (average of day 50.3 after AI). The P4 concentration (ng/mL) was high and did not differ in cows that did not undergo early (11.1 ± 0.6) or late (10.4 ± 0.8) CL regression. Circulating P4 of cows that had either early or late luteolysis was similar on days -2, -1 and on the day of the onset of luteolysis (8.5 ± 3.3) determined by CL size using ultrasound. However, 1, 2 and 3 d after the onset of luteolysis (early or late luteolysis) P4 was lower for cows with CL regression than for cows without luteolysis (5.6 ± 2.9 vs 10.5 ± 0.8). The oxytocin challenge on D70 of pregnancy increased PGFM concentrations at 30, 60 and 90 min after oxytocin compared to time 0 or 120 min after oxytocin (19.5 ± 4.3^{a}, 40.3 ± 8.8^{b}; 35.0 ± 6.2^{b}, 33.5 ± 4.6^{b}, and 17.5 ± 4.2^{a}; for 0, 30, 60, 90, and 120 min, respectively). Despite PGF release after oxytocin, no cow underwent luteolysis of the original CL or had pregnancy loss after the oxytocin challenge. In summary, ipsilateral ACL did not regress during the first 70 days of pregnancy, whereas most contralateral CL regressed during this period, especially with late luteolysis, providing evidence for the involvement of local mechanisms in regression of ACL and in protection of the ipsilateral CL during the first and second month of pregnancy. In addition, oxytocin-induced release of PGF without regression of the original CL or loss of pregnancy at 70 d of pregnancy provides evidence that the pregnancy maintains the ipsilateral CL via mechanisms that prevent either transport or action of PGF on the CL. Wisconsin Experiment Station (WIS01240), BARD (IS-4799-15), FAPESP, CNPq, and CAPES.

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Fractal analysis is a useful tool to evaluate bovine luteal development

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The *corpus luteum* (CL) is a temporary endocrine gland that is formed after ovulation and is the structure responsible for the initial maintenance of gestation. The tissue remodeling by which CL is formed is poorly described, particularly with respect to extracellular matrix components. To assist in evaluating and quantifying tissue changes, fractal dimension (FD) becomes a useful method, being used as a diagnostic tool for retinopathies, histopathological studies of neoplasms, hepatocyte morphometry, liver fibrosis and cardiac studies. Furthermore, it is a useful technique for quantifying the organization in an image from fractals that describe the amount of space and the self-similarity of the structure, once FD detects subtle morphological changes and performs functional quantitative measures. Based on this, we hypothesized that fractal analysis will be different between functional and regressing bovine CLs. For this, c*orpora lutea* was morphologically classified into two developmental stages as previously described (Ireland *et al*.,1980): functional CL (mid-cycle CL) showed well developed vasculature often visible at the apex and were completely orange or yellow, measuring from 1.6 to 2.0 cm; regressed CL showed no visible vasculature at the surface and were pale yellow to incolor measuring less than 1 cm diameter. Ten CLs were collected from a local abattoir (SP): five of functional CLs and five of regressed CL. The CLs were dissected, fixed in methacarn and processed for light microscope. The slides stained with H&E were designed to fractal analysis. One section of each CL was used to acquire five images/ section (totalizing 25 images per CL stage). After, images were binarized for reading and were the estimated by the box-counting method, through the software Image J. The result can be quantitatively expressed as FD = (Log Nr / log r-1) and, for this reason, the dimension will always quantify between 0 and 2. The means were compared with t-test using JMP software (SAS InstituteCary, NC). Differences were considered significant when $P \leq 0.05$. The results demonstrated a higher fractal dimension in functional CL (1.79±0.009) compared with regressed CL (1.66±0.02; P=0.0016). In conclusion, the higher FD observed during luteal regression showed that this analysis is an effective method to evaluate the tissue changes observed during luteal development in cattle. The authors are grateful to grant #2013/11480-3, São Paulo Research Foundation (FAPESP).

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Hormonal treatment post insemination for induction of accessory corpora lutea and production of progesterone in sheep

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Different therapeutic strategies have been used with the objective of increasing the concentration of progesterone (P_4) and improving luteal function to reduce embryonic losses. Hormonal treatments in sheep were carried out in different breeds, animal categories and seasons of the year, using gonadotrophin releasing hormone (GnRH) or human chorionic gonadotrophin (hCG) in the early or late luteal phase. The aim of the study was to evaluate the effect of the administration of GnRH or hCG at day 4 post fixed-time artificial insemination (FTAI) on the induction and maintenance of accessory corpora lutea (acc-CL) and on the production of serum P_4 concentration. Multiparous Merino ewes (n= 36) were treated for estrus synchronization using intravaginal progestogen sponges (60 mg of MAP; Progespon®, Syntex, Argentina), during 14 days and a single dose of equine Chorionic Gonadotropin (200 IU of eCG, i.m.; Novormon®, Syntex, Argentina) was administered at the end of progestagen treatment. At 53-56 h after sponge removal, FTAI was performed vaginally with a dose of 100 million spermatozoa of fresh semen. The ewes were assigned randomly to three groups on day 4 post FTAI: 1. GnRH group (n= 12, 4 μg of GnRH, i.m., Buserelin, Receptal®, Intervet, Argentina), 2. hCG group (n= 12, 300 IU of hCG, i.m., Gonacor®, Ferring, Argentina) and 3. Control group (n= 12, 1 mL of saline solution, i.m.). Laparoscopic observation of the ovaries at day 4, 10 and 21 post FTAI was performed to determine the presence of ovulatory CL (days 4, 10 and 21) and acc-CL (days 10 and 21). Serum P_4 concentration was assessed by chemiluminescence on days 4, 7, 10, 14, 17, 21, 28 and 35 post FTAI. The number of acc-CL was compared by one-way ANOVA. The serum P_4 concentration in pregnant ewes was analyzed by a generalized linear model with time-repeated measurements. Statistical significance was accepted from $P<0.05$. The hCG group showed higher mean concentrations of P_4 on days 7, 10, 14, 21, 28 and 35 post FTAI compared with the GnRH group and the Control group ($P<0.05$), while no differences were observed between these two latter groups ($P>0.05$). In all groups, an increase of serum P_4 concentration was observed on day 35 post FTAI ($P<0.05$). The presence of an acc-CL was observed in 85 and 50 % of ewes treated with hCG or GnRH, respectively, whereas no ewes with an acc-CL were observed in the Control group ($P<0.05$). The hCG group had higher concentration of P_4 in the animals that had an acc-CL compared to those that did not generate acc-CL ($P<0.05$), while no differences were observed in the GnRH Group ($P>0.05$). The acc-CL were maintained until 21 days post FTAI in pregnant sheep treated hormonally. In conclusion, administration of hCG or GnRH at 4 days post FTAI induced and maintained the formation of an acc-CL. However, serum concentration of P_4 increased only in the hCG group and maintained until day 35 post FTAI. Differences in the pharmacodynamics of these two hormones might induce acc-CL with different steroidogenic capacity. Further research should be done to assess the effect of these hormones on the histological and functional characteristic of acc-CL. Funded by Projects PNSA 1115053 (INTA) and PICT 2012-2238 (FONCyT).

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Dietary restriction in sheep: uterine functionality in ewes with different body reserves during early gestation

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The aim of this work was to study the reproductive response to a food restriction in ewes with different body condition score (0, emaciated-5, obese) at the beginning of the experiment (BCSi). During the breeding season, 36 Rasa Aragonesa ewes were divided into 2 groups with different BCS: BCS> 2.75 (high, H, 2.9 ± 0.04) and BCS <2.25 (low, L, 2.1 ± 0.04). Both groups received a diet to cover energy and protein maintenance requirements for 20 days, after which they were randomly assigned to two nutritional treatments: 1.5 (control, C) or 0.5 (undernourishment, S) times the daily maintenance requirements establishing four groups: high BCSi fed the control diet (HC, n = 9), high BCSi undernourished (HS, n = 10), low BCSi control (LC, n = 9) and low BCSi undernourished (LS, n = 8). The first day of the experimental diet, ewes were estrous synchronized with intravaginal sponges for 12 days and mated by ten rams (estrus=Day 0). Embryos were recovered on Day 5, and embryo viability was estimated *in vitro* after incubation for 48 h. Only ewes that had embryos were included in this study. Gene expression of uterine receptors of progesterone (PR), estrogen (ER), growth hormone (GHR), insulin (INSR), leptin (LEPR), adiponectin (ADIPOR2) and IGF-I, IGF-II and IGF binding proteins (IGFBP2-6), were determined by RT-PCR. All the variables were analyzed by ANOVA using a mixed procedure that included in the model BCSi, the nutritional treatment and their interaction. Embryo data was evaluated using Proc Genmod. Undernutrition decreased liveweight and insulin concentration but had no effect on leptin and IGF-I levels. BCSi affected all hormones, but the effect was more pronounced on IGF-I (395 *vs* 261 ng/mL for H and L groups respectively, P<0.05). Embryos from ewes with greater BCS tended to have higher viability rates (83.3 *vs* 58.3%, P=0.09) and *in vitro* embryo development (75.0 *vs* 48.4%, P=0.1). Uterine gene expression of most of the studied genes was not affected by BCSi or nutritional treatment. Ewes with low BCSi tended to have more *INSR* mRNA (P=0.07) than ewes with high BCSi, and LC ewes tended to have more *GHR* mRNA than HC ewes (P=0.10). *GHR* mRNA expression was higher in undernourished ewes than in control ewes (P=0.02). The changes in uterine gene expression suggest a compensatory mechanism that increases hormone tissue sensitivity when the hormone is decreased. Besides, *BP2* mRNA tended to be higher in undernourished than in control group (P=0.08). Overall, BCSi – but not undernutrition- effect on embryo viability is consistent with the marked differences found in IGF-I concentrations, which is a main promoter of embryo development and uterine function. Surprisingly, even if aligned in a similar pattern, BCSi and undernutrition affected few uterine genes. It should be taken into account that gene expression does not always reflect actual protein content. In conclusion, in the present study, embryo viability depended mainly on metabolic history (body reserves) than on more recent feeding level.

