



063 Environment and Reproduction

**Effect of different feeding strategies on reproductive parameters in dairy cows**

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The objective of this study was to investigate the effect of different feeding strategies on the reinitiation of ovarian cyclicity, pregnancy rates, and metabolic profiles in spring calving Holstein dairy cows. Feeding strategies involved total mixed rations (TMR) or a combination of TMR and pastures (PMR). Multiparous cows (n=90) were assigned to the following treatments: TMR+TMR (100% TMR *ad-libitum* in spring and summer), PMR25+TMR (grazing + 25% TMR in spring and 100% TMR in summer), PMR25+PMR35 (grazing + 25% TMR in spring + 35% TMR in summer). In treatments that included pasture, cows grazed the same pasture but in separate groups. Individual milk production was determined weekly and milk for progesterone was collected twice a week to estimate first postpartum ovulation. The voluntary waiting period was 45 days after calving. Blood samples were obtained every 15 days during the experimental period to determine non-esterified fatty acids (NEFA) and betahydroxybutyrate (BHB). Body condition score (BCS) was determined fortnightly. Response variables were analyzed using the GLIMMIX procedure of SAS (SAS 9.2, 2008). Milk production was affected ( $P<0.0001$ ) by the feeding strategy and also by the interaction ( $P=0.02$ ) between treatment and season. In spring, milk production was  $33\pm 0.9^a$ ,  $26\pm 0.9^b$ , and  $26\pm 0.9^b$  for TMR+TMR, PMR25+TMR and PMR25+PM35 cows, respectively. In summer, milk production was  $31\pm 0.7^a$ ,  $28\pm 0.7^b$  and  $25\pm 0.7^c$  for TMR+TMR, PMR25+TMR and PMR25+PM35 cows respectively. The BCS was greater in TMR+TMR cows ( $2.9\pm 0.05^a$ ) compared with PM25+TMR cows ( $2.7\pm 0.05^b$ ), presenting no difference with PM25+PM35 cows. The concentration of NEFA was not affected by treatment the feeding strategy ( $P>0.05$ ), but the concentration of BHB tended ( $P=0.06$ ) to be affected by the treatments: PMR25+PMR35 had greater ( $P<0.05$ ) BHB concentration than the TMR+TMR group ( $0.70\pm 0.05$  vs.  $0.54\pm 0.05$  mmol/L), presenting no difference with PMR25+TMR, probably related to the increased pasture intake in PMR25+PM35 cows. For NEFA and BHB concentrations, there was an effect of the day postpartum ( $P<0.05$ ) but there was no interaction between treatment and day postpartum. No effect ( $P>0.05$ ) of feeding strategy on the probability of reinitiation of ovarian cyclicity was observed. The calving to first ovulation intervals were  $46.7\pm 17$ ,  $46\pm 22$  and  $49\pm 28$  days for TMR+TMR, PMR25+TMR and PMR25+PM35. Calving to first service intervals were  $62.6\pm 17$ ,  $55.8\pm 20$  and  $57\pm 21$  days for TMR+TMR, PMR25+TMR and PMR25+PMRD35. There was no effect of the treatments on the probability of pregnancy at 120 days postpartum ( $0.46\pm 0.1$ ,  $0.35\pm 0.1$  and  $0.46\pm 0.1$  for TMR+TMR, PMR25+TMR and PMR25+PMRD35, respectively). Data show that even if the different feeding strategies affected milk production, they did not affect the reproductive parameters measured. The greater milk production and similar reproductive performance of TMR+TMR cows may be the result of increased density of dietary nutrients and increased intake. Agencia Nacional de Investigación e Innovación (ANII) of Uruguay for financial support. Project N° FSA\_1\_2013\_1\_12442.



064 Environment and Reproduction

**Effect of maternal nutrition on embryo survival, uterine environment and embryo transfer in sheep**

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Maternal nutrition is an important factor that influences the processes of implantation and embryonic development. The objective of this study was to evaluate the effect of nutritional status of donor and recipient ewes on embryo survival and uterine gene expression. The study was carried out in the Laboratory of Small Ruminants Reproduction of INTA Bariloche during the breeding season. Merino donor (n = 36) and recipient (n = 75) ewes were randomly assigned to two treatment diets, receiving 1.5 (Supplemented, [S]) or 0.5 (Restricted, [R]) times daily maintenance requirements during 21 days before recovery and transfer of embryos. Estrous synchronization was performed with intravaginal sponges containing progesterone for 14 days and an i.m administration of equine chorionic gonadotropin (eCG) at time of sponge removal. Donor ewes received a superovulatory treatment, which consisted of the administration of 100 mg of follicle stimulating hormone (FSH) in 6 decreasing doses every 12 hours. Intrauterine artificial insemination with frozen semen (100 million spermatozoa) was performed 12 hours after detection of estrus. On day 7 post-estrus, embryos were recovered and evaluated from S and R donors and were transferred by semi-laparoscopy procedure to S and R recipients, defining the following groups: SS, SR, RS and RR. Embryo survival rate was determined by ultrasonography on day 22 post embryo transfer. At embryo recovery, endometrial tissue was collected for biopsy from donors (n = 26) and recipients (n = 10) and stored in liquid N<sub>2</sub> until analysis. The embryo survival rate was analyzed using the CATMOD procedure of SAS. Two-way ANOVA, followed by post- hoc Tuckey tests, was performed to compare relative mRNA expressions. Data are presented as least square means ± pooled standard errors. Means were considered different when  $P \leq 0.05$ , and tendency to differ when  $P < 0.10$ . The uterine gene expression of the receptors for progesterone (PR), insulin-like growth factor 1 (IGF-1R) and leptin (LEPR) was determined by real-time PCR. At the time of embryo transfer, R donors ( $0.14 \pm 0.06$ ) and R recipients ( $0.08 \pm 0.03$ ) had less ( $P < 0.05$ ) uterine relative mRNA levels for PR compared with S donors ( $0.39 \pm 0.15$ ) and S recipients ( $0.86 \pm 0.0$ ). Likewise, IGF-1R and LEPR relative mRNA levels on day 7 post-estrus were greater ( $P < 0.05$ ) in S (IGF-1R:  $0.15 \pm 0.03$ ; LEPR:  $0.17 \pm 0.04$ ) than in R (IGF-1R:  $0.05 \pm 0.01$ ; LEPR:  $0.04 \pm 0.01$ ) females. The embryo survival rate after transfer tended ( $P < 0.10$ ) to be less in R recipients that received embryos from S donors (SR, 27%) compared with the other treatments (64, 64 and 57% for SS, RS and RR, respectively). In conclusion, nutritional status of donors and recipients during 21 days before embryo transfer affected uterine gene expression, which might have modified the uterine environment of both donor and recipient females leading to changes in embryo survival. Funded by Projects PNSA 1115053 and PRET 1281102 (INTA), PICT 2012-2238 (FONCYT).



065 Environment and Reproduction

**Impact of postpartum locomotion score on reproductive performance in grazing dairy cows**

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The association between lameness in the first 50 days in milk (DIM) and calving-to-first service interval was evaluated in grazing Holstein cows to test the hypothesis that lame cows in early lactation have a longer calving to first service interval. A prospective observational study was carried out on a commercial dairy farm located in Carlos Casares (35°37' S, 61°22' W), Argentina. A total of 96 cows were included in the study. Locomotion score (LS) was evaluated four times 15-d apart in early lactation by using a 5-point scale: 1 = normal, 2 = presence of a slightly asymmetric gait, 3 = the cow clearly favored 1 or more limbs (moderately lame), 4 = severely lame, to 5 = extremely lame (nonweight-bearing lame). A cow was considered lame if she had a LS over 3 in the last scoring before the end of voluntary waiting period (50 DIM). After the voluntary waiting period, cows were detected twice daily for estrus and artificially inseminated (AI) using the AM-PM rule. Cows were considered in estrus when over 50% of tail paint was removed. Pregnancy was checked by transrectal ultrasonography approximately 30 d after AI. Calving to first service interval was registered for all cows until 200 DIM. The association between lameness and interval from calving to first service was assessed using survival analysis by fitting Kaplan-Meier survival curves to the data and using the Cox's proportional hazard regression model (PROC LIFETEST and PROC PHREG, SAS ver. 9.4). Lame cows had a median (95% confidence interval; CI) calving to first service interval 7 days longer ( $P \leq 0.05$ ) than non-lame cows (75 [73-82] vs. 68 [59-74], respectively). In addition, non-lame cows had greater ( $P \leq 0.05$ ) hazard of first service than lame cows (hazard ratio = 1.65, 95% CI = 1.06-2.58). Therefore, lameness in early lactation grazing dairy cows extends the interval postpartum to first service likely because it delays ovarian cyclicity.



