124 Characterization of endometrial immune cells adjustments along pregnancy in the cow. Ana Carolina Furlanetto Mançanares[1], Gabriela de Paula Figueiredo[2], Rodrigo da Silva Nunes Barreto[1], Daniele dos Santos Martins[2], Flávio Vieira Meirelles[2,1], and Lilian J. Oliveira*[1], *[1]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, SP, Brazil, [2]Faculdade de Zootecnia e Engenharia de Alimentos, São Paulo, SP, Brazil.*

Successful of embryo implantation requires synchronization between maternal and fetal factors to promote endometrial receptivity. In this scenario, the maternal immune system may play an important role in the establishment as well in the development of conceptus until onset of parturition in eutherian mammals. Our study aimed to identify and quantify the subpopulations of immune cells in the bovine pregnant endometrium. We hypothesized that the endometrial immune cells pool change as pregnancy progresses. Samples from early (33 ± 1.5 d), mid (170 ± 16.9 d) and late pregnancy (227 ± 10.8 d) were obtained from a local abattoir (n = 5, per group). Cryostat sections were labeled for CD4 (T helper), CD8 (T cytotoxic), CD14 (macrophage), CD25 (activation T cells) and WC1 (γd). The samples were analyzed under epifluorescence microscope. The number of positive cells was counted and normalized to the total number of cells per mm^2 in 5 random fields for each group/marker. Data were analyzed separately for each marker by least square ANOVA using the GLM procedure of SAS. The $WC1^+$ cells were most prominent in the endometrial stroma, with greater cell number in mid than in early and late stages of pregnancy ($P < 0.01$). Moreover, $CD4^+$ ($P = 0.09$) and $CD8^+$ ($P < 0.01$) decreased in number in late pregnancy, whereas $CD25^+$ cells were low only in mid-pregnancy ($P = 0.02$). $CD14^+$ cells increased, respectively, 3- and 4-fold in mid and late stages of pregnancy ($P < 0.01$). Our results show that there is a deviation of endometrial immune cells subpopulations during pregnancy in the cow. In early pregnancy, there is a predominance of immune cells involved in regulation of immune response (CD3, CD4 and CD8) to allow embryo attachment and survival; whereas in mid-pregnancy, number of cells with trophic function are increased (CD14 and WC1) likely to allow placental development. In late pregnancy, there is a recruitment of cells involved in inflammatory response to induce placental detachment and parturition (CD14 and CD25).

Key Words: endometrial immune cell, pregnancy, bovine

125 Expression of nonclassical MHC-I isoforms is deregulated in cloned placenta. Rodrigo Silva Nunes Barreto[1], Felipe Perecin[2], Flávio Vieira Meirelles[2], Flávia Thomaz Verechia Pereira[3], Maria Angelica Miglino[1], and Lilian J. Oliveira*[1], *[1]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brazil, [2]Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga, SP, Brazil, [3]Campus Experimental de Dracena, Universidade Estadual Paulista, Dracena, SP, Brazil.*

Trophoblast cell evades the maternal immune recognition by expressing non-classical (NC) MHC-I isoforms, in humans and mice. In the cow, MHC-I proteins are expressed from 120 d of pregnancy, however in clones MHC-I expression can begin as early as d 35. This abnormal expression of MHC-I in clones may play a role on high rates of embryo/fetal losses in this model. Our study aimed to determine expression of MHC-I and its NC isoforms in normal and cloned bovine pregnancy. Samples of normal and cloned bovine placenta in early (n = 5) and term (n = 6) pregnancy were used for immunolocalization of MHC-I by IHC, using antibodies for bovine MHC-I (IL-A88) and murine NC isoform (Qa-2). Also, the expression of classical MHC-I (JSP-1) and NC isoforms (NC1–4) was analyzed by qRT-PCR. Data were analyzed by least square ANOVA using the GLM procedure of SAS. The model included main effects (group and animal) and standard errors. In normal placenta, NC1 was equally expressed in early and late pregnancy, whereas in clones, NC1 tended to increase expression in late pregnancy ($P = 0.07$). NC2 was higher expressed during early pregnancy in clones than early normal pregnancy ($P = 0.03$) and both normal and clone late pregnancies ($P = 0.04$). For NC3 expression showed interaction between type and stage of pregnancy ($P = 0.01$). The expression of NC3 was higher in later in normal pregnancy ($P < 0.01$), while in clones the NC3 expression was higher in early stages ($P = 0.04$). Neither NC4 and JSP1 showed changes in expression among groups. Immunohistochemistry analysis showed that IL-A88 stained the maternal epithelium and non-invading trophoblast but not trophoblast giant cells. In early pregnancy, IL-A88 staining was weak in normal and almost absent in cloned placenta. At term, IL-A88 staining increased in normal placenta. For Qa-2, no staining was detected in early pregnancy in normal whereas the cloned placenta showed strong staining. Furthermore, in the term pregnancy the Qa-2 staining did not differ between normal and cloned placenta. Altogether, our data suggest that the MHC-I expression is dysregulated in clone pregnancy, which may contribute to their low pregnancy rates and success.