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Testosterone concentration profiles in bucks treated with a GnRH-agonist implant or immunized against GnRH

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The chronic use of GnRH-agonists or the immunization against GnRH are reversible contraceptive methods that inhibit temporarily the hypothalamic-pituitary-testicular axis activity. The aim of this study was to compare the changes in testosterone profile in bucks treated with a GnRH-agonist or immunized against GnRH. Seven bucks were treated with deslorelin (4.7 mg) subcutaneous implants (GnRH agonist that is released continuously; group AGO), 7 with an anti-GnRH vaccine s.c. (300 µg GnRH-protein conjugate, two doses, group IMM), and 9 bucks remained untreated as controls (group CON). The study lasted 18 months, beginning the treatments during late spring (Week 0). Blood samples for measuring testosterone concentration (Tc) were weekly collected from Week -4 to 3 (Month 1), and monthly from Month 2 to 17. Data were analyzed using a mixed model considering as main effects the treatment, time, and the interaction between treatment and time, and are presented as LSmeans ± SEM. Bucks from AGO group had greater Tc than CON and IMM bucks in Months 1 (19.6±2.2 nmol/L, 12.5±1.8 nmol/L and 7.6±2.1 nmol/L, respectively, $P<0.05$ for all comparisons) and 2 (24.0±3.4 nmol/L, 5.9±2.9 nmol/L and 1.0±3.4 nmol/L, respectively, $P<0.05$ for all comparisons). Bucks from CON group had greater Tc than AGO and IMM bucks in Months 3 (31.8±2.9 nmol/L, 11.0±3.4 nmol/L and 7.9±3.4 nmol/L, respectively, $P<0.05$ for all comparisons) and 4 (19.2±2.9 nmol/L, 7.3±3.4 nmol/L and 7.9±3.4 nmol/L, respectively, $P<0.05$ for all comparisons). From Months 5 to 14 there were not differences in Tc between groups. Bucks from CON and IMM groups had greater Tc than AGO bucks in Months 15 (25.1±3.6 nmol/L, 29.3±3.7 nmol/L and 10.4±3.7 nmol/L, respectively, $P<0.05$ for all comparisons) and 16 (19.4±2.9 nmol/L, 23.5±3.7 nmol/L and 6.7±3.7 nmol/L, respectively, $P<0.05$ for all comparisons). Bucks from IMM group had greater Tc (33.6±3.7 nmol/L) than CON (16.4±3.1 nmol/L, $P<0.05$) and AGO (8.4±3.7 nmol/L, $P<0.05$) bucks in Month 17. At the beginning of the study AGO bucks had greater Tc due to the administration of the GnRH agonist ("flare up" effect). However, it later decreased and remained low, probably due to the inhibition of the pituitary or the testes, effect that was not overcome in 17 months. On the other hand, Tc decreased in IMM bucks but recovered in coincidence with the increase in CON bucks, during the onset of the breeding season. However, at the end of the study IMM bucks had greater Tc than CON bucks. The anti-GnRH vaccine probably lost the effect due to a decrease of the immune response, followed by a rebound effect. In sum, the effects elicited by the chronic use of a GnRH agonist lasted longer and produced a more pronounced decrease in Tc than the immunization against GnRH.

011 Reproductive Endocrinology

Follicle and hormone profiles during selection of the dominant follicle under different physiologic conditions in Holstein heifers

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Diameter deviation during selection of the future dominant follicle (F1) from the future largest subordinate follicle (F2) is defined as continued growth rate of F1 and decreased growth rate of F2. Expected diameter deviation in Holstein heifers begins when F1 is 8.5 mm and has been classified as conventional (F2 ≥ 7.0 mm at deviation) or undersized (F2 < 7.0 mm). Our overall objective was to understand the circulating hormones and ovarian physiology associated with conventional and undersized deviation. This study compared circulating FSH, LH, and P4 with the follicle dynamics during three different physiological conditions (spontaneous wave 2 vs a wave 2 induced by aspiration of wave-1 follicles, wave 1 vs 2, and conventional vs undersized deviations). Holstein dairy heifers (N = 24) were evaluated during wave 1 and randomized 6 days after ovulation into an induced wave 2 and a spontaneous wave 2. Values were normalized to the day of expected diameter deviation (day 0) and compared for days −2 to 0 and 0 to 2 using the SAS PROC MIXED procedure. Hypothesis 1 was supported that an induced wave 2 and spontaneous wave 2 have similar follicle dynamics. However, the peak of the FSH surge was more prominent at the emergence of an induced wave 2 ($P < 0.003$). Hypothesis 2 was supported that waves 1 and 2 differ in follicle and hormone events. Circulating P4 was less and LH was greater ($P < 0.01$) encompassing deviation in wave 1 than wave 2. Diameter of F1 did not differ but diameter of F2 was greater ($P < 0.01$) on day 0 in wave 1 (7.3 ± 0.2 mm) than in wave 2 (6.6 ± 0.2 mm). Differences between waves were not found when deviation in each follicular wave was classified as conventional vs undersized deviation and analyzed separately. Hypothesis 3 was supported that hormone differences occur between conventional and undersized deviations. Growth rate of F2 differed ($P < 0.0005$) during days −2 to 0 (conventional, 2.6 ± 0.2 mm/2d; undersized, 1.4 ± 0.3 mm/2d). However, circulating FSH and P4 concentration on days −1 and 0 tended to be greater ($P < 0.06$) in undersized deviations. Thus, the effect of different hormonal conditions on follicle dynamics was observed for F2 and not for F1. Understanding the physiology that produces conventional vs undersized deviations accounted for most differences in follicle dynamics and circulating FSH in these different physiological conditions. In addition, study of wave 2 may be facilitated by inducing a wave 2 since it was similar in follicle dynamics to a spontaneous wave 2. The authors thank the funding support from Eutheria Foundation, University of Wisconsin Experiment Station, CNPq, CAPES, and COLFUTURO.

012 Reproductive Endocrinology

The Periovulatory Endocrine Milieu Affects the Metabolomic Profile of the Oviductal Fluid in Beef Cows

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Oviductal secretions regulate the environment in which sperm storage and capacitation, fertilization, and early embryo development occur; however, molecular control of oviduct receptivity to the embryo is poorly understood. A model for receptivity based on the manipulation of the size of the pre-ovulatory follicle (POF) was used to compare oviductal fluid (OvF) composition on day 4 of the estrous cycle. The central hypothesis was that the size of POF modulates the periovulatory endocrine milieu and affects the composition of the OvF. Cycling, non-lactating, multiparous Nelore cows were presynchronized prior to receiving cloprostenol (large follicle [LF] group) or not (small follicle [SF] group), along with a progesterone (P4) device on Day (D) -10. Devices were withdrawn and cloprostenol administered 42–60 h (LF) or 30-36 h (SF) before GnRH agonist treatment (D0). As a result, greater proestrus estrogen concentrations, corpora lutea and early diestrus progesterone concentrations were also observed in LF group in comparison to SF group. Four days after GnRH-induced ovulation, the oviduct was dissected and lumen was flushed using 2 mL of sterile PBS to obtain OvF. The OvF was centrifuged to remove cells and debris. Next, the supernatant was frozen in liquid Nitrogen, and stored at -80°C for further analysis. Quantitative mass spectrometry was used to determine the concentration of 21 amino acids (AA), 21 biogenic amines (BA), 40 acylcarnitines (AC), 76 phosphatidylcholines (PC), 14 lysophosphatidylcholines (LP), 15 sphingomyelins (SM), 29 hexoses (HX), and 17 prostaglandins and related compounds (PGC). Multivariate analyses using the software MetaboAnalyst 3.0 were performed to identify which metabolites better explained the separation of experimental groups and which could be potentially used as markers of receptivity. Analytes with 50% or more of missing data were excluded from analyses. Partial Least Squares Discriminant Analysis (PLS-DA) was used to create a scores plot between the two groups and to identify the most important explanatory variables. The PLS-DA showed that the overall metabolite profiles of the LF-LCL and SF-SCL groups were significantly different and that samples from each group were divided clearly into two non-overlapping clusters. The most influential variables to separate the two groups included AAs, PCs, LPs and arachidonic acid. These results were further confirmed by univariate analyses. There were statistical differences in the concentration of 31 metabolites ($P \leq 0.05$) between groups. We concluded that the composition of the OvF is different between cows with contrasting receptivity and fertility status. Although further studies and analyses are needed, it could be assumed that molecules in OvF presenting different concentrations between groups can be used as biomarkers of receptivity. Additionally, it will be critical to identify the function of each of these compounds during early embryo development and to evaluate their potential use as supplements for in vitro production of embryos. The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq): AMGD grant number 150844/2017-4 and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): MB grant number 2011/03226-4.