066 Environment and Reproduction

### Seasonal changes in melatonin concentrations in female guanaco (*Lama guanicoe*)

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The guanaco is a wild South American camelid characterized by a strategy of seasonal reproduction of long days. In many ungulates, photoperiod is known as a major factor for the onset of the breeding season, but in Camelids this effect is not well described. The translation of the environmental light signal to hormonal changes is mediated through the secretion of melatonin from the pineal gland. Our previous study showed that these animals have a circadian pattern of melatonin secretion with maximum concentrations during the night and minimums during the day. The aim of this work was to evaluate the seasonal changes in plasma melatonin concentrations in female guanaco comparing short-day versus long-day photoperiods. The study was performed in a Mediterranean climate (33°28' S) under a short-day (10 h daylight/14 h darkness) and long-day (14 h daylight/10 h darkness) photoperiod. The experimental group was formed by 9 adult, captive-bred, non-pregnant non-lactating and healthy guanacos. Blood samples were collected once a week during the morning (10 am), for 3 weeks. Melatonin was measured by competitive ELISA assay. Concentrations of melatonin during the short- and long-day photoperiods differed ( $P < 0.001$ ) and averaged  $23 \pm 10$  and  $9 \pm 3$  pg/mL, respectively. Furthermore, a negative correlation between duration of light hours and melatonin concentration was observed ( $r = -0.714$ ;  $P < 0.001$ ). This pattern resembled that observed in other ungulates in different latitudes. Therefore, it is possible that changes in melatonin concentrations modulated by exposure to natural illumination might play a role in the reproductive seasonality in adult female guanacos. We suggest that secretion of melatonin is an important endocrine signal influenced by daylight exposure between short- and long-days in guanacos. This work was funded by CONICYT, BECA DOCTORADO NACIONAL 2016.



067 Environment and Reproduction

**Effect of controlled heat stress on follicular dynamics and steroid production of dairy cows**

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Heat stress (HS) has an enormous economic impact on the dairy industry. Reproductive losses in dairy cows associated with HS include reduction in duration and intensity of expression of estrus and increased incidence of anestrus and silent ovulations. This study aimed to investigate the effects of continuous and severe HS on follicular dynamics and steroidogenesis in Holstein cows. Healthy non-lactating Holstein cows were synchronized with the Ovsynch protocol concurrent with an intravaginal progesterone device. On the day of ovulation, cows were contemporaneously and randomly assigned to thermoneutral (TN; n=12) or HS (n=12) treatments. The temperature and relative humidity in the climate chamber were, respectively, 25.9°C and 73.0% for TN, and 36.3°C and 60.9% for HS. Transrectal ultrasonography of ovarian structures was performed twice daily over two follicular waves. On day 9 of the first follicular wave, the largest follicle (F1~12.0 mm) was individually aspirated and all remaining visible follicles were ablated. Subsequently, at expected deviation time (F1~8.5 mm), both the F1 and the second largest follicle (F2) were individually aspirated. Follicular estradiol and progesterone concentrations were determined by ELISA. Blood samples were collected daily to determine concentrations of FSH, inhibin, and estradiol by radioimmunoassay. The diameters of follicles and hormone concentrations were analyzed using ANOVA with repeated measures followed by Tukey post-hoc test with significance declared at  $P \leq 0.05$ . Exposure to HS had deleterious effect on follicle dynamics. During the first follicular wave, the diameter of the F1 was smaller ( $P < 0.001$ ), whereas the F2 was larger ( $P > 0.01$ ) for HS than TN cows. An enhanced growth of follicles in this wave in HS cows was associated with increased ( $P < 0.05$ ) circulating FSH concentration and coincided with decreased ( $P < 0.02$ ) concentration of inhibin in serum. In the second follicular wave, the follicular diameters for F1 and F2 were smaller ( $P < 0.01$ ) in HS than TN cows. Although greater ( $P < 0.01$ ) serum concentrations of FSH were observed in HS than TN cows, no difference was detected in serum concentrations of inhibin between treatments. In both follicular waves, treatment did not affect serum concentrations of estradiol. The intrafollicular concentrations of estradiol and progesterone differed ( $P < 0.001$ ) across the stages of follicle development, but no effect of treatment was detected or interaction between treatment and stages of follicle development. These results indicate that HS may impair follicular development by altered concentrations of FSH and inhibin, contributing with the low fertility observed in dairy cattle during the summer. This study was supported by FAPESP (Grant #2012/18297-7, Grant #2013/20083-8, and Grant #2014/21257-2).



068 Environment and Reproduction

### **Effects of temperament on reproductive performance of *Bos taurus* heifers enrolled in a 7 d CO-synch + CIDR protocol**

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It has been reported previously that cattle temperament significantly impacts production traits such as reproduction. The objective of the present study was to assess the association between temperament and pregnancy to fixed-timed artificial insemination (FTAI) in *Bos taurus* beef heifers. A total of 297 Angus influenced heifers from 3 locations were evaluated for temperament based on chute score and exit velocity on the first day of the estrous synchronization protocol (d-9) and at FTAI (d0). Individual exit velocity score was calculated by separating exit velocity results into quintiles and assigning heifers with scores from 1 to 5 (1 = slowest; 5 = fastest). Temperament scores were determined by the average of individual chute scores and exit velocity scores. Heifers were then classified by temperament type according to the temperament score (score less than or equal to 3: calm; score > 3: excitable). Pregnancy status was determined by transrectal ultrasonography approximately 40 days after FTAI. Hair from the tail switch was collected on d-9 and on the day of FTAI for cortisol evaluation. Overall, 71% of heifers were classified as calm whereas 29% were classified as excitable. Pregnancy to FTAI was less ( $P = 0.042$ ) in excitable compared with calm heifers (36% vs. 55%, respectively). Mean concentration of cortisol in the hair was reduced ( $P < 0.001$ ) from d-9 ( $3.5 \pm 0.3$  pg/mg of hair) to d0 ( $1.74 \pm 0.3$  pg/mg of hair) in all heifers, independently of temperament. In addition, no differences for mean hair cortisol concentrations were found between calm ( $2.91 \pm 0.2$  pg/mg of hair) and excitable heifers ( $2.67 \pm 0.2$  pg/mg of hair). We conclude that heifer temperament is associated with fertility and excitable heifers have reduced pregnancy to FTAI. Also, the reduction in concentrations of cortisol in hair from d-9 to d0 suggests that cattle handling during the estrous synchronization protocol might have acclimated cattle to handling and minimized potential stress.



069 Environment and Reproduction

**Dose-dependent effect of astaxanthin on bovine spermatozoa exposed to heat shock**

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Heat stress affects spermatogenesis and compromises sperm quality; however, the direct effect of elevated temperature on spermatozoa is not fully understood. Insemination of cows under heat stress exposes the mature spermatozoa to elevated temperature in the female reproductive tract. This can compromise sperm motility and fertilizing ability. Previous studies in our laboratory demonstrated that heat shock increased reactive oxygen species (ROS) production in bovine spermatozoa. Therefore, the objective of this study was to determine the dose-response effect of the carotenoid antioxidant astaxanthin on motility of bovine spermatozoa subjected to moderate heat shock. For each replicate, semen straws from 3 different bulls were thawed and subjected to Percoll gradient. Sperm concentration and motility was evaluated immediately after Percoll purification (0 h control group). Sperm cells ( $1.5 \times 10^6$  sperm/mL) were incubated in SP-TALP and SP-TALP-Ethanol (astaxanthin vehicle control: 0.15% ethanol) at 38.5 and 40°C for 4 h, or incubated at different astaxanthin concentrations (150, 200, 250, 300 and 350 nM) in SP-TALP-Ethanol at 40°C for 4 h. Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS. This experiment was replicated five times. Sperm motility at 0 h control ( $69.0 \pm 2.3\%$ ) was greater ( $P < 0.0001$ ) than all other treatments. Sperm incubation at 38.5 and 40°C reduced motility ( $P < 0.0001$ ) compared with 0 h control. However, such drop in motility was greater for sperm exposed to 40°C than 38.5°C. Heat shock at 40°C for 4 h reduced ( $P < 0.0001$ ) sperm motility in both SP-TALP ( $20.0 \pm 2.3\%$ ) and SP-TALP-Ethanol ( $19.0 \pm 2.3\%$ ) compared with 38.5°C ( $39.0\%$  for SP-TALP and  $41.0\%$  for SP-TALP-Ethanol; SEM=2.3). Supplementation of heat-shocked spermatozoa with astaxanthin concentrations of 200 ( $36.0 \pm 2.3\%$ ) and 250 nM ( $33.0 \pm 2.3\%$ ) recovered motility to values similar to 38.5°C control. Therefore, astaxanthin recovered sperm motility and attenuated the negative effect of heat shock possibly by counteracting heat-induced ROS in bovine spermatozoa. This research was supported by Coordination for the Improvement of Higher Level Education – Personnel (CAPES # 168277/2017-4).