Key Words: nonclassical MHC, bovine, cloned placenta

126 Behavioral and hormonal pattern around estrus and the characteristics of preovulatory follicles of repeat breeder dairy cows. Uzi Moallem*[1], Maya Zachut[1], and Pankaj Sood[1,2], *[1]Department of Ruminants Science, Volcani Center, Bet Dagan, Israel, [2]Department of Veterinary Gynecology and Obstetrics, Palampur, India.*

Repeat breeding in dairy cows results in large economic loss; however, the etiology of this phenomenon remains elusive. The objectives were to examine the behavioral and endocrine patterns around estrus, and the characteristics of preovulatory follicles in repeat breeder (RB) vs. normal (CTL) cows. The CTL cows were >60 d in lactation, cycling and not inseminated, and a cow was considered as RB if it did not become pregnant after at least 4 successive inseminations (average 7.0 ± 2.0), with normal intervals. A total of 27 and 31 cycles in 12 RB and 18 CTL cows, respectively, were synchronized. Data were analyzed as repeated measurements by the MIXED procedure of SAS, and the model included the effects of treatment, cow and cluster. Behavioral estrus and ovulation were observed in 81.5 and 83.8% of the synchronized cycles in the RB and CTL cows, respectively. The RB and CTL cows had similar estrus durations of 21.4 and 19.6 h, respectively, but estrus was more intense in the RB, as indicated by numerically higher overall activity indexes and higher peak neck activity. The interval from PG injection to estrus onset (i.e., proestrus) was 8.2 h shorter in RB than in CTL cows (47.9 and 56.1 h, respectively; $P < 0.007$), but the average preovulatory follicle size was similar. Plasma estradiol (E_2) concentration at peak was numerically higher (21%) and the AUC tended to be higher in the RB than in the CTL cows. Also, LH secretion during the period from 18 to 3 h before the LH peak was lower in RB than in CTL: 2.5 and 4.6 ng/mL, respectively ($P < 0.01$). In another study, 16 preovulatory follicles (8 CTL and 8 RB) were aspirated 48 h after PG injection. No differences were observed in androstenedione and progesterone (P_4) in follicular fluid, but concentrations were 1.7 fold higher ($P < 0.005$)

and E_2/P_4 ratio tended to be higher ($P < 0.07$) in RB than in CTL cows. In conclusion, better estrus expression, similar follicular diameter and higher E_2 in RB indicate that the etiology of repeat breeding lies beyond these parameters. However, short proestrus and subdued LH concentrations before the LH peak, which could impair oocyte competence and development, are first reported in RB cows.