013 Reproductive Endocrinology

Local effect of the corpus luteum (CL) on reproductive tract functionality in the ewe

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This study was designed to evaluate the effect of the laterality of CL on embryonic development and oviductal-uterine functionality in the ewe. For this purpose, *in vitro* produced embryos (Day 0: IVF) were transferred on Day 1 into oviducts ipsilateral (ILAT) and contralateral (CLAT) to the CL (18 to 20 embryos per oviduct, 38 to 40 embryos per ewe) in 12 estrous synchronized ewes one day after ovulation. On Day 6, the reproductive tract was obtained at slaughter, and the uterine horns were flushed to collect the embryos. A greater recovery rate, better embryo development, and less degenerated embryos were obtained when transferring zygotes to the ILAT than the CLAT oviduct (de Brun *et al*., 2016. Proc 30th Annu Mtg of Brazilian Embryo Technol Soc (SBTE 2016), p.599). Progesterone, estradiol, insulin, IGF1, leptin and adiponectin concentrations were determined in plasma, uterine fluid, and uterine and oviduct tissue, ILAT and CLAT to the CL. Gene expression of *ERa*, *PR, IGF1, IGF1R, INSR, IGFBP2-6, LEPR*, *AdipoR1y 2* were determined in ILAT and CLAT uterine horns. All statistical analysis were performed using the Statistical Analysis System (SAS), PROC MIXED, where the model was the side relative to ovulation-CL. The ILAT oviductal tissue presented 4 times higher P4 concentrations compared to the CLAT oviduct (57.3 ± 15.1 vs. 11.6 ± 4.8 ng/g of tissue, $P<0.05$); nevertheless, no other differences according to the side of CL were found in hormones concentrations in uterine or oviductal tissues. On the other hand, ILAT uterine fluid presented lower insulin concentrations compared to the CLAT (5.9 ± 0.3 vs. 4.6 ± 0.2 µIU/mL, $P=0.05$), perhaps associated with higher uptake of insulin by the embryo in the ILAT horn. The ILAT uterine horn also had greater expression of *ERa* (1.8 ± 0.2 vs. 0.9 ± 0.3, $P<0.05$), which may be related to a greater previous exposure to preovulatory estradiol. In addition, *LEPR* (1.3 ± 0.2 vs. 0.7 ± 0.2, $P<0.05$) and *IGFBP3* (1.1 ± 0.1 vs. 0.7 ± 0.1, $P=0.08$) mRNA expression was greater or tended to be greater in the ILAT vs. the CLAT uterine horn, and both factors have been positively associated with embryo growth in ruminants (Cerro and Pintar, 1997. Dev Biol, 184:278-95). In conclusion, we suggest a local effect of CL, which is evidenced by the concentrations of progesterone in oviductal tissue, insulin in uterine fluid and the differential gene expression of some target genes according to the side of the reproductive tract with respect location of the CL, which is associated with greater embryo recovery and development in the ipsilateral horn.

014 Reproductive Endocrinology

Functional transitions in the corpus luteum are associated with changes in NR5A2 abundance, which regulates luteal progesterone production

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Nuclear receptor subfamily 5 group A member 2 (NR5A2) has roles in ovulation and early luteal development. However, little is known about its function in the bovine corpus luteum (CL) during acquisition of luteolytic capacity (ALC), luteolysis, or luteal rescue. To identify key regulators of luteal regression or survival, differentially abundant mRNA from an RNAseq study of CL on day 17 of the estrous cycle and pregnancy were compared to a microarray dataset accessed via the NCBI Gene Expression Omnibus that compared luteal response to $PGF_{2\alpha}$ on day 4 and day 11 of the estrous cycle. NR5A2 was among mRNA that were both greater in early pregnancy and lesser after $PGF_{2\alpha}$ on day 11, but not on day 4. The abundance of NR5A2 during functional transitions in the CL was investigated using qPCR and western blot. Luteal abundance of NR5A2 mRNA increased on day 6 compared to day 4 of the estrous cycle ($P < 0.05$), while abundance of its protein was unchanged. Both mRNA and protein for NR5A2 decreased with time during induced luteolysis ($P < 0.05$), but protein decreased earlier than mRNA. NR5A2 mRNA declined by 8 hr, whereas NR5A2 protein tended to decrease by 0.5 hr and decreased by 2 hr after $PGF_{2\alpha}$ injection. During early pregnancy, NR5A2 mRNA changed ($P < 0.05$), with both day 20 and day 23 having lesser mRNA than day 14, but no days differing from day 17; protein abundance did not change. Discrepancies between NR5A2 mRNA and protein during ALC, luteal regression, and early pregnancy indicate that protein abundance may be regulated by miRNA. Thus, miRNA expression during early pregnancy and ALC was measured by Nanostring technology. Twelve miRNA that differed between day 4 and 6 CL and five miRNA that changed (padj < 0.1) during early pregnancy (day 14-23) were predicted to target NR5A2. Most notably, miR-432-5p fit the expression pattern expected for inhibition of NR5A2 during ALC and early pregnancy. Because of the decrease in abundance of NR5A2 during luteolysis and stabilization during early pregnancy, a role in luteal progesterone production was hypothesized. Cultured luteal cells were treated with the NR5A2 antagonist Calbiochem # 505601 (10 μM). There was no effect of treatment on cell viability. However, the NR5A2 antagonist decreased progesterone production ($P < 0.05$). Overall, these data demonstrate a role for NR5A2 as a regulator of progesterone production and implicate miRNA as regulators of NR5A2. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2012-67015-30212 from the USDA National Institute of Food and Agriculture to JLP, Multistate Project NE 1227 (Hatch WV 476), USDA NIFA predoctoral fellowship no. 2017-67011-26062 to CKH, and the Erickson Discovery Grant to AR.

015 Reproductive Endocrinology

The involvement of resistin in the regulation of gonadotropin secretion in sheep

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Relationships between energy metabolism and fertility have been observed in many species. However, there are several metabolic signaling pathways, including those involving the adipokine resistin, that have not been fully explored. Work in cattle and rodents has shown that resistin, in addition to its roles in insulin resistance and inflammation, is involved in the regulation of gonadal and testicular steroidogenesis and gametogenesis. However, its role in the regulation of reproductive processes in other species such as the seasonal breeding sheep is unknown. Herein, we tested the hypothesis that resistin can influence secretion of anterior pituitary hormones and that its effect is dependent on day-length in ewes. Thirty ewes of the Polish Longwool breed, a breed that exhibits strong seasonal reproduction, were ovariectomized with estrogen replacement using subcutaneously inserted estradiol implants. Ewes were fed at libitum and housed in natural photoperiod (longitude: 19°57' E, latitude: 50° 04' N). Intravenous treatments consisted of control or recombinant bovine resistin (rbresistin) in saline: 1) Control (saline; n = 10), 2) Low resistin (1.0 µg/kg BW; n = 10), and 3) High resistin (10.0 µg /kg BW; n= 10). Experiments were conducted during both short (SD) and long days (LD). Blood samples were collected every 10 minutes during 4 h. Blood plasma concentrations of FSH and LH, were assayed using RIA. Pulse parameters of LH and FSH secretion were calculated using the Pulsar Computer Program. A season x dose interaction was observed for all hormonal variables measured. Greater concentrations ($P < 0.001$) of LH and FSH were observed during SD compared for LD in all groups. During SD, the High dose (10 µg/kg BW) decreased ($P<0.001$) basal LH and amplitude ($P < 0.05$) of LH pulses and increased ($P < 0.001$) circulating concentrations of FSH. However, the lower dose of resistin decreased ($P < 0.001$) FSH concentrations compared to Controls. During LD, both the Low and High resistin doses increased mean concentrations of LH ($P < 0.001$ and $P < 0.05$, respectively) and FSH ($P < 0.001$). These results demonstrate for the first time that resistin is involved in the regulation of pituitary gonadotropin secretion and this effect is differentially mediated during LD and SD. Further studies are underway to clarify the potential roles of resistin in modulating GnRH/gonadotropin secretion in seasonally-breeding sheep. Research supported by NCN 2015/19/B/NZ9/01314 to DAZ.

016 Reproductive Endocrinology

Expression of inhibin α subunit in bovine theca cells: does inhibin α contribute to the regulation of ovarian androgen production?

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Ovarian follicular fluid contains considerable amounts of 'free' inhibin α subunit but its functional significance remains obscure. Here, we report the unexpected finding of comparable expression levels of INHA mRNA in theca interna and granulosa layers of small/medium size bovine antral follicles (1-8 mm in diameter). Immunoreactive inhibin α subunit protein was also evident in the follicular theca layer and in cultured theca cells. Different molecular weight forms of inhibin α were secreted by cultured theca cells, including pro-αC isolated previously from bovine FF. These observations suggest that the theca interna layer may contribute significantly to the inhibin α subunit content of peripheral blood and antral follicular fluid, hitherto considered to be solely of granulosal origin. In vitro experiments revealed that RNAi-mediated knockdown of thecal INHA inhibited INSL3 and CYP17A1 expression and androgen production ($P < 0.01$) while INSL3 knockdown reduced INHA and inhibin α secretion ($P < 0.01$). These findings suggest a local inhibitory role of thecal inhibin α on androgen production. Despite this, treatment of cultured theca cells with purified pro-αC had no effect on basal or LH-induced androgen production. BMP treatment reduced thecal INHA expression, inhibin α protein secretion and androgen production in an inhibin-reversible manner. However, an in vitro experiment to test the hypothesis that free inhibin α subunit acts locally to modulate the effects of BMP and/or inhibin on androgen production yielded inconclusive results with no significant effect observed. Furthermore, neither circulating nor intrafollicular androgen concentrations were found to differ between control heifers and heifers actively immunized against inhibin α subunit, casting further doubt on thecal inhibin α subunit as having a significant physiological role in modulating androgen production. Further research is required to establish what intra-ovarian or peripheral endocrine role(s), if any, are played by theca-derived inhibin α subunit. supported by BBSRC.