070 Environment and Reproduction

**Effect of seminal plasma on the interval to ovulation, dominant follicle and corpus luteum size in Alpacas (*Vicugna Pacos*) and Llamas (*Lama glama*)**

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Alpacas and llamas as other camelids are induced ovulators and require an external stimulus, mounting by the male, for ovulation. Nerve growth factor beta (NGF-β), a protein present in the seminal plasma (SP) is reported as being responsible for stimulating ovulation; however, limited information exists on the dynamics of the follicular wave in alpacas and llamas post induction of ovulation with SP. To determine the effect of two external stimulations on the interval to ovulation and dominant follicle and corpus luteum size, adult female alpacas and llamas, 5-6 years old were assigned to one of two treatments when a dominant follicle  $\geq 7$  mm was present: SP (n = 6): 1 mL of SP i.m; GnRH (n = 6): 0.05 mg acetate of busereline. Animals were examined by ultrasonography using a 5.0 MHz transducer at 1- to 2-h intervals between 22 to 40 h after treatment or until ovulation occurred. All animals were evaluated by ultrasonography every day from day 2 to day 7 post-treatment, and again on days 9, 12 and 15 post-treatment. Data were analyzed using ANOVA. Interval from treatment to ovulation did not differ between SP and GnRH in alpacas ( $26.7 \pm 0.8$  vs  $28.0 \pm 1.7$  h) or llamas ( $30.5 \pm 1.4$  vs  $31.0 \pm 1.5$  h), but a significant difference ( $P < 0.05$ ) was observed between the two species. Interval to determination of follicle  $\geq 7$  mm did not differ between SP or GnRH treatment in alpacas ( $12.3 \pm 2.5$  vs  $13.3 \pm 1.4$  h) or llamas ( $8.6 \pm 2.5$  vs  $7.9 \pm 2.0$  h), but it differed ( $P < 0.05$ ) between species. Corpus luteum diameter was smaller ( $P < 0.05$ ) in SP than GnRH treatment in alpacas ( $8.2 \pm 0.8$  vs  $10.3 \pm 0.5$  mm), but not in llamas ( $9.8 \pm 1.0$  vs  $9.2 \pm 0.8$  mm). In conclusion, treatment did not influence interval to ovulation, but affected CL diameter only in alpacas. Interval from treatment to ovulation was longer in alpacas than llamas. Research funded by Project "Role of seminal plasma in reproductive physiology and application of biotechnologies in camelids" -149-2017- CIENCIACTIVA – CONCYTEC.



071 Environment and Reproduction

**Ewe fertility in group mating: Does the number of rams the ewe mates with affect fertility?**

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In commercial sheep meat production, ewes are often mated in large groups with multiple rams present in each group. We hypothesised that ewe fertility would vary according to the number of rams the ewe mated with in the first breeding cycle. To test this hypothesis, yearling ewes  $\approx$  8 months of age or adult ewes 1.5 years of age were joined with rams in a group mating situation during two breeding seasons for each age. For the yearling ewes, 4 groups of ewes ( $n=159-177/\text{group}$ ) were joined with 3 rams/group for a total of 35 days. For the adult ewes, 3 groups of ewes ( $n=287-300/\text{group}$ ) were joined with 3 rams/group for a total of 33-38 days. In each group, each ram wore a marking harness with a different colour crayon. For the yearling ewes, the number of rams (#R) the ewe mated to in her first mating was determined using the crayon marks. For the adult ewes, #R the ewe mated to in the first 17 days of the mating season were recorded. The effects of #R a ewe mated with on ovulation rate (OR; measured by laparoscope) and the number of lambs born (NLB) were analysed with restricted estimated maximum likelihood. The model included flock and weight at 4 weeks before (adults only) and at the start of the mating season. In the yearlings, of the 577 ewes with mating marks,  $25.4 \pm 4.1\%$ ,  $48.3 \pm 4.0\%$  and  $26.2 \pm 5.0\%$  mated to 1, 2 or 3 rams, respectively. The #R the ewe mated to was not associated ( $P > 0.05$ ) with differences in OR (1.89, 1.85, and 1.76 for #R 1, 2 or 3, respectively;  $SED = 0.08$ ) or NLB (1.58, 1.39, and 1.44 for #R 1, 2 or 3, respectively;  $SED = 0.11$ ). For the adult ewes,  $19.0 \pm 2.2\%$ ,  $19.2 \pm 3.5\%$ ,  $34.4 \pm 1.3\%$ , and  $27.4 \pm 3.3\%$  were marked by 0, 1, 2 or 3 rams, respectively. In general, as #R the ewe mated with increased, NLB increased (1.24, 1.54, 1.79, and 1.80 for 0, 1, 2 or 3 #R, respectively;  $P < 0.01$ ,  $SED = 0.09$ ), but OR did not change (2.14, 2.10, 2.19, and 2.18 for 0, 1, 2 or 3 #R, respectively;  $SED = 0.07$ ). Limiting the data for the outcomes from the first breeding, overall fertilization/embryo survival increased ( $P < 0.05$ ,  $SED = 5\%$ ) as #R mated to increased (60%, 77%, and 80% of oocytes ovulated represented by a lamb at birth for 1, 2 and 3 #R, respectively). In summary, although no associations were found between #R a yearling ewe mated to and her fertility, in adult ewes, as #R increased, ewe fertility increased. For ewes that failed to mate to any rams in the first breeding cycle, this was likely driven by having one less chance to become pregnant and thus an increased number of ewes that were not pregnant at the end of the breeding season as well as reduced embryo survival. However, decreased fertility was still observed in ewes mating to a single ram versus multiple rams, and decreased embryo survival likely underlies this observation. Funding provided by AgResearch's Strategic Science Investment Fund from NZ Ministry of Business, Innovation & Employment.



072 Environment and Reproduction

**Ovarian characteristics and somatic development in 2 to 5 months old female Nelore (*Bos taurus indicus*) calves**

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The herd's genetic gain could be faster and the generation interval shorter by including prepubertal bovine females in assisted reproductive programs. However, *in vitro* embryo production from calves is still challenging, and factors affecting recovered oocyte quality and developmental potential are not fully understood. In this regard, the relationship between somatic development and age at puberty is well known, but the relationship with reproductive development during the prepubertal period is unclear. Thus, the aim of the present study was to evaluate ovarian characteristics in 2 to 5-month old Nelore calves and their possible association with somatic development endpoints. Eight females were evaluated by transrectal ultrasonography (MyLab 30 VetGold, Esaote, 5 to 7.5 MHz probe, Genoa, Italy) once a week, during 12 weeks, to measure ovarian diameter, follicle population, diameter of the largest follicle present, and diameter of uterine horns. Calves were also evaluated for the following biometrics endpoints: height at the withers and hip, body length, depth, thoracic perimeter, rump width at ischium and ilium, rump length, head length, head width, and body weight. The possible associations were analyzed by Spearman correlation test, and a value of  $P < 0.05$  was considered significant. There was a moderate correlation between ovarian diameter and follicle population ( $r = 0.45$ ;  $P < 0.0001$ ) and uterine horn diameter ( $r = 0.31$ ;  $P = 0.002$ ). There was a strong correlation among all biometric parameters ( $0.85 < R < 0.98$ ;  $P < 0.05$ ). Ovarian diameter was positively correlated with all biometric parameters, with  $R$  values ranging from 0.31 to 0.48 ( $P < 0.05$ ). The diameter of the largest follicle present also was associated with thoracic perimeter ( $P = 0.039$ ;  $R = 0.32$ ). However, there was no significant correlation between follicle population and any biometric endpoint. The present results show an association between somatic development and reproductive tract development during the initial prepubertal period. Thus, we can hypothesize that biometric parameters can also be associated to oocyte development potential in calves, as well as to the potential as oocyte donors in adulthood. Financial support: EMBRAPA, CAPES, FAPEMIG e FAP-DF.



073 Environment and Reproduction

**Milk yield, periparturient diseases and body condition score as factors affecting the risk of fetal losses in high-yielding Holstein cows**

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Non-infectious causes of abortion play an important role in premature pregnancy termination. The infectious causes of abortion have been a primary focus of attention, because they may be controlled to some extent by vaccination. The cause of non-infectious abortions is difficult to diagnose, but in many herds non-infectious abortions are more prevalent than infectious ones. The objective of this study was to assess various risk factors affecting fetal losses in high-yielding Holstein cows in a hot environment. In a retrospective observational study, 14,384 records from Holstein cows from a large dairy herd in northern Mexico (25°N; 23.5°C mean annual temperature) and vaccinated against some agents of reproductive diseases were used. Fetal loss between 43 and 260 days affected 23.8% of the pregnancies. Multivariable logistic models indicated that dry period >60 d was associated ( $P \leq 0.05$ ) with less incidence (odds ratio, OR = 0.8; 95% confidence interval, CI = 0.8-0.9) of fetal loss compared with cows with dry periods <60 d. Cows with BCS >3.0 at calving and 15 days postpartum were at half the odds of fetal loss compared with similarly aged cows with BCS <3.0. Cows with peak milk yield > 38 kg had 5.5 times greater ( $P < 0.01$ ) odds of having fetal loss than cows with peak milk yield < 38 kg (36.9 vs. 9.6%). Fetal loss increased when cows had 305-d milk yield >9000 kg (OR = 2.1) compared with cows with milk yield < 9000 kg. Retained placenta was associated ( $P \leq 0.05$ ) with increased fetal loss (OR = 1.2; 95% CI = 1.1-1.4). Cows suffering premature parturition were greater ( $P \leq 0.05$ ) risk of suffering fetal loss than cows with normal parturition (OR = 1.2; 95% CI = 1.0-1.4). A bimodal distribution of detection of fetal loss was observed with peaks around 50 and 220 days of pregnancy, likely because when pregnancy diagnoses were performed with more frequency. It was concluded that, in this particular farm with cows under hot environment, increased milk yield, long dry period, reduced BCS at calving, and incidence of retained placenta were all associated with increased risk of fetal loss.