017 Reproductive Endocrinology

Development of a physiological model of proestrus in cows using exogenous hormones

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The aim of this study was to develop a model of proestrus similar to the physiological status using only exogenous hormones. Two total doses of estradiol benzoate (EB) were used to determine the protocol that would more closely mimic the peak circulating E2 (6-12 pg/mL) and morphological and behavioral changes observed during natural proestrus (evaluated in a parallel experiment). Nonlactating multiparous Holstein cows (n = 18) were pre-synchronized with EB (2 mg) and an intravaginal P4 device (1 g) on D-7. On D0 the device was removed, PGF was administrated (0.526 mg) and 2 new P4 devices (2 g each) were introduced. On D1 another PGF was administered. On D4 and D5 all follicles =5 mm were aspirated (OPU). After OPU on D5, one of the P4 devices was removed and cows were randomized into 2 groups (n = 9/group): Low (L) E2 (total EB = 0.4 mg) or High (H) E2 (total EB = 0.8 mg). The EB total doses were divided into 8 treatments given 6 h apart (from 0 to 42 h) using increasing EB amounts (first 2 doses = 10%, 3rd and 4th = 20%, 5th and 6th = 30%, and the last 2 doses = 40% of the total). The second P4 device was removed 18 h after withdrawal of the first one. Blood samples were collected for E2 analysis every 6 h, from D5 to 7, just before each EB treatment. The uterus was examined using transrectal ultrasound to evaluate changes in endometrial thickness (ET) at 0, 12, 24 and 48 h after the beginning of EB treatments. Expression of estrus was observed using tail chalk. Continuous variables were analyzed using PROC MIXED and binomial data using Chi-Square analysis in SAS (P = 0.05; tendency = 0.05 < P < 0.1). Circulating E2 (pg/mL) was very low in all cows from both groups at time 0 (L = 0.1 ± 0.04 vs H = 0.03 ± 0.01). When data were normalized to the time of E2 peak, there was a progressive increase in circulating E2 over time with approximately double the E2 in H than L resulting in greater peak circulating E2 in H than L cows (7.2 ± 0.5 vs 3.5 ± 0.4). Similarly, when data were normalized to the time of EB treatment, circulating E2 increased progressively with an effect of time in both L (greater E2 at 36, 42 and 48 h than time 0) and H (greater E2 at 24, 30, 36, 42 and 48 h than time 0). Further, there was time*treatment interaction, with group H having higher E2 at time 42 (5.6 ± 0.8 vs 3.0 ± 0.3) and 48 (6.8 ± 0.6 vs 2.9 ± 0.5) than L. There was no effect of treatment on ET (mm) but there was an effect of time in both groups with greater ET at time 24 and 48 [L (0 h = 6.6 ± 0.3^{a}; 12 h = 7.4 ± 0.4^{a}; 24 h = 9.3 ± 0.2^{b}; 48 h = 10.6 ± 0.3^{b}); H (0 h = 6.5 ± 0.3^{a}; 12 h = 7.0 ± 0.4^{a}; 24 h = 9.2 ± 0.4^{b}; 48 h = 10.2 ± 0.4^{b})]. Group H tended to have greater expression of estrus (100% vs 66.7%). In conclusion, the model of proestrus using exogenous hormonal treatments had similar hormonal dynamics, changes in ET, and expression of estrus as reported during natural proestrus. Both groups had similar ET changes, however, the group treated with higher EB doses had greater circulating E2 with peak concentrations that more closely mimicked physiological concentrations with all cows expressing normal behavioral estrus. Therefore, a protocol using a total dose of 0.8 mg of EB seems to represent the natural circulating E2 dynamics during proestrus and may be useful for future studies on the mechanisms involved in the physiological changes that occur during proestrus. FAPESP, CAPES and CNPq.

018 Reproductive Endocrinology

Intensity of estrous expression detected by automated monitor and its relationship with concentration of pregnancy-associated glycoprotein

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The aim of the current project was to assess the relationship between intensity of estrous expression, detected by an automated activity monitor system, and the concentration of pregnancy-associated glycoprotein (PAGs) at 24 and 31 days post artificial insemination (AI) in serum and milk. A total of 442 events from 256 lactating Holstein cows were enrolled. Cows were continuously monitored for activity by an AAM (Afimilk). At the time of an alert of increase in activity, all animals had their body condition scored and activity data recorded to determine the intensity of estrous expression. Cows had a sample of milk and blood harvested at 24 and 31 days post AI, for the analysis of the concentration of PAGs. All animals were examined by ultrasonography on day 31 ± 3 and 60 ± 3 days post-AI for the detection of a viable embryo. Pregnancy losses were determined as the percent animals that lost their pregnancy between 31 and 60 days post-AI. Pregnancy data were analyzing using logistic regression with the GLIMMIX procedure and continuous variables were analyzed using ANOVA with the MIXED procedure of SAS. Activity was classified as high or low using the median of the peak activity. Animals that had high activity had higher fertility when compared with animals that had low activity (48.5% vs 35.2%, retrospectively; $P < 0.05$). Pregnancy losses tended to be higher in animals that had low activity at the moment of AI (18.2% vs 8.9%, $P = 0.07$). Animals that had higher activity had higher PAGs in serum at day 24 (2.05 ± 0.2ng/mL vs 2.84 ± 0.3ng/mL; $P = 0.05$) but not milk ($P = 0.18$). PAGs at day 31 post AI were higher in both serum and milk in animals that had a higher increase in activity at the moment of AI than those that did not (serum: 10.41 ± 0.6ng/mL vs. 8.89 ± 0.6ng/mL; $P = 0.04$; milk: 1.24 ± 0.06ng/mL vs 1.01 ± 0.05ng/mL; $P = 0.01$). When we considered only pregnant animals into the analyses animals that had high activity had higher concentration of PAGs in serum (3.43 ± 0.3ng/mL vs. 2.54 ± 0.3ng/mL; $P < 0.01$) but not in milk ($P = 0.29$). PAGs at day 31 post AI were higher in both serum and milk in animals that had a higher increase in activity at the moment of AI than those that did not (serum: 10.18 ± 0.6ng/mL vs. 8.77 ± 0.5ng/mL; $P < 0.05$; milk: 1.18 ± 0.06ng/mL vs 0.97 ± 0.05ng/mL; $P = 0.01$). No difference in PAG concentration at day 24 post-AI were found in serum or milk for those that later lost their pregnancy, however, those that went on to lose their pregnancy had lower PAG concentration at day 31 post-AI both serum and milk (serum: 8.45 ± 1.04ng/mL vs. 9.80 ± 0.4ng/mL; $P = 0.02$, and milk: 0.85 ± 0.12ng/mL vs. 1.10 ± 0.05ng/mL; $P = 0.02$). In conclusion, estrus expression detected by the automated activity monitors were associated with concentration of PAGs at day 24 in serum but not in milk and at day 31 in both serum and milk. Pregnancy losses were found to occur in animals with lower activity and lower concentrations of PAGs at day 31. Authors would like to thank Dairy Farmers of Canada, NSERC and BC Dairy Association.

019 Reproductive Endocrinology

Administration of endothelin-1 induces complete luteolysis in cyclic ewes

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Luteal regression is initiated in domestic ruminants by surges of prostaglandin F-2α (PGF-2α) from the uterus. Studies have demonstrated that ET-1 is required for the manifestation of the luteolytic effects of PGF-2α. A series of experiments was performed to determine if multiple injections of ET-1 would induce luteolysis and reduce the length of the estrous cycles in ewes. Ewes were treated (5/group) with saline, or varying amounts and frequencies of ET-1 as follows: 100 μg (single injection - 1x), 25 μg (4x), 50 μg (2x) and 50 μg (4x), on d 9 of the estrous cycle. Multiple injections of ET-1 were administered at 2 h intervals and jugular venous blood samples were collected before and at frequent intervals through 24 h after treatment and twice daily until ewes returned to estrus and analyzed for concentrations of progesterone. Treatments with ET-1 (100 μg-1x; 25 μg-4x; 50 μg-2x) resulted in a transient decrease ($P<0.05$) in progesterone; levels were similar to control values by 24 h and lengths of the estrous cycle were not affected. Complete luteolysis was induced in all ewes treated with 50 μg (4x) and the length of the estrous cycle was reduced (11.4 d; $P<0.01$). In a second experiment, ewes were treated with 50 μg ET-1 (4x) or saline (n=6/group) on d 9 and concentrations of progesterone and PGFM were determined for 24 h after treatment. ET-1 induced luteolysis in all 6 ewes treated and there was an increase ($P<0.05$) in PGFM from 2 through 18 h after treatment. Exogenous ET-1 induces ovine luteolysis and is associated with concommitant increases in PGFM.