074 Environment and Reproduction

**The tail in estrous tropical hair ewes (*Ovis aries*) is used as a proceptive signal and favors copulation**

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Docking tails is a painful procedure, that also can affect possible tail functions related to estrus and copulation. Two experiments were performed to determine if the tail has an active role in: 1) estrous ewes' proceptive behavior, and 2) facilitating copulation in hair sheep. In the first study, the movements and position of the tail of estrous (ES) and non-estrous (NE) ewes in response to male presence and courtship were compared in 20 mature ewes. In the experiment 2, the rams' courtship and mating behavior toward intact (INT) or docked (DOCK) ewes, being ES or NE, were compared using 5 ewes/treatment arranged as a 2 x 2 factorial, with main factors as ES vs. NE, and INT vs. DOCK ewes. In both experiments, 25 rams were individually tested with restrained ewes during 3 min. In Experiment 1, tail movements occurred in short episodes in response to the physical contact of the male, but the rest of the time the tail of NE ewes was drawn inward to the body; in ES ewes the tail simply hung naturally straight downward. Ewes in ES performed more tail moving episodes with more movements per episode, movements that had greater amplitude and higher elevation angle from the body than in NE ewes ( $16.0 \pm 1.3$  vs.  $4.1 \pm 0.6$ ;  $6.2 \pm 0.3$  vs.  $2.1 \pm 0.3$ ;  $90.5 \pm 4.0^\circ$  vs.  $7.5 \pm 0.9^\circ$ ;  $49.0 \pm 2.3^\circ$  vs.  $17.3 \pm 0.9^\circ$ ;  $P < 0.001$  for all the comparisons; ES and NE ewes, respectively). In Experiment 2, the reaction time (RT) of DOCK ewes was delayed but only when they were in ES ( $19.9 \pm 5.5$  vs.  $47.5 \pm 11.2$  seconds;  $P < 0.05$ , for INT and DOCK ewes, respectively), with no difference in NE ewes ( $137.1 \pm 17.0$  vs.  $138.8 \pm 12.8$  seconds;  $P > 0.05$ , for INT and DOCK ewes, respectively). There were less failed intromissions to INT than to DOCK ewes when they were ES ( $4.2 \pm 1.2$  vs.  $9.5 \pm 1.8$ ;  $P < 0.05$ ), but not if they were NE ( $18.9 \pm 5.3$  vs.  $17.7 \pm 4.9$ ;  $P > 0.05$ ). There were also more failed intromissions before ejaculation to ES ( $7.6 \pm 0.9$  vs.  $3.4 \pm 0.5$ ;  $P < 0.05$ ), but not to NE ewes ( $7.1 \pm 0.8$  vs.  $5.6 \pm 0.5$ ;  $P > 0.05$ ). In addition, there were no differences according to the physiological status in those with docked tail ( $7.6 \pm 0.9$  vs.  $7.1 \pm 0.8$ ;  $P > 0.05$ , for ES and NE ewes, respectively). There were more anal intromissions to DOCK ewes (52% vs 0% in INT ewes) when they were in estrus, with intermediate values for NE ewes regardless of the presence of the tail (9 and 33%, for INT and DOCK ewes, respectively). In addition, there was an interaction between being or not in ES and having or not the tail ( $P < 0.05$ ) in the RT, and the number of failed and anal intromissions. Movements and position of the tail in ES ewes is a main component of the proceptive behavior, and facilitates mating and avoids failed and anal intromissions in hair sheep. Authors acknowledge Reyes Vázquez for his help with animal management.



075 Environment and Reproduction

**Pasture allowance in pregnant ewe: Effect on the development of the reproductive tract of their male offspring**

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The aim of the work was to compare the effect of natural pasture allowances (PA) offered to pregnant ewes on scrotal circumference (SC), anogenital distance (AG), penis size (PS), testes weight (TW), diameter of seminiferous tubules (DTS) and percentual volume of seminiferous tubules (VOL) of their male offspring. Single-bearing multiparous Corriedale ewes were randomly assigned to two PA from 30 to 143 d of gestation: i) High (HPA, n = 6): 14, 15 and 20 kg DM/100 kg body weight (BW)/d during 30-60, 61-110 and 111-143 d of gestation, respectively; ii) Low PA (LPA, n = 7): 6, 5 and 10 kg DM/100 kg BW/d on the same intervals as of HPA, respectively. Each treatment had three replicates in one of three independent paddocks (randomized block design with three replicates). From 100 d of gestation until weaning, ewes were supplemented daily with 300 g/animal of rice bran (88% DM, 14% crude protein, 9% acid detergent fiber, and 24% neutral detergent fiber) and from lambing to weaning all ewes were kept in the same paddocks with grass *ad libitum*. After weaning until slaughter at 200 d of age, lambs were located in individual pens where they were offered 6% of their individual BW adjusted every 15 d of a mixed diet: alfalfa hay and a complete commercial mixed ration (16% protein; 2% ether extract, 13% moisture, 19% crude fiber, 9% minerals). All lambs were slaughtered following standard procedures (electrical stunning, bleeding, skinning and evisceration) in a motor slaughterhouse. Mother's BW were registered fortnightly during gestation. The lamb's BW were registered at birth, 45 and 90 d of age, and fortnightly during fattening. The SC, AG, PS were measured at 180, 190 and 200 d of age. After slaughter, tests were weighed and tissue fixed in Bouin's solution. The histological sections stained in hematoxylin-eosin were analyzed (light microscope, video camera, pc with software Infinity®). Body weight, SC, AG and PS were analyzed as repeated measures using the MIXED procedure of SAS with day as repeated effect. The model included treatment, day, and interaction between treatment and day as fixed effects, and the block as random effect. Other variables were analyzed using mixed models with treatment as fixed effect and block as random effect. Statistical differences were considered when  $P \leq 0.05$ , and tendencies when  $0.05 < P \leq 0.10$ . Data were expressed as least square means  $\pm$  s.e.m. Ewes of HPA had greater ( $P = 0.0002$ ) BW ( $44.7 \pm 0.7$  kg) than LPA ewes ( $42.7 \pm 0.7$  kg). An interaction ( $P = 0.028$ ) between treatment and day was detected because treatment did not affect lamb's BW at birth and 45 d, but HPA lambs were heavier ( $P < 0.05$ ) than LPA lambs at 90 d of age. Treatment tended ( $P = 0.07$ ) to influence SC (HPA:  $31.7 \pm 2.0$  vs. LPA:  $26.6 \pm 1.9$  cm), influenced ( $P = 0.04$ ) AG (HPA:  $40.9 \pm 1.2$  vs. LPA:  $37. \pm 1.2$  cm), but did not affect PS. Treatment influenced ( $P \leq 0.05$ ) DTS (HPA:  $369.7 \pm 19.0$  vs. LPA:  $313.4 \pm 17.6$   $\mu$ m) and VOL (HPA:  $51.6 \pm 1.7$  vs. BOF  $46.2 \pm 1.7$  %), but did not affect TW (0.6). Pasture allowance of ewes during gestation affects the development of the male offspring at 200 d of age.



076 Environment and Reproduction

**The permanent presence of ovariectomized, steroid-treated goats does not prevent the seasonal decrease of LH and testosterone in male goats**

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Male goats exposed to females in estrus during the non-breeding season exhibit increased plasma luteinizing hormone (LH) and testosterone concentrations. Objectives were to determine whether the permanent presence of ovariectomized (OVX), steroid-treated goats, prevents the seasonal decrease in plasma concentrations of LH and testosterone observed during the non-breeding season. One group of bucks ( $n = 5$ ) was kept in contact with 3 OVX goats (OVX-only), whereas the other group ( $n = 5$ ) was kept in contact with 3 OVX treated with 1 mg of estradiol preceded by 2 i.m. injections of progesterone 72 h apart (10 and 5 mg, respectively) labeled as OVX-steroid. In both groups, females were exchanged once a week throughout the study. Plasma LH concentrations were determined every 20 min during 6 h (08:00-14:00) in October, February, March and June, 72 h after exchanges of females in each group of males. Plasma testosterone concentrations were determined once a week, 48 h after exchanges of females in each group of males from October to July. Plasma LH and testosterone concentrations were analyzed by a two-way ANOVA with repeated measurements to assess the effects of treatment and time. Plasma LH and testosterone concentrations varied over the study (time effect:  $P = 0.0001$ ), but these variations differed between bucks in contact with OVX-only or OVX-steroid (interaction between treatment and time:  $P = 0.0001$ ). However, plasma concentrations of LH from both treatments were less than 1 ng/mL throughout the study. Plasma testosterone concentrations in bucks in contact with OVX-steroid and OVX-only decreased from October ( $12 \pm 3$  and  $12 \pm 4$  ng/mL, respectively), and reached basal concentrations in January ( $4 \pm 1$  and  $3 \pm 1$  ng/mL), which continued until June. Thereafter, testosterone concentrations increased in July in bucks in contact with OVX-steroid and OVX-only ( $11 \pm 2$  and  $10 \pm 3$  ng/mL, respectively). We concluded that the permanent presence of OVX, steroid-treated goats does not prevent the seasonal decrease in LH and testosterone in bucks. This study was supported by CONACYT-México, Ciencia Básica (254176).