020 Reproductive Endocrinology

The response of ovarian follicle, at the early static phase, to eCG-GnRH in Holstein heifers

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Equine chorionic gonadotropin (eCG) is a potent hormone to enhance follicle growth in cattle. More recently, we have found that 84 h after administrating eCG at the early static phase of follicle development, there is a dominant follicle at growing phase available on the ovary (Hosseini, A. *et al*. 2018 IJVR 19:15-21). The objective of this study was to investigate the effect of ovulating agent (GnRH) on ovarian follicles at the early static phase that is primed with eCG. Holstein heifers were synchronised using two injections of prostaglandin F_2a analogue, 14 days apart. On the second day in which ovarian follicle remained nearly similar size (approximately Day 8 of oestrous cycle, early static phase; Day 0 of experiment), heifers were randomly assigned into two experimental groups (n=5 in each group). On Day 0, Heifers in the treatment group received an i.m. injection of eCG (500 IU; Folligon®; Intervet, Holland). At the same time, heifers in the control group received saline. Heifers in both groups received GnRH analogue (100µg Gonadorelin acetate, GONAbreed®, Parnell, Australia) 84 hrs after receiving eCG/saline. On a daily basis, ultrasound examination was carried out to investigate follicle development and blood sampling was conducted to measure progesterone concentrations. Data were analyzed using GLM procedure. On Day 0, the diameter of the ovarian follicle was similar between control (13.5±0.29 mm) and treatment groups (13±0.32 mm; P>0.05). However, at the time of GnRH administration, the diameter of the ovarian follicle reached at 12.2±0.75 mm and 17.2±0.45 mm in control and treatment group, respectively (P<0.05). From Day 0 to GnRH administration, in the treatment group, follicles were in the growing phase, with the growth rate of 1.2±0.14 mm/day; however, at the same period, ovarian follicles in the control group were in the regressing phase with the growth rate of -0.4±0.18 mm/day. Following GnRH administration, all heifers in the treatment group ovulated, whereas no heifers in the control group ovulated (P<0.05). The average progesterone concentrations between Day 8 to 15 of oestrous cycle were 5.1±0.1 and 6.2±0.26 ng/mL, in control and treatment groups, respectively (P<0.05). In conclusion, administration of eCG in the early static phase of follicle development could change the fate of the ovarian follicle from a regressing to a growing follicle, responsive to an ovulating agent. The authors expressed their sincere appreciation for the financial and academic support of Faculty of Veterinary Medicine, University of Tehran. Special thanks to the Institute of Veterinary Research, Faculty of Veterinary Medicine, University of Tehran, for their kind collaboration for smooth running of this study.

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Effect of IGF2 on bovine luteinising follicular angiogenesis and progesterone production *in-vitro*

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Insulin-like growth factor (IGF) 2, acting through the IGF type 1 receptor (IGF1R), is likely to play an important role in the follicle-luteal transition in the cow. Importantly, IGF2 promotes granulosa cell proliferation and differentiation and is present in greater concentrations than IGF1 in follicular fluid. *IGF2* mRNA is localised to both granulosa and theca cells with *IGF2* mRNA levels in theca cells greater in dominant follicles than other follicles. Therefore, this study tested the hypothesis that IGF2 will promote angiogenesis and steroid production in bovine luteinising follicular cells *in vitro*. Granulosa and theca cells were dispersed from abattoir-derived bovine antral follicles (10-16 mm, n=4 cultures) and co-cultured under serum-free conditions. In Expt 1, cells were treated with IGF2 (0, 10 or 100 ng/mL) in the absence and presence of FGF2/VEGFA (both 1 ng/mL). On culture day 5, endothelial cell (EC) networks were quantified by von Willebrand factor immunohistochemistry and image analysis. Spent media was assayed for progesterone by ELISA while cell growth/viability was determined by MTT assay. In Expt 2, cells were treated with picropodophyllin (PPP, specific IGF1R inhibitor) in the presence and absence of IGF2 (10 ng/mL). Organised intricate EC networks were formed in the absence and presence of angiogenic-stimulation. In Expt 1, IGF2 decreased total EC network area ($P<0.05$) and branch points ($P<0.05$) under both basal and angiogenic-stimulated conditions ($P>0.05$). Progesterone concentrations were 4-fold greater ($P<0.001$) on day 5 than 3 of culture and were unaffected by treatment with IGF2 ($P>0.05$). The number of viable cells was increased by 15% by IGF2 treatment ($P<0.001$) on day 5 of culture. In Expt 2, IGF2 had no effect on EC network area but PPP reduced total EC area and perimeter, degree of branching, number of EC islands and branch points by 60-70% ($P<0.001$) irrespective of IGF2 treatment. Additionally, PPP decreased progesterone production by 12% on day 3 ($P<0.05$) and by 65% on day 5 ($P<0.001$) in the presence and absence of IGF2 treatment. In conclusion, exogenous IGF2 had limited effect on luteinising follicular angiogenesis, but inhibition of IGF1 receptor reduced luteal endothelial cell network formation and progesterone production in luteinising follicular cells. This suggests that endogenous production of either IGF1 or IGF2 stimulates follicular-luteal angiogenesis *in vitro*. Funded by TETFund, Nigeria.

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Luteal regression is compromised in high producing lactating Holstein cows independent of embryonic mortality

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The aim of this study was to compare the length of the luteal phase in lactating Holstein cows that were previously exposed to artificial insemination (AI) or not (Control). Non-pregnant cows (n=714) were submitted after the voluntary waiting period to a synchronization protocol: an intravaginal progesterone device (CIDR), 100 μg of GnRH and 2.0 mg of estradiol benzoate on d -11; 25 mg of PGF2α on d -4; and CIDR removal, 1.0 mg of estradiol cypionate and PGF2α on d -2. Cows were then randomly assigned at estrus (d 0) to the AI or the Control (sham) treatments. Only cows observed in estrus, captured by activity monitors prior to treatment, and with ovulation confirmed by ultrasonography 72 h after estrus were enrolled in the study. An ovarian map, using ultrasonography, of each individual cow was performed to evaluate the corpus luteum (CL) presence on d 17, 24 and 31, and to determine its regression status. For the analysis of length of luteal phase, only cows detected with a CL on d 17 and later not diagnosed pregnant were included in the analysis (n = 413; Control = 122; AI = 291). Cows with the original CL (d 17, 24, 31), not rebred during the period and diagnosed non-pregnant on d 31 were classified as having an abnormally long luteal phase. Pregnant cows that were retrospectively removed from the luteal phase calculations and rebred cows between d 17 and 31 were assumed to have a normal luteal regression. The body condition score (BCS) and a size and position score of the uterus (SPS) were measured by per rectum palpation at d 0 and included in the analyses of risk factors associated with pregnancy and CL dynamics. Binomial data were analyzed using PROC LOGISTIC of SAS with a backward stepwise elimination procedure. Statistical models included treatment, parity, BCS, SPS and interactions. Proportions were collected through frequency tables (PROC FREQ of SAS). Cows on both treatments were similarly distributed by parity, BCS and SPS (P>0.42). Pregnancy per AI at d 31 and 60 in the AI group was 21.0% and 17.2%, respectively, whereas pregnancy loss was 18.2%. Major risk factors for a successful pregnancy were parity (P<0.02) and SPS (P=0.04), as primiparous and cows with a SPS of 1 and 2 had greater pregnancy per AI. Presence of a CL was similar between Control and AI groups (P>0.24) prior to initiation of the synchronization protocol (57.3 vs 51%) and at d 24 (70.2 vs 69.7%) and 31 (50.8 vs 45.7%). Similarly, the frequency of spontaneous or induced re-breedings after d 17 of the treatments was not different between Control and AI groups (50.0 vs 46.4%; P=0.88). Treatment did not affect the proportion of cows with a persistent CL at d 31 (41.1 and 38.8% for Control and AI groups, respectively) and none of the explanatory variables were significant as risk factors to explain the presence of a long luteal phases. In conclusion, this study demonstrated, as shown by the lack of differences between the AI and control groups, that a large proportion (~ 40%) of lactating Holstein cows failed to properly regress the CL in a timely manner and consequently endured an abnormally long luteal phase that does not seem to be associated with the presence of an embryo. Authors would like to thank Colorado Dairy, Conapec Jr., CNPq and NSERC.

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Proinflammatory cytokine gene expression in endometrium and fertility in timed AI postpartum beef cows

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The objective of this study was to evaluate the uterine health and fertility of postpartum beef cows subjected to TAI protocols at different moments in the postpartum. Multiparous lactating Nelore cows (*Bos indicus*; *n* = 244) from two comercial beef farms in Rondônia - Brazil, were used in this study. The TAI protocols were initiated between 20 and 60 days postpartum (DPP). Cows were given 2 mg of estradiol benzoate i.m. and received an intravaginal progesterone-releasing device (1.9 g progesterone, CIDR®) to synchronize follicular wave emergence on Day 0. The CIDR was removed and cows were given 150 µg of d-Cloprostenol i.m. (PGF2α-analogue; Croniben®), 1 mg of ECP im (E.C.P.®), and 300 IU of eCG (Novormon®) i.m. on Day 8. All cows were TAI 48 h after CIDR removal. The cows were divided into three groups according to the days postpartum (PP) that the hormonal treatment was initiated, as follows: 1) Early PP (n=64), cows between 20 to 30 DPP; 2) Middle PP (n=115), cows between 31 to 45 DPP; and 3) Late PP (n=65), cows between 46 to 60 DPP, on the Day 0 of the TAI protocol. At Day 0 of the protocol, endometrial cytobrush samples were collected from a subset of cows (n=148). Slides for citology were prepared before the same cytobrush was transfered to a microtube containing 1 mL of RNA later reagent. Total RNA was extracted from 40 cytobrush samples (14, 11 and 15 from Early, Middle and Late Group, respectively) and analysis of *il1*, *il6*, *il8*, *tnf*, *GAPDH* and *βactin* gene expression was performed using quantitative real-time PCR. Cows from the Early group had lower (P<0.05) pregnancy per AI (P/AI) than cows from Middle and Late groups; 29.7% (19/64), 45.2% (52/115), and 52.31% (34/65), respectively. Accordingly, the Early group had higher (P<0.05) proportion of polymorphonuclear cells in the uterus than Middle and Late groups; 9.0%, 3.8%, and 2.2%, respectively. Relative expression of *il1* and *il8* were higher (P<0.05) in the Early group than Middle and Late groups. In contrast, expression of *il6* and *tnf* did not change among groups. These results demonstrated that cows subjected to TAI protocols early (< 30 DPP) in the reproduction season are less likely to become pregnant. Moreover, proinflammatory cytokines *il1* and *il8* are less active in the uterus after 30 DPP. Jéssica Andrade, Paulo Marcos Neves, and Izabela Lemos have scholarship from CAPES. Elizangela Moreira has scholarship from FAPERO/CNPq. George Silva has scholarship from CNPq. This research project is supported by Universal CNPq/2016 from MCT and by Macroprograma MP1 project from Embrapa.