### **Effect of somatic cell count and its temporal association with service date on conception rate in grazing dairy cows**

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Reproductive efficiency is one of the most important factors associated with dairy farm profitability and is negatively affected by diseases such as clinical and subclinical mastitis. High somatic cells count (SCC) are used as indicators of subclinical mastitis and has been reported as risk factor for reproduction success (Fuenzalida et al., 2015). Although several studies have been done in dairy cows under confinement systems, to our knowledge only few have been done in grazing dairy cows. The objective of this study was to assess SCC and its temporal association with service date on pregnancy per insemination in grazing dairy cows. A retrospective longitudinal study was conducted using a dataset including records from lactations started between January 1, 2000 and December 31, 2014 (1,930,376 lactations) from 867 dairy herds located in the Province of Buenos Aires, Argentina. Four categories of SSC were define from SCC records measured 43 days before and 30 days after each service (only the first four services were used). The categories (SSC\_C) were: 1) Healthy when both SCC records had <150K cells/mL; 2) New Case: when the first SCC record had <150K cells/mL and the second had >150K cells/mL; 3) Cured when the first SCC record had >150K cells/mL and the second had <150K cells/mL; and 4) Chronic when both SCC records had >150K cell/ml (Fuenzalida et al., 2015). Data were analyzed with logistic model using mixed models (PROC GLIMMIX, SAS ver. 9.4) and the probability of pregnancy was modeled. The model included the fixed effects of SSC\_C, number of services (1 to 4), and their interaction, days in milk at every service (DIM), parity (1, 2, 3 and ≥4), calving season [Summer (Dec 21 to Mar 20), Autumn (Mar 21 to Jun 20), Winter (Jun 21 to Sep 20) and Spring (Sep 21 to Dec 20)], and calving year (2000 to 2014). Model also included the random effect of lactation to take into account the correlation between services within lactation. Pregnancy was associated with SSC\_C, number of service, and their interaction. Healthy cows were reference for comparison. The odds ratios (OR) for pregnancy at first service in cows classified as Cured, New Case or Chronic were 0.92 (95% CI: 0.90-0.94), 0.87 (95% CI: 0.85-0.88), and 0.84 (95% CI: 0.83-0.86) respectively. The odds of pregnancy were less for Cured, New Case, and Chronic than for Healthy cows in all the successive services, but the effect size decreased with service number. For example, after the first service, the OR for pregnancy were 0.95 (95% CI: 0.88-1.03), 0.91 (95% CI: 0.84-0.98), and 0.91 (95% CI: 0.86-0.98) for Cured, New Case and Chronic cows, respectively. Somatic cell count and its temporal association with date of service are important risk factors for pregnancy in grazing dairy cows. This work was supported by UNLP Incentive Program V11/230 grant to RLS. Fuenzalida, M.J. et al, 2015. The association between occurrence and severity of subclinical and clinical mastitis on pregnancies per artificial insemination at first service of Holstein cows. *J. Dairy Sci.* 98:3791-3805.



078 Environment and Reproduction

### **Aerobic metabolism is maintained in rams under testicular hyperthermia due to increased testicular blood flow**

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Heat stress is an important factor affecting reproduction of male animals. Increasing global temperatures elicit concerns regarding male fertility, since in most mammals, testes must remain ~3-4°C cooler than body temperature for production of morphologically normal and motile sperm. The classical dogma is that when testes are exposed to increased temperatures, there is no increase in blood flow and therefore the resulting hypoxia affects spermatogenesis. However, in our previous studies regarding impacts of oxygen (O<sub>2</sub>) concentration on sperm quality, damage caused after warming the testis was not replicated by hypoxia nor prevented by hyperoxia. The objective of this study was to understand impacts of increased testicular temperature on testicular blood flow, conductance (blood flow/aortic pressure), O<sub>2</sub> delivery and extraction, metabolic rate, and evidence of hypoxia. Nine crossbred yearling rams were maintained under general anesthesia and their testicular temperatures were increased in a step-wise fashion, at 33, 37 and 40°C (±0.5°C). Effects of temperature were analyzed by one-way analysis of variance for repeated measures, followed by a Dunnett's *t*-test. Testicular blood flow increased (P<0.05) as testicular temperature increased from 33 to 40°C (13.2 ± 2.7 vs 17.7 ± 3.2 ml/min/100 g of testes; mean ± SEM), with more profound (P<0.01) increases in conductance (1.08 ± 0.20 vs 1.60 ± 0.27 ml/min/g/mmHg 10<sup>3</sup>). Furthermore, an increase (P<0.0001) in metabolic rate (0.35 ± 0.04 vs 0.64 ± 0.06 mL O<sub>2</sub>/min/100 g of testes) was followed by an elevation (P<0.0001) in O<sub>2</sub> extraction (31.2 ± 5.0 vs 33 47.3 ± 3.1%). Lastly, there were no significant differences in hypoxia or impaired metabolism markers such as lactate, pH, HCO<sub>3</sub><sup>-</sup> or base excess, indicating no evidence of anaerobic metabolism. This is apparently the first report that heating testes of rams with an intact scrotum increased testicular blood flow. Furthermore, these results were in accordance with our previous findings that hyperthermia and not hypoxia apparently caused impaired spermatogenesis after increased testicular temperature. In conclusion, successive increases in testicular temperature nearly doubled testicular metabolic rate. Remarkably, there were no indications of testicular hypoxia or anaerobic metabolism, due to increases in blood flow (25%), conductance (48%) and, in particular, O<sub>2</sub> extraction (52%). Therefore, these data, in combination with other reports, challenged the paradigm that testicular hyperthermia fails to increase testicular blood flow and the ensuing hypoxia disrupts spermatogenesis.



079 Environment and Reproduction

**Associations between ovarian cyclicity, uterine health, body condition score, metabolic status and parity during the postpartum period in seasonal calving grazing dairy cows**

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The objective was to evaluate the associations between ovarian cyclicity (OC), uterine health status (UHS), body condition score (BCS), metabolic status (MS), and parity during early lactation in seasonally-calving, pasture-based dairy cows. First and second lactation spring calving dairy cows (n=2,602) from 35 dairy farms located in Ireland were enrolled in the study. All farms were visited every 2 weeks, and at each visit animals that were at week 3 (wk3; range 14 to 27 DIM) and week 7 (wk7; range 42 to 55 DIM) post-calving were examined. The BCS was measured using a 1 to 5 scale in 0.25 increments. Transrectal ultrasound examinations were conducted to determine OC and UHS. Blood samples were collected at each visit and the concentration of glucose, beta-hydroxybutyrate (BHB) and nonesterified fatty acids (NEFA) were analysed by enzymatic colorimetry. Cows were grouped into three BCS categories: low (less or equal to 2.50), target (between 2.75 and 3.25) and high (> 3.25); 3 OC categories: cycling (CyC, corpus luteum (CL) present), anestrus (AN, CL absent and dominant follicle (DF) present) deep anoestrus (DA; CL and DF absent); 4 UHS categories based on ultrasound findings: healthy (H), low infection (LI), mild infection (MI), severe infection (SI); and 3 MS categories based on glucose, BHB and NEFA: good, moderate and poor. Fisher's Exact Test was used to test associations between these different categorical variables and was supplemented by logistic regression to calculate odds ratios and predicted probabilities. At wk3 and wk7, 45.0% and 92.4% of cows had resumed cyclicity, respectively, and 96.4% and 73.6% of cows had a uterine infection, respectively. On wk3, there was association between likelihood of having resumed OC and UHS, BCS and parity; cows with low BCS (OR=0.73, P=0.05), uterine infection (both MI and SI; OR=0.17 and 0.04, respectively; both P <0.0001) and parity 1 (OR=0.63, P=0.001) had lower likelihood of having resumed OC compared with cows at target BCS, absence of uterine infection (H) and parity 2, respectively. Correspondingly, cows with low BCS (OR=1.16, P<0.01), anestrus (both AN and DA; OR=7.9 and 7.1, respectively, both P=0.0001), and parity=1 (OR=1.19, P=0.05) had greater likelihood of having uterine infection compared with cows with target BCS, CyC and parity=2, respectively. There was an association between BCS and MS (P=0.05); cows with good MS were more likely to be in the target BCS category than cows in the moderate or poor MS categories. Similar associations were observed at wk7. In conclusion, OC, UHS, BCS and parity were associated with each other throughout the postpartum period. Supported by Irish Department of Agriculture, Food and the Marine (Grant 13S528).