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Health status and resumption of ovarian ciclicity in dairy cows

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The objectives were to assess if health status interacted with parity to affect the resumption of ovarian ciclicity (ROC) and haptoglobin (Hp), insulin-like growth factor-I (IGF-I) and insulin profiles during the transition period of primiparous (PP, n=116) and multiparous (MP, n=172) Holstein cows. Cows were fed a total mixed ration according to requirements, milked 3 times daily and bled once a week from -2 to +7 weeks relative to calving. A prospective observational cohort study was followed including only healthy cows at the beginning of the study. Peripartum diseases were diagnosed by a trained veterinarian and cows were classified in 3 categories: healthy, 1 event or 2 events. For statistical analysis PROC FREQ, PROC MIXED, and multivariable logistic regression (MLR) were performed. From 288 cows, 45.8% become sick and 1.9% (n=6) were discarded or died within 30 DIM. The proportion of cows and their health status by parity were: MP: 53.5%, 29.4% and 17% and PP: 55%, 31.3% and 13.5% for healthy, 1 event and 2 events cows respectively (Ruprechter *et al*., 2018). Primiparous produced less milk than MP cows (6,866 ± 272 *vs* 8,341 ± 213 L, P<0.01) and healthy produced more than sick cows (8,165 ± 199, 7,547 ± 276 and 7,098 ± 387 L) for healthy, 1 and 2 events cows respectively (P<0.05). Insulin, IGF-I and Hp profiles were affected by the interaction among parity, health status and week. Concentrations of Hp were greater in sick than healthy cows at weeks +1 and +3 (P<0.01), as expected for this acute phase protein, being also higher in 2 events MP than 1 event MP cows (P<0.01). Overall, IGF-I and insulin concentrations were greater in PP than MP cows, since PP are still growing animals. While healthy MP cows presented a sharp IGF-I decrease after calving, healthy PP cows maintained IGF-I concentrations, being this consistent with the lower milk production and the reported attenuated uncoupling somatotropic axis of PP cows. Independent of parity, all sick cows had a sharp IGF-I decrease around calving suggesting a lower dry matter intake during the transition period (greater net energy balance). From 288 cows, 59.7% (n=172) become cyclic during the first 7 weeks postpartum while 40.3% (n=116) did not. Health status and parity affected ROC (P<0.05). More MP cows reinitiated than PP cows (64%, 110/172 *vs* 52%, 60/116 respectively). A greater percentage of healthy cows reinitiated ovarian ciclicity (68%, 106/156) when compared to 1 event (53%, 46/87) and 2 events cows (44%, 20/45). After performing MLR, insulin and IGF-I resulted predictive for ROC (P<0.05); Insulin: (OR [IC]); 1.09 [1.01-1.17] at week -1 and IGF-I: 1.01 [1.00-1.02] at week +1. In conclusion, health status interacted with parity to model ROC. Besides IGF-I and insulin concentrations around calving were both predictive for ROC arguing for the importance of these anabolic hormones signaling the metabolic status to the reproductive axis.

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Protein supplementation control on luteal progesterone and expresion of its receptor in ovarian tissues of Boer goats

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Nutritional effects on reproductive performance such as ovulation rate and embryo survival in sheep are well documented, but there are still debates among researchers and farmers regarding this matter in goats. There are reports in the literature showing in goats that high energy-protein supplementation has no influence on ovulation rates but improves embryo survivability and maintenance of pregnancy. Therefore, we examined the effects of short-term protein supplementation (commercial pellet; double maintenance) on ovulation rate, mRNA expression of progesterone receptor gene in corpus luteum and the concentration of plasma progesterone. In this study, seventeen female Boer goats were divided into two groups; 1) Control group (n=9) received maintenance diet (commercial protein pellet and Napier grass) and 2) Treated group (n=8) received double maintenance diet (commercial protein pallet-2x M and Napier grass). The feeding treatment begun 5 days before CIDR removal (Day 0) for 25 days. The body weight and BCS were recorded every two weeks until all animals were slaughtered (Day 27). The ovaries of all animals were collected, and the number of CL was counted and kept in RNAlater for analysis of gene expression through qPCR. Results show that short-term supplementation of high protein does not affect ovulation rate (Control = 1.00 ± 0.24; Treated = 1.25 ± 0.24; $P>0.05$) or change body weight or body condition during the experimental period. However, the plasma progesterone concentration (Day 27; Control = 7.88 ± 0.26; Treated = 10.77 ± 0.28 ng/mL) and the expression of progesterone receptors in corpus luteum (Control = 1.00 ± 0.48; Treated = 3.79 ± 0.38) were higher in treated does ($P<0.05$). In conclusion, we suggest that protein supplementation appears to control the structural and functional integrity of CL tissue, thus increasing the production of progesterone. I would like to acknowledge the Fundamental Research Grant Scheme (01-01-15-1713FR) from Ministry of Education (MOE) Malaysia and Putra Grant (GPIPS/2016/9493100) from Universiti Putra Malaysia for financial support. Also, we appreciate the team members of Animal Sciences Research & Technology (AnSTECH) for their kind participation in these experiments.

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Resistin regulates prolactin concentrations from the ovine adenohypophysis depending on season

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Adipokines are hormones that are mainly produced by white adipose tissue, an endocrine organ involved in energy homeostasis. However, there are several metabolic signaling pathways, including those involving the adipokine resistin, that have not been fully explored. One prominent feature of season in animals is its effects on the neuroendocrine regulation of pituitary hormone release, including prolactin (PRL). This axis is highly sensitive to photoperiod in all seasonally breeding mammals, with marked activation of PRL secretion by long days. However, no studies in seasonally-breeding sheep have examined the role of resistin in regulating the secretion of PRL, particularly in the context of the effect of day length. Thus, in the present in vivo study, we investigated the effect of different doses of resistin on PRL secretion during contrasting photoperiods. Thirty Polish Longwool ewes, a breed that exhibits a strong seasonal reproductive pattern, were ovariectomized with estrogen replacement using subcutaneously inserted estradiol implants. Ewes were fed ad libitum and housed under natural photoperiod (longitude: 19°57' E, latitude: 50° 04' N). Within season and replicate ewes were assigned randomly to one of three treatments (five ewes/treatment/season) and infused intravenously once at time 0. Treatments consisted of control or recombinant bovine resistin (rbresistin) in saline: 1) Control (saline; n = 10), 2) Low resistin dose (R1; 1.0 µg/kg BW; n = 10), and 3) High resistin dose (R2; 10.0 µg/kg BW; n = 10). Jugular blood samples were collected at 10-min intervals beginning immediately before the start of infusions and continuing for 4 hr. Experiments were conducted during both short (SD) and long (LD) days. Plasma concentrations of PRL were assayed using RIA. All data are expressed as the mean ± standard error of the mean [SEM]. Hormone data were analyzed by a series of 2-way analyses of variance (two-way ANOVA) using SigmaPlot statistical software (version 11.0; Systat Software Inc., Richmond, CA, USA) for repeated measures. A season x dose interaction was observed for PRL concentrations. Within both control and treated groups (R1 and R2), mean PRL concentrations were greater ($P < 0.001$) during LD than during SD. However, only the high dose of rbresistin increased PRL concentrations during both LD – 120.4 ± 2.1 ng/mL ($P < 0.001$) and SD – 23.05 ± 0.6 ng/mL ($P < 0.05$) compared to controls (LD – 78.1 ± 0.9 ng/mL and SD – 12.04 ± 0.8 ng/mL) the R1 group ($P < 0.001$ and $P < 0.05$, respectively for LD – 59.3 ± 0.3 ng/mL and SD – 16.02 ± 0.5 ng/mL). Results suggest that there may be a link between adipose tissue production of resistin and the control of PRL release. Such an effect would likely involve a direct influence of resistin at the pituitary depending on the length of the day. In summary, the current data indicate that resistin may serve as an additional adipokine engaged in the regulation of PRL, an essential hormone for mammary gland development and milk production in ruminants. Research supported by NCN 2015/19/B/NZ9 01314 to DAZ.