080 Environment and Reproduction

**Expression of COX, PPARGC1-A and NRF-1 genes in granulosa cells of goats after short term dietary supplementation with high lipid levels**

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In ruminants, the level of lipid inclusion used in diets does not exceed 5% of dry matter (DM) to control nutritional and reproductive consequences. High concentrations of lipids can lead to a decrease in oocyte quality due to the formation of cytotoxic highly reactive lipid peroxides, which are detrimental to organelles. Short dietary supplementation with lipids alters granulosa cells function in goats. Thus, the objective of this work was to verify in goats the effect of high lipid dietary supplementation for a short period on cholesterol (CHOL) and triglycerides (TG) concentration in follicular fluid (FF) and gene expression of mitochondrial function in granulosa cells (GCs). Twenty adult crossbred goats, with homogenous body condition ( $3.0 \pm 0.2$ ; mean  $\pm$  SD) and age ( $28.2 \pm 3.4$  months), were fed chopped elephant grass and concentrates (corn 60.3%, wheat bran 32.6%, and soybean meal 3.1%). Seven days before ovarian recovery, goats were assigned to a control group (CG, n=10) which received the diet described previously, or the lipid group (LG, n=10), in which ground flaxseed replaced 30% of the ingredients in the concentrate DM. The dietary fat contents were 2.6% and 6.8% on DM basis for CG and LG, respectively. Estrus was synchronized in all goats by insertion of a vaginal progesterone device on day 0. On day 6, the device was removed, and 0.075 mg of prostaglandin F<sub>2 $\alpha$</sub>  and 150 IU of equine chorionic gonadotropin were injected i.m. At 36 h after prostaglandin treatment, goats received 0.125 mg of gonadotropin-releasing hormone. Starting on day 9 until day 11, 200 mg of follicle-stimulating hormone was injected i.m. with doses divided in application with 12 h intervals. Goats were slaughtered on day 12 and ovaries were collected and follicular contents aspirated. Granulosa cells were collected and stored until RNA extraction. Cholesterol and TG concentrations of FF were analyzed by spectrophotometry (Mindray® BS-120; Guangdong, China). Enzymatic activity of glutathione peroxidase from FF was measured using commercial kit (Ransel, Randox, Crumlin, UK). Analysis of *COX*, *PPARGC1-A* and *NRF-1* genes expression in CGs was performed using SYBR Select Master Mix (Life technologies, USA). Total RNA was extracted using the kit CellsDirect™ One-Step qRT-PCR and the RPS9 reference gene was selected as endogenous control. Data log-transformed (log<sub>10</sub>), were subjected to ANOVA using the GLM. Diets type (CG and LG) was the main effect tested. Pairwise comparisons were performed by student t test. No significant effect of diet was found for concentrations of CHOL and TG in FF, which averaged  $28.1 \pm 7.8$  and  $13.7 \pm 3.8$  mg/dL, respectively. Glutathione peroxidase activity was greater (P=0.005) in LG than CG ( $432.0 \pm 18.7$  vs.  $355.0 \pm 15.9$  U/L). All genes were expressed in GCs, but treatment did not affect gene expression. Increasing the dietary lipid content fed for a short period increased glutathione peroxidase activity in follicular fluid, but it did not influence expression of mitochondrial genes of granulosa cells.



081 Environment and Reproduction

### **Evaluation of the leucocitary response with the use of Pegbovigrastim in Holstein cows in the peripartum**

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Pegbovigrastim is a modified form of bovine granulocyte colony stimulating factor (bG-CSF), which is a group of cytokines necessary for the proliferation and differentiation of hematopoietic stem cells, conjugated to a polyethylene glycol (PEG). Treatment of cows with pegbovigrastim induces a transient leukocytosis with neutrophilia that might influence innate immune response. The objective of this study was to evaluate the leukocyte response with the use of pegbovigrastim in postpartum Holstein cows. The experiment was carried out at the experimental station of the Agronomic Research Institute located in São Bento do Una, in the state of Pernambuco. Twelve pregnant cows assigned to two treatments, control untreated cows (CG), and treated cows (TG) in which pegbovigrastim was administered at 7 days before and within 24 hours of calving. The CG received injections of saline solution (0.9% NaCl, wt/vol). Total and differential leukocyte counts were evaluated every 7 days from the first treatment application to 21 days postpartum. Endometrial cytology was evaluated every 7 days in the first 21 days postpartum. Blood samples were collected by puncture of the jugular vein using a 25 x 8 mm (21G) needle attached to evacuated tubes containing an aqueous solution of ethylene diamine tetracetate tripotassium (K3-EDTA) at 15%. The leukocyte count was performed by the manual method in Neubauer's chamber. Two blood smears were made for differential leukocyte counting, these smears after drying were stained using the rapid pantype kit according to the manufacturer's recommendation. In each blood smear 100 leukocytes were identified and classified according to their morphological and dyeing characteristics. The experiment followed a completely randomized design with two treatments and six replicates per treatment, and data were analyzed for prepartum period, day of calving and postpartum period. The data were tested for distribution of residuals using the Kolmogorov-Smirnov test, being expressed in central tendency measurements. Data were analyzed by ANOVA with repeated measures using the GLM procedure of SAS. In the case of significance of ANOVA, means were partitioned by the least significant difference of the Student- Newman-Keuls test. A P-value of 0.05 was considered significant. Cows in TG had increased ( $P < 0.0001$ ) count of leukocytes, mainly because of increased segmented neutrophils. The concentration of segmented neutrophils in blood averaged 10,467 cells/ $\mu\text{L}$  in TG and 4,278 cells/ $\mu\text{L}$  in CG ( $P < 0.004$ ). The reference values for segmented neutrophils in cattle is between 600 and 4,000 cells/ $\mu\text{L}$  of blood. There was no change in the percentage of segmented neutrophils present in the endometrium. It is concluded that the treatment is effective in increasing the concentration of leukocytes in blood, mainly segmented neutrophils. Support for this project was provided by Elanco (donation of Pegbovigrastim), Facepe (scholarship), and IPA (cows).



082 Environment and Reproduction

**Maternal nutrient restriction followed by realimentation in beef cows alters maternal and fetal circulating amino acid profiles**

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Inadequate maternal nutrition impairs offspring growth, fertility and carcass quality. A practical strategy for ameliorating these developmental programming consequences is through realimentation of the undernourished dam. Our lab has previously demonstrated that realimentation of nutrient restricted gestating beef cows increases uterine blood flow to the conceptus. The objective of this study was to evaluate the effects of realimentation on amino acid profiles in maternal and fetal circulation. On d 30 of pregnancy, multiparous cows (initial body weight [BW] = 620.5 ± 11.3 kg and body condition [BCS] = 5.1 ± 0.1) were assigned to one of three dietary treatments: control (CON; 100% NRC; n = 18) and restricted (RES; 60% NRC; n = 30). On d 85, cows were slaughtered (CON, n = 6; R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12), or were realimented to control (RC; n = 11). On d 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254, all remaining cows were slaughtered. Maternal serum, fetal serum, and amniotic fluids were collected at slaughter. Amino acid concentrations were determined using ultra performance liquid chromatography. Data were analyzed with generalized least squares using the mixed procedure of SAS to examine the effects of dietary treatment at each day of slaughter. On day 85, maternal serum from RES cows contained less (P = 0.06) histidine, isoleucine, alanine, and valine, and more (P = 0.09) glycine compared to CON. Day 85 fetal serum from CON cows had more (P = 0.01) tryptophan compared to RES, while amniotic fluid from CON cows had more (P = 0.09) glutamate, but less (P = 0.07) phosphoserine, tyrosine, and phenylalanine than RES cows. Minimal changes were observed on day 140 of gestation, with maternal serum from RC cows having greater aspartate (P = 0.03) than CC and RC cows. By day 254, RRC cows had greater (P = 0.04) arginine concentrations in maternal serum compared to RCC and CCC cows, while fetal serum from RRC cows contained greater (P < 0.06) citrilline, phenylalanine, and tryptophan compared to RCC and CCC cows. Amniotic fluid from RRC cows had greater (P < 0.05) concentrations of histidine and lysine compared to RCC and CCC cows. In conclusion, it appears that amino acid circulation in pregnant beef cows and their fetuses may be sensitive to maternal diet, including the duration of nutrient restriction and realimentation. Realimentation at the right time during pregnancy may be a viable feeding strategy for producers to improve pregnancy outcomes of malnourished beef dams. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2009–65203–05812 from the USDA National Institute of Food and Agriculture to KAV and KCS.



083 Environment and Reproduction

**Introduction of androgenized steers during the late luteal phase triggers an advancement of luteolysis**

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The sudden introduction of males (biostimulation or bull effect) induces an increase of estrogen concentration, which may trigger the mechanisms resulting in luteolysis. The objective of the present experiment was to determine if the introduction of androgenized steers to beef heifers during their late luteal phase advances the luteolytic process. The trial was performed in a private farm in Uruguay (35° S) during May (autumn) with 12 Angus based heifers. Heifers remained isolated from any contact with males more than 4 weeks before beginning the trial (minimum distance = 500 m). Heifers' ovulation was synchronized with two doses of 500 µg i.m. of chlorprostenol (Ciclast DL, Syntex, Argentina) separated 11 days, and a dose of 100 µg i.m. of gonadorelin acetate (Gonasyn, Syntex, Argentina) 2 days after the second dose of chlorprostenol. The day in which each animal ovulated (day 0 of the estrous cycle) was determined by transrectal ultrasonography. Transrectal Doppler ultrasound observations of the corpus luteum (CL) were performed daily from day 10 to day 12 of the estrous cycle of all animals. On day 12, the animals were separated into two groups of 6 animals each that remained in paddocks of the same size and with the same pasture availability. While 2 androgenized steers were joined with the biostimulated heifers (group BT), the other heifers remained continuously isolated from males (group CON). From day 13 to 19 of the estrous cycle, CL were observed with Doppler ultrasound every 12 h. There was no effect of biostimulation on CL volume, but the area of the CL perfused was lower in BT than CON heifers ( $0.09 \pm 0.02 \text{ cm}^2$  vs  $0.16 \pm 0.02 \text{ cm}^2$  respectively ( $P = 0.015$ ), indicating an earlier luteolysis. Furthermore, the percentage of the CL area that was perfused was also lower in BT than CON heifers ( $2.4 \pm 0.4\%$  vs  $4.2 \pm 0.4\%$   $P = 0.011$ ). Overall, it was concluded that the introduction of androgenized steers during heifers' late luteal phase advanced the luteolytic process. Support provided by Javier Meilán, from Zoetis, for the donation of hormones; Victor Mayorga and Augusto Ryonosuke, for their help in the field work; Comisión Sectorial de Investigación Científica (CSIC), Universidad de la República, for the financial support.



084 Environment and Reproduction

### **Timing and duration of nutrient restriction and its impacts on placental development and umbilical blood flow in sheep**

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Nutrient-restriction beginning on day 50 of gestation in nulliparous ewe lambs decreased umbilical blood flow by day 80 (Lemley et al., 2012; AJP.302:R454). We hypothesized that ewes would experience a decrease in umbilical blood flow upon nutrient restriction (day 50 to 90) and that blood flow would recover to control values upon realimentation during late gestation (day 90 to 130), or remain reduced in ewes that continued to be nutrient restricted. On day 50 of gestation, young nulliparous white face ewes (6 to 8 mo; n = 41) carrying singletons were randomly assigned to two dietary treatments where ewes received 100% of NRC recommendations (CON) or 60% of CON (RES). On day 90 of gestation, ewes either remained on CON or RES until day 130, or CON ewes were RES from day 90 to 130, or RES ewes were realimented to CON from day 90 to 130. This resulted in 4 treatment groups: CON-CON, CON-RES, RES-RES, RES-CON. On day 50, and every 10 days until day 110, umbilical blood flow and placentome measurements were obtained via ultrasonography. The study had an end point of day 130 (data not shown). The study was conducted as a completely randomized design arrangement with repeated measures. Data were analyzed using the MIXED procedure of SAS. Non-significant interactions and main effects were removed from the models when  $P \geq 0.25$ . There were no three way interactions or main effect of late gestational treatment on umbilical blood flow or placentome size ( $P \geq 0.25$ ). However, there was a significant interaction of mid gestational nutrition and day ( $P < 0.01$ ) on umbilical blood flow with CON ewes having greater blood flow compared to RES by day 90. Similarly, placentome size was affected by mid gestational nutrition and day ( $P \leq 0.05$ ). Placentomes from CON ewes were larger than placentomes from RES ewes by day 80. However, placentome sizes were similar among treatments on day 90 ( $P \geq 0.22$ ). We reject our hypothesis that realimentation would return umbilical blood flow to control levels, and we were surprised CON-RES ewes had similar blood flow compared to CON-CON ewes. The majority of placental development occurs during the first two thirds of pregnancy. Our findings suggest that an adequate placental development during mid gestation could potentially “protect” the fetus from a decreased umbilical blood flow later on gestation when nutrients were limited by 40%. We are uncertain why realimentation did not alter umbilical blood flow by day 110 of gestation, but further analyses at day 130 may provide some insight into our study. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016-67016-24884 from the USDA National Institute of Food and Agriculture.



085 Environment and Reproduction

**Effect of post-insemination intrauterine treatment with cephalosporin on the subsequent reproductive performance of dairy cows with mild endometritis**

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Cows in estrus but with signs of clinical endometritis (CE) are often not inseminated or undergo an intrauterine treatment. Decades ago, the so-called Åström method was described as intrauterine infusion of an iodine-potassium solution a few days after artificial insemination (AI). Nowadays, the use of antibiotics instead of iodine solution is common and the treatment is performed a few hours after AI. The objective of this study was to evaluate the effect of this modified Åström method on pregnancy per AI (P/AI; proportion of cows pregnant after AI). The study was conducted on a large dairy farm in Slovakia. A total of 323 Holstein Friesian cows were included in the study at the day of AI. Before enrollment, vaginal discharge was evaluated with the Metricheck device. Animals with clear discharge were assigned to a healthy comparison group (HE=112) and animals with cloudy discharge or fleck of pus in the mucus were divided into a treatment and a control group. The treatment group (MET; n=108) received an intrauterine treatment with 500 mg cephalosporin (Metricure<sup>®</sup>, Intervet Deutschland GmbH) 6±1 hours after AI. The control group (CO; n=103) remained untreated. From a subset of cows with CE (n=62), bacteriological samples were taken from the uterus with the cytobrush technique and subjected to Fourier-transform infrared spectroscopy. The statistical evaluation was carried out with the software SPSS (version 24.0, IBM SPSS Inc., Munich, Germany). For the comparison of the P/AI between the three groups HE, MET and CO, Chi-square tests were performed. The level of significance was set at P<0.05. Pregnancy at first AI was did not differ among groups (HE 37%; MET 32%; CO 31%). Non-pregnant cows were re-inseminated at the next estrus. For cows with a second AI, P/AI was greater in the MET (59%) compared with CO (44%). Interestingly, the lowest P/AI was observed in HE (32%; P<0.05). Cumulated results of the first and second AI showed a similar trend with the highest proportion of pregnant cows in MET (73%) followed by CO (63%) and HE (58%), although differences were not significant. Most of the examined cows (56/62) were bacteriologically positive. The uterine microflora was highly diverse and 234 isolates were found, mainly Gram-positive bacteria. Most frequently detected genera were *Staphylococcus* (15%), *Bacillus* (9%) and *Corynebacterium* (7%). Pathogenic species, such as *Trueperella pyogenes* and *Escherichia coli* were not or rarely isolated (0 to 2%). In summary, post-insemination treatment with cephalosporin had a significant positive effect on P/AI at the second AI but not for the AI immediately before treatment. One reason for this delayed effect could be that the time for recovery of the inflamed endometrium after treatment was too short before the embryo entered the uterus. This finding and the fact that typical uterine pathogenic bacteria were rarely detected questions the indication of an intrauterine antibiotic treatment in cows with mild CE at breeding.



### **Determinants of cytological endometritis in multiparous Holstein cows**

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Cytological endometritis (CE) significantly reduces the reproductive performance of cows. Our objective was to establish the determinants that predispose dairy cows to CE. Multiparous Holstein cows (n = 119) from one 1,200-cow dairy herd were used. Body condition score (BCS) was evaluated at 2 weeks before expected calving and cows were classified as thin ( $\leq 2.75$ ), moderate (3.00 and 3.25) and moderately fat (3.50 and 3.75). Blood was collected at wk -2 prepartum, and at wk +1 and +3 postpartum. Endometrial samples were taken at 40 d postpartum using an endometrial brush attached to a stainless steel device for use in cows (EP2029026B1). The first 45-d cumulative milk yields were categorized as low ( $< 1,314$  kg), moderate ( $\geq 1,314$  to  $< 1,780$  kg), or high ( $\geq 1,780$  kg). Diseases were recorded. Plasma insulin and insulin-like growth factor 1 (IGF1) and serum amyloid A (SAA) were analysed by ELISA; all other plasma variables were measured using an autoanalyser. Optimal criterion values were obtained from receiver operating characteristic curve analysis. Data were analysed with logistic regression and multiple correspondence analysis. The CE prevalence was 30.3% with a cut-off of  $> 8\%$  polymorphonuclear neutrophils. Multiple correspondence analysis revealed two distinct clusters of risk factors for CE. The determinants of CE corresponding to the first cluster were associated with metabolic imbalance indicated by low BCS ( $\leq 2.75$  vs. 3.50 and 3.75, odds ratio [OR] = 5.76,  $P = 0.027$ ), high non-esterified fatty acids ( $> 108$   $\mu\text{ekv/L}$ , OR = 3.65,  $P < 0.011$ ), high haptoglobin ( $> 0.08$  mg/mL, OR = 3.38,  $P < 0.005$ ) and low IGF1 ( $< 74.6$  ng/mL, OR = 5.96,  $P < 0.001$ ) levels at wk -2 prepartum; low IGF1 ( $< 36.5$  ng/mL, OR = 3.88,  $P = 0.012$ ), low insulin ( $< 0.33$  ng/mL, OR = 2.59,  $P = 0.025$ ) and low Ca ( $< 2.43$  mmol/L, OR = 3.08,  $P = 0.009$ ) levels at wk +3 postpartum; a high cumulative milk yield over the first 45 d of lactation ( $\geq 1,780$  kg vs.  $\geq 1,314$  to  $< 1,780$  kg, OR = 10.54,  $P < 0.001$ ) and an increased parity ( $\geq 3$  lactations vs. 2 lactations, OR = 2.32,  $P = 0.044$ ). The determinants of CE corresponding to the second cluster were related to a high degree of systemic inflammation indicated by high haptoglobin ( $> 0.81$  mg/mL, OR = 6.39,  $P < 0.001$ ), high SAA ( $> 128$  ng/mL, OR = 9.13,  $P < 0.001$ ), low albumin ( $< 36.5$  g/L, OR = 2.96,  $P = 0.009$ ), low cholesterol ( $< 2.15$  mmol/L, OR = 2.33,  $P = 0.044$ ) and low IGF1 ( $< 13.2$  ng/mL, OR = 5.96,  $P < 0.001$ ) levels at wk +1 postpartum. This cluster included a low albumin level ( $< 38.1$  g/L, OR = 3.92,  $P = 0.002$ ) at wk +3 postpartum, ill health (OR = 2.93,  $P = 0.008$ ) and a low cumulative milk yield ( $< 1,314$  kg vs.  $\geq 1,314$  to  $< 1,780$  kg, OR = 7.81,  $P < 0.001$ ) over the first 45 d of lactation. In conclusion, two distinct clusters of cows experienced high risk for the development of CE: 1) cows with metabolic imbalance and a high milk yield over the first 45 d of lactation; 2) cows with a high degree of systemic inflammation, ill health and a low milk yield over the first 45 d of lactation. Supported by IUT8-1.