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Association of antral follicle count and peripheral anti-Müllerian hormone concentrations with fertility in beef cows

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While it has been reported that reproductive performance in cows with high antral follicle count (AFC) was improved compared with those with low AFC, other research groups have demonstrated that there was no difference in conception rate between cows with high AFC and those with low AFC. On the other hand, anti-Müllerian hormone (AMH) may be a useful biomarker for evaluating fertility in cattle. Objectives of this study were to clarify effect of the stage of estrous cycle on AFC and to elucidate whether there is an association between AFC, peripheral AMH concentrations and fertility in beef cows. A total of 71 Japanese black cows (33.0 ± 2.7 days postpartum) were treated with Ovsynch protocol on day -11 and ovulation was confirmed on day 0. The cows were divided into three groups; cows treated with prostaglandin $F_{2\alpha}$ (PG) on day 7 (Day 7 group, n = 24), those treated on day 11 (Day 11 group, n = 23) and those treated on day 15 (Day 15 group, n = 24). All cows received GnRH 56 hours after PG administration and were inseminated at fixed time (16-20 hours after GnRH administration). AFC was defined as the total number of antral follicles (2 mm or larger in diameter, detectable by ultrasonography) in the both ovaries. AFC and size of the largest follicle were recorded by ultrasonography at PG, at GnRH and one week after timed AI, and blood samples were collected at PG and one week after timed AI for determination of plasma AMH and progesterone concentrations. Plasma AMH concentrations were measured by ELISA and plasma progesterone concentrations were measured by Enzyme-Linked Fluorescent Assay. All the cows were also divided into two groups based on the median AFC: cows with high AFC (equal to or higher than the median; High AFC) and cows with low AFC (lower than the median; Low AFC). Pregnancy diagnosis was made at 30 to 50 days after timed AI. There were no significant differences in AFC at PG, size of the largest follicle at GnRH, plasma progesterone concentrations and volume of corpus luteum one week after timed AI and conception rate among the three groups. The median AFC was 37. Low AFC group had higher ($P < 0.05$) conception rate (24/35, 68.6%) than High AFC group (13/36, 36.1%). Plasma AMH concentrations in Low AFC group (854 ± 460 pg/mL) were lower ($P < 0.05$) than those in high AFC group (1625 ± 905 pg/mL). In conclusion, although the stage of estrous cycle does not seem to affect AFC, both AFC and peripheral AMH concentrations had an association with subsequent fertility in beef cows. We are grateful to the staff of the farms for their care and management of the cows. We would also like to thank Aska Animal Health for providing the hormonal drugs and MTF for providing the ultrasound equipment used in this study.

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Use of FSH in FTAI protocol in lactating dairy cows

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The aim of the present study was to evaluate the use of follicle stimulating hormone (FSH) as an alternative to equine chorionic gonadotropin (eCG) to improve pregnancy rates in fixed-time artificial insemination (FTAI) protocols in high producing dairy cows. It was also evaluated the effect of the hormonal protocol over the dairy production. For that, 111 crossbred multiparous cows (*Bos taurus* vs *Bos indicus*), lactating, 45-100 days postpartum, with body condition score between 3 and 4 (1 to 5 scale), milk production from 20 to 40 liters per day and randomly distributed in three groups were used. All females were evaluated ultrasonographically for the presence of a corpus luteum (CL) at day zero and were randomly distributed in experimental groups. Group1 (n=34) were submitted to a FTAI protocol and the cows were treated with a progesterone device (PRIMER™, Agener União, Brazil) for 8 days, two estradiol benzoate doses (EB; RIC-BE™, Agener União, Brazil) applied intramuscularly (IM), the first one (2 mg) were administered at the time of implant placement – D0 and the second dose (1 mg), 24 hours after progesterone device removal – D9, a dose (0.500 mg) of PGF2α (Prolise™, ARSA, Argentina) that was administered at the time of progesterone device removal – D8 and a dose of GnRH (25 µg buserelin acetate) (Gestran Plus™, Agener União, Brazil) administered 24 hours after removal of the device – D9. The animals were inseminated 52 hours after progesterone device removal with freezing semen from the same bull. Group 2 (n=37) were submitted to the same FTAI protocol plus the application of 0.75 mL (15 mg) of FSH (Folltropin™-V, Vetoquinol, Canada) IM at D8. At the time of FTAI all cows had their ovary ultrasonographically scanned for the presence of dominant follicle (diameter ≥ 10mm). The third group (n=40) was not submitted to a hormonal protocol. Cows were milked thrice daily and were maintained in feed lane with natural and artificial shadow and with access to water and mineral mixture *ad libitum*. Pregnancy was diagnosed 40 days after IA by ultrasonography. For statistical analysis frequencies (presence of CL at D0, presence of dominant follicle at D10 and pregnancy rate) were compared by Chi-square test with 5% of significance. For milk production comparison among the three groups, data were evaluated by the Kruskal-Wallis test followed by Dunn test with 5% of significance. There was no difference in pregnancy rate between Group 1 (44.11%; 15/34) and Group 2 (45.94%; 17/37) ($P > 0.05$). Considering the presence of a dominant follicle at D10, it was present in 67.64% (23/34) of the cows in Group 1 and in 97.30% (36/37) of the cows in Group 2. It was also observed that milk production did not vary throughout the evaluated moments in Groups 1, 2 and 3 and that milk production did not vary among evaluated groups. Based on the results obtained it can be concluded that treatment with 15 mg of Folltropin® showed a larger number of animals with dominant follicles on the day of AI, but the use of this hormone was unable to increase pregnancy rate. Moreover, the hormonal protocol and more handlings did not affect the dairy production, and the presence of a CL at the beginning of the protocol did not improve pregnancy rate. The authors thank Cariocão Farm, CNPq-Brazil and Fapemig-Brazil.

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Creep feeding has no effect on antral follicle count at weaning

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Creep feeding (CF) is a nutritional tool that improves growth and development of calves, is associated with an increase in IGF1 concentrations at weaning and reduces the age at puberty (Guggeri *et al*., 2014. Livestock Science 167:455-462). Primordial follicles develop during pre-natal life, but nutrition increases the number of gonadotrophin responsive follicles that can be assessed by ultrasonography (Scaramuzzi *et al*., 2011. Reprod Fertil Dev, 23:444-467). Since antral follicle count (AFC) is a phenotypic marker of fertility (Evans *et al*., 2010. Soc Reprod Fertil Suppl, 67:421-429), we hypothesized that high nutrition in early life would have a positive effect on AFC. Fifty-four Hereford cows and their calves of 73 ± 1.5 days of age were assigned to two groups, with two replicates: 1) Without CF (-CF; n=27); 3) With CF (+CF; n=27). The cows grazed on *Campos* natural grassland with initial adjustment on forage allowance higher than 8 kg DM/kg BW for all plots. Calves supplementation was performed during 98 days with distiller's dried grains with solubles supplied to 40% of the diet daily and adjusting the supplement every 2 weeks, according to the body weight evolution and the respective requirements. Body weight and daily weight gain were evaluated in all calves. At the end of the supplementation period, females calves (n=26 +CF and n=22 –CF) were submitted to transrectal ultrasonography for 4 consecutive days and follicles > 3 mm counted using an Aloka 500 with a transrectal 7.5 MHz probe. Body weight and daily gain were analysed by ANOVA, using the mixed procedure in SAS. An average of the 4 days of follicle count was obtained, and analysed using the GLM procedure of SAS. Values were considered significant if $P < 0.05$. Creep feeding had a positive impact on average daily gain (+CF: 0.982±0.02 kg/d vs –CF: 0.832±0.02 kg/d; $P<0.05$) and weaning weights (+CF: 205±1.6 kg vs –CF: 191±1.6 kg; $P<0.05$). However, it was not associated with differences in AFC, that were similar in +CF (14.3±1.6 follicles) and –CF calves (16.8±1.8 follicles; $P>0.05$). We concluded that CF does not modify ACF of calves at weaning. The authors would like to thank the team working at Glencoe Research Station, INIA, Tacuarembó.

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Mechanism of LH release after peripheral administration of kisspeptin in cattle

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Kisspeptin (Kp) elicits LH release in cattle but whether the effect is mediated by activation of GnRH neurons in the hypothalamus is unknown. Experiments were done to determine if administration of Kp will increase LH secretion through activation of GnRH neurons and to determine if pre-treatment with a GnRH receptor blocker will alter the pattern of Kp-induced LH release and ovulation. In Expt1, Holstein cows were assigned randomly to two groups (n = 3 per group) 24 h after administration of PGF2α and given either Kp (3 doses of 15 mg hKp10 iv at 60-min intervals), or control (saline). Blood samples were collected every 15 min from -30 min to 150 min (0 min = treatment). Cows were euthanized at 150 min. The head was perfused with 4% paraformaldehyde via the carotid artery to fix the brain *in situ*. The mid-brain (pre-optic area to mammillary body) was excised, cryoprotected in saturated sucrose solution, frozen at -80ºC and sectioned serially at a thickness of 50 mm using a cryostat microtome. Every 20th free-floating section was processed for double immuostaining for cFos (Nickel-DAB) and GnRH (DAB) using sequential immuno-peroxidase reactions. The number of neuron cell bodies was counted in the pre-optic area and the hypothalamus by bright-field microscopy. In Expt 2, pubertal heifers (n = 5 per group) were assigned randomly to 1) hKp10 group: 3 doses of 15 mg hKp10 iv at 60-min intervals, 2) Cetrorelix+hKp10 group: pretreatment with a GnRH antagonist (Cetrorelix, im) before hKp10 treatment or 3) control group: 3 dose saline iv at 60-min intervals. Treatment was initiated 6 days after emergence of a follicular wave induced by ultrasound-guided follicle ablation (performed 3 days after ovulation). A CIDR was placed in vagina at the time of ablation and heifers were given PGF2α at 4.5 and 5 days after follicle ablation. Blood samples were collected at 15 min intervals from -60 min to 240 min of treatment to measure plasma LH concentrations. Ovaries were examined daily by transrectal ultrasonography. Data were compared among groups by ANOVA for repeated measures. In Expt1, hKp10 induced higher plasma LH concentrations from 15 to 150 min after treatment than in controls (P=0.01), but the proportion of GnRH cells expressing cFos did not differ between hKp10 and control groups (5.8% and 3.5%, respectively; P=0.11). In Expt2, a rise in plasma LH concentration was detected from 15 to 240 min in the hKp10 group but not in the groups treated with Cetrorelix or saline (P<0.001). Similarly, ovulation was detected in the hKp10 group but not in the groups treated with Cetrorelix or saline (4/5, 0/5, and 0/5, respectively; P=0.02). In summary, treatment with hKp10 induced LH release and ovulation in cattle, but was not associated with GnRH neuron activation, and the effect was blocked by a GnRH antagonist. Results support the hypothesis that the effect of kisspeptin is mediated downstream of GnRH synthesis, perhaps by inducing release of pre-synthesized GnRH from the nerve terminals in the median eminence. Research supported by the Natural Sciences and Engineering Council of Canada.