## **Resistin acts as a link between reproduction and energy metabolism in sheep**

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Resistin exerts a regulatory influence on numerous reproductive and metabolic processes, including maintenance of energy homeostasis during different reproductive states. It is particularly important in seasonally breeding sheep which must adapt their metabolic state to the changing environment. Metabolic factors such as insulin, leptin, and glucose affect various aspects of reproduction and are related to activity of resistin-secreting adipocytes; however, information regarding the interactions among them in sheep is limited. Numerous metabolic abnormalities can lead to the development of pathophysiological conditions such as the polycystic ovarian syndrome (PCOS) and recent studies indicate that resistin may have a role in PCOS development. Moreover, obesity is significantly related to declines in fertility. Females with obesity have abnormal plasma resistin profiles, which may help explain some types of infertility. Herein we examined the interaction of season and recombinant bovine resistin (rbresistin) on plasma concentrations of leptin, insulin and glucose in experiments conducted during both short (SD) and long (LD) days. Thirty ewes of the Polish Longwool breed, which exhibits strong seasonal reproduction, were ovariectomized and given estrogen replacement using subcutaneously inserted estradiol implants. Ewes were housed in natural photoperiod (longitude: 19°57' E, latitude: 50° 04' N). Animals were 2 to 3 years of age and had a mean body weight of 60±2 kg. Intravenous treatments at the beginning of experiment (Time 0) consisted of control or rbresistin in saline: 1) Control (C; saline; n = 10), 2) low resistin (R1; 1.0 µg/kg BW; n = 10), and 3) high resistin (R2; 10.0 µg /kg BW; n= 10). Blood samples were collected every 10 minutes during 4 h. Blood plasma concentrations of resistin, leptin and insulin were assayed using RIA and ELISA kits. Glucose was measured using a Roche AccuChek Active device. Plasma leptin concentrations were greater ( $P < 0.05$ ) in C during LD compared to SD. The R1 and R2 resistin treatments resulted in 2- and 3-fold increases, respectively, in circulating concentrations of resistin ( $P < 0.05$ ) during LD. Resistin infusion decreased ( $P < 0.001$ ) mean circulating concentrations of leptin in a dose- dependent manner during both seasons. However, only R2 increased ( $P < 0.05$ ) plasma concentrations of insulin and did so during both seasons. Both R1 and R2 decreased ( $P < 0.05$ ) glucose concentrations in both seasons. There was no time by treatment interaction in response to resistin injection. To the best of our knowledge, this is the first study to report a role for resistin in modulating circulating leptin, insulin and glucose, and indicate that the ability of resistin to create this effect is somewhat seasonally-dependent. Further studies investigating the interaction of resistin and other adipokines such as leptin are warranted. Research supported by NCN 2015/19/B/NZ9/01314 to DA.



088 Environment and Reproduction

### **Estrous expression improves the success of timed artificial insemination and embryo transfer**

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Detection of estrus is important for reproductive performance. Use of automated activity monitors as tools for detection of estrus has become popular on dairy farms. This study aimed to evaluate the associations between estrous responses measured with automated activity monitors at the end of a timed artificial insemination (TAI) or embryo transfer (ET) protocol and fertility outcomes. In study 1, lactating Holstein cows had their estrous cycles synchronized using a protocol based on estradiol and progesterone and bred by TAI using conventional semen (n=1411 events from 1040 cows). In study 2, lactating Holstein cows had their estrous cycles synchronized using a protocol based on estradiol and progesterone and 7 d post-estrus received an embryo (n=1147 events from 657 cows). Embryos were transferred as either *in vivo* (fresh or frozen) or produced *in vitro* (only frozen). Pregnancy was confirmed at 31 ± 3 d post-TAI or estrus; for study 1, pregnancy losses were determined by a second pregnancy diagnosis at 60 d post-TAI. In both studies, the expression of estrus was monitored through a leg-mounted activity monitor (Afimilk Pedometer Plus, Afikim, Israel). Estrus was determined to have occurred when the relative increase (RI) in activity of the cow exceeded > 100% of their baseline activity. At estrus, physical activity was categorized as high or low intensity using the median. Data were analyzed with the GLIMMIX procedure of SAS and cow was used as a random effect. In study 1, 82.0% of cows expressed estrus on the day of TAI or the preceding evening. Cows expressing estrus around TAI had greater (P < 0.01) pregnancy per AI (P/AI) than those that did not express estrus (32.7 vs. 6.2%). Cows that expressed estruses of high intensity had greater (P < 0.01) P/AI than those with low intensity of estrous expression or that did not express estrus at all (35.1 vs. 27.3 vs. 6.2%). Cows with high intensity of estrous expression had less (P < 0.05) incidence of pregnancy loss compared with cows with low intensity of estrous expression (13.9 vs. 21.7%). In study 2, 89.1% of cows expressed estrus before ET. Cows expressing estrus before ET had greater (P < 0.01) pregnancy per ET (P/ET) than those that did not express estrus (35.8 vs. 5.9%). Of the cows that expressed estrus, those with high intensity of estrous expression had greater (P < 0.01) P/ET than those with low intensity of estrous expression (41.5 vs. 30.6%). No interaction was found between estrous expression and source of embryo (in vivo fresh, in vivo frozen or in vitro frozen), but cows receiving *in vitro* frozen embryos had less (P = 0.02) P/ET than those receiving embryos produced *in vivo* and transferred fresh or frozen (30.0 vs. 38.7 vs. 39.0%). Expression of estrus is associated with improved pregnancy in dairy cows bred by timed AI and timed ET. Furthermore, cows with more intense estrous expression have improved maintenance of pregnancy either following AI or ET. Authors would like to thank Colorado Dairy, Conapec Jr., Dairy Farmers of Canada and NSERC.



089 Environment and Reproduction

**Heat stress induces proteomics changes in the follicular fluid of dairy cows**

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Reduced fertility associated with heat stress (HS) is a complex and multifactorial problem involving hormone secretion, follicular development, oocyte and embryo quality, and uterine function. The aim of this study was to investigate the effects of experimentally induced HS on follicular fluid (FF) proteome of cows at key stages of follicular development. Nonlactating Holstein cows were synchronized with the Ovsynch protocol that incorporated an intravaginal progesterone device. On the day of ovulation, cows were contemporaneously and randomly assigned to thermoneutral (TN; n = 12) or HS (n = 12) treatments. The temperature and humidity in the climate chamber were, respectively, 25.9°C and 73.0% for TN, and 36.3°C and 60.9% for HS. Transrectal ultrasonography was performed every 12 h to monitor follicular dynamics. On day 9 of the first follicular wave, the largest follicle (F1~12 mm) was individually aspirated and all remaining visible follicles were ablated. At expected deviation time (F1~8.5 mm), both the F1 and the second largest follicle (F2) were individually aspirated. After albumin depletion, triplicates of FF were reduced, alkylated, and digested with trypsin. The resulting peptides were labelled with TMTsixplex (Pierce, Rockford, USA) and quantified using LC-MS/MS (Orbitrap Elite, Thermo, San Jose, USA). Quantitative proteomic data were compared by PROC GLM (SAS Institute, Cary, USA). A total of 158 unique proteins were identified in the FF, mainly as plasma-matched proteins. Twenty-eight differentially ( $P < 0.05$ ) expressed proteins were found in FF of cows exposed to HS versus TN, of which seven were affected by the interaction between the stage of follicle development and treatment. Data analysis using IPA software (Qiagen, Redwood City, USA) revealed that the most significant canonical pathways associated with the proteins identified in the FF were acute phase response, liver X receptor (LXR), retinoid X receptor (RXR) and farnesoid X receptor (FXR) activation, complement system, and coagulation system. Many components of the immune system (IgGs, inter-alpha inhibitor H4, alpha-2-macroglobulin, transthyretin, complement components C4, C6, C7, C8, C9, complement factor B, and factor I) were up-regulated by HS, while components of the coagulation cascade (plasminogen, hemopexin, prothrombin, and vitronectin) were down-regulated in response to HS. In conclusion, these findings demonstrate that HS alters the protein expression of FF, which might affect follicular function as well as oocyte quality and could explain in part the reproductive failure of heat stressed dairy cows. FAPESP (Grants #2012/18297-7, #2013/20083-8, and #2014/21257-2).