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Estrous cycle stage-specific actions of exogenous prostaglandin F_{2a} on angiogenic and cell-death pathways in bovine Corpus Luteum may depend on its local or systemic administration

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Prostaglandin F_{2a} (PGF) and its analogues are extensively used to induce regression of the bovine corpus luteum (CL). This process consists of functional and structural luteolysis. Although apoptosis was considered as the most potent mechanism of cell death, a caspase-independent cell death pathway-necroptosis was found as alternative process of CL regression in cows. However, the newly formed bovine CL (Days 1-5 of the cycle) is resistant to the luteolytic actions of PGF. Moreover, local luteal PGF may play a role in angiogenesis and CL formation after ovulation. The mechanisms underlying these differential PGF effects are poorly understood. The goal of this study was to examine differences in expression of genes related to: angiogenesis (FGF2, VEGF and their receptors), necroptosis (RIPK1, RIPK3, CYLD, and MLKL) and apoptosis (CASP3, CASP8, Bax, and Bcl-2) in response to the local or the systemic administration of PGF in early (Day 4 of the cycle) versus mid (Day 10) CL. Cows at Day 4 (n=24, 6/treatment) or Day 10 (n=24, 6/treatment) were treated as follow: (1) intramuscular (i.m.) Saline injection (control), (2) i.m. PGF injection (25 mg; Dinoprost), (3) intra-luteal Saline injection (control) or (4) intra-luteal PGF injection (2.5 mg). CLs were collected by ovariectomy 4-h after treatment. Gene and protein expression was investigated by qRT-PCR and Western Blotting, respectively, and localization of select proteins was evaluated by immunohistochemistry. Intra-luteal and i.m. PGF injections up-regulated FGF2 expression, but decreased expression of VEGF and its receptors in the early and mid CL ($P<0.05$). In mid CL, whereas both local and systemic PGF injections increased the expression of necroptosis related genes RIPK1 and RIPK3 ($P<0.05$), induction of CYLD was responsive only to local PGF administration ($P<0.05$). In early CL, intra- luteal PGF induced upregulation of RIPK1 and MLKL ($P<0.05$) but downregulated RIPK3 ($P<0.05$). Intramuscular PGF administration resulted in a decrease in RIPK1 and RIPK3 expression, but in upregulation of CYLD ($P<0.05$). In mid CL, both routes of PGF administration resulted in an increase in pro-apoptotic Bax and a decrease in anti-apoptotic Bcl-2 expression ($P<0.05$). In early CL, whereas intra-luteal PGF resulted in increased Bax expression, systemic PGF resulted in decreased Bax expression but induced expression of Bcl- 2. In mid CL, whereas both routes of PGF treatment increased CASP3 expression, CASP8 induction was responsive only to systemic PGF ($P<0.05$). In the early CL, both routes of PGF administration resulted in a decreased expression of CASP3 but upregulation of CASP8. In conclusion, genes and proteins related to angiogenesis, necroptosis and apoptosis reveal stage- specific responses to PGF administration depending on whether it was administered locally or systemically. Although local PGF may play a luteoprotective role by inhibiting necroptosis and apoptosis pathways in the early CL, RIPK-depending necroptosis is a potent mechanism responsible for structural CL regression during luteolysis in cattle induced by PGF. Supported by NSC grant DEC-2017/X/NZ9/00363. DJS was supported by KNOW Consortium "Healthy Animal - Safe Food", MS&HE Decision No. 05-1/KNOW2/2015.

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Elevated free fatty acid concentrations alter gene expression, cell proliferation and steroid hormone production in cultured bovine granulosa cells

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Following the onset of lactation, high-yielding dairy cows enter a state of negative energy balance (NEB) when the energy demand for maintenance and lactation exceeds that of dietary energy intake (Bauman & Currie 1980). One of the major metabolic consequences of NEB is the increased concentration of non-esterified fatty acids (NEFA) in the serum due to fat mobilization. Elevated NEFA levels have been found detrimental for bovine (Vanholder *et al*., 2005) and human (Mu *et al*., 2001) granulosa cell growth and function *in vitro*. Elevated concentrations of saturated fatty acids result in deleterious effects on cell survival by induction of the apoptotic signaling cascade, while mono-unsaturated fatty acids like oleic acid have shown to rescue cells from such toxic effects either by channeling fatty acids towards lipid storage in lipid droplet or by increasing β-oxidation in mitochondria (Listenberger and Brown 2008). In the present study, we investigated the effects of physiological concentrations of three main NEFAs on granulosa cell viability, proliferation, steroid production and gene expression in a bovine E2 active granulosa cell culture model. Initially, granulosa cells from small to medium sized follicles (2-6 mm) were cultured for 48 h. Subsequently, the spent media were replaced with media containing different concentration of Palmitic acid (C16:0) (PA), Stearic acid (C18:0) (SA) and Oleic acid (C18:1) (OA) during the next 6 d of culture with regular media change every 48 h. Treatment with all three NEFAs increased the transcript levels of the fatty acid translocase *CD36* ($P < 0.05$) indicating the active uptake of free fatty acids by granulosa cells. In addition, both PA and SA treatment at 200 μM upregulated the of the gonadotrophin receptors *FSHR, LHCGR*, aromatase *CYP19A1* and the cell cycle regulator *CCND2* whereas OA treatment at 400 μM ($P < 0.05$) downregulated the transcript abundance significantly. Also, Oestradiol 17β production was stimulated with both PA (200 μM) and SA (200 μM) ($P < 0.05$), while reduced with OA treatment (400 μM) ($P < 0.05$). Thus, our results indicate that elevated free fatty acid concentrations specifically alter the granulosa cell functionality *in vitro*. Suggestively, this could be a possible mechanism through which free fatty acids influence folliculogenesis during the early postpartum period in high-yielding dairy cows.

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Prostaglandin profile in pregnant cows during initiation of active placentation

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In cattle, the incidence of late embryonic mortality (EM) varies from 3.2 to 42.7% of pregnancies. Relatively little is known about the causes or mechanisms associated with late EM, most of which occurs around the time of placentation. Usually associated with negative reproductive outcomes, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) may play a role in the initiation of placental attachment and proper placentome formation. The objective of the study was to evaluate $PGF_{2\alpha}$ secretion during active placentation from day 29 to 38 in pregnant cows that maintained pregnancy or experienced EM. Non-lactating cows were artificially inseminated to high fertility bulls (n=40) or sham inseminated (control; n=5) with heat treated semen at day 0. Control cows received a CIDR at day 17, which was replaced with a new CIDR at day 27, to maintain elevated circulating progesterone (P4) concentrations. Pregnancy diagnosis was performed at day 29 via ultrasound. Pregnant cows (n=23) and control cows (n=4) underwent coccygeal vein cannulation at day 29. A polyethylene catheter (BD Intramedic) was inserted 65 cm into the caudal vena cava via the coccygeal vein of all cows for sampling of utero/ovarian drainage. Blood samples were collected every 6 hours until day 38. Uteri of pregnant cows were examined by ultrasonography daily to monitor pregnancy until catheters were removed at day 38. Final pregnancy diagnosis occurred at day 70 of gestation. Cows pregnant at day 29 but lacking a fetus with a viable heartbeat at day 70 were considered to have undergone embryonic mortality (n=3). Serum concentrations of prostaglandin $F_{2\alpha}$ metabolite (PGFM) and $PGF_{2\alpha}$ were measured with a validated commercial ELISA and P4, a validated commercial RIA. Pregnancy associated glycoprotein (PAG) levels were measured daily using an in house ELISA. Data were analyzed using repeated measures in SAS 9.4 and pulses were identified using AutoDecon Pulse2. Concentrations of PGFM were significantly elevated in pregnant cows compared to control cows ($P<0.05$) across the sampling period as well as between sampling periods ($P<0.01$) during days 29 to 38 of gestation. Number of PGFM pulses did not differ between control cows (1.60±0.40 pulses) and cows that lost (2.25±0.63 pulses) or maintained pregnancy (1.67±0.18 pulses) but, in cows with 2 or more PGFM pulses, cows that maintained pregnancy had an increased pulse interval compared to cows that lost pregnancy (61.8±12.4 hours vs 26.4±9.5 hours). However, there was no difference in $PGF_{2\alpha}$ levels between control and pregnant cows. Control cows and pregnant cows had similar levels of P4 during the sampling period ($P>0.05$); however, the pregnant cows had increased PAG in circulation ($P<0.001$). There was no difference between circulating PGFM, $PGF_{2\alpha,}$ or P4 in cows that maintained a pregnancy until day 70 of gestation versus those that experienced EM ($P>0.05$); however, PAGs were decreased at day 29 of gestation ($P<0.05$). Based on these results pregnant cows have increased circulating concentrations of prostaglandin, but it remains unclear if prostaglandin is playing a role in cows undergoing embryonic mortality. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26457 from the USDA National Institute of Food and Agriculture.