Key Words: repeat breeders, estrus intensity, LH secretion

127 Effects of dry period length on onset of ovarian activity and ovarian cyclicity in the subsequent lactation. Juncai Chen*[1], Nicoline M. Soede[1], Gerrit J. Remmelink[2], Bas Kemp[1], and Ariette T. M. Van Knegsel[1], *[1]Adaptation Physiology Group, Wageningen University, Wageningen, the Netherlands, [2]Livestock Research, Wageningen University and Research Centre, Wageningen, the Netherlands.*

Negative energy balance (NEB) caused by high milk yield and insufficient feed intake in early lactation has been related to compromised cow health and fertility. Recent studies show that the NEB in early lactation could be alleviated by omitting or shortening dry period in dairy cows. It can therefore be hypothesized that omitting or shortening dry period improves fertility in dairy cows. The objective of this study was to evaluate the effects of dry period length on onset of ovarian activity and ovarian cyclicity in Holstein-Friesian dairy cows within 100 d in milk (DIM). The cows (60 primiparous and 108 multiparous) were randomly assigned to one of 3 dry period lengths (0, 30, and 60 d). Milk samples were collected 3 times a week for progesterone concentration analysis until 100 DIM after calving. Onset of luteal activity (OLA) was defined as the occurrence of at least 2 succeeding milk samples with progesterone concentrations ≥2 ng/mL. Normal resumption of ovarian cyclicity was defined as onset of first luteal activity occurring within 45 DIM, and followed by regular ovarian cycles of 18 to 24 d in length. Data are expressed as percentage or LSMEANS ± SEM. Within 100 DIM postpartum, cows with a 0-d dry period had greater incidence of normal resumption of ovarian cyclicity compared with cows with a 60-d dry period (53.2% vs. 26.0%; $P = 0.01$). Cows with a 30-d dry period tended to have greater incidence of normal resumption of ovarian cyclicity compared with cows with a 60-d dry period (47.7% vs. 26.0%; $P = 0.09$). In addition, cows with a 0-d dry period tended to have shorter interval from calving to first commencement of luteal activity (23.1 vs. 28.9 ± 2.0 d; $P = 0.07$) compared with cows with a 60-d dry period. Overall, our results demonstrate that omitting dry period improves resumption of ovarian cyclicity within 100 DIM in the subsequent lactation.

Key Words: continuous milking, progesterone, ovulation

128 Effect of extending the duration of the postpartum voluntary waiting period on reproductive performance of lactating dairy cows. Matias L. Stangaferro*[1], Robert Wijma[1], Magdalena Masello[1], Mark J. Thomas[2], and Julio O. Giordano[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Dairy Health & Management Services, Lowville, NY.*

Our objective was to investigate the effect of extending the duration of the voluntary waiting period (VWP) on reproductive performance of lactating dairy cows. Holstein cows [n = 1,082; 434 primiparous (PP) and 648 multiparous (MP)] from 3 commercial dairy farms in New York were blocked by parity and total milk production in their previous lactation (MP only) and were randomly assigned to receive first timed AI (TAI) service at 60 ± 3 (SVWP) or 88 ± 3 DIM (LVWP) after synchronization of ovulation with the Double-Ovsynch protocol (GnRH-7 d-PGF-3 d-GnRH-7 d- GnRH-7 d-PGF-56 h-GnRH-16 h-TAI). Pregnancy diagnosis was conducted by rectal palpation or transrectal ultrasound 39 and 90 d after AI. Data for pregnancies per AI (P/AI) at 39 d after TAI, pregnancy loss (PL) from 39 to 90 d after AI, and percentage of cows pregnant up to 150 DIM were analyzed by logistical regression using PROC GLIMMIX of SAS. Models included the effect of treatment, parity, and their interaction. Farm was included as a random effect in all models. At 39 d after TAI, P/AI were greater ($P < 0.01$) for cows in the LVWP (46.0%; 228/496) than cows in the SVWP (38.4%; 225/586) group. Pregnancies per AI were greater ($P < 0.01$) for PP (49.5%; 215/434) than for MP (36.7; 238/648) cows. Pregnancy loss, was similar ($P = 0.88$) for cows in the LVWP (4.5%) and the SVWP (4.7%) group, and for PP (4.6%) and MP (4.6%) cows ($P = 0.97$). The percentage of cows pregnant by 150 DIM was similar ($P = 0.23$) for cows in the LVWP (62.6%; 278/444) and the SVWP (69.6%; 261/375) group but were greater ($P < 0.01$) for PP (74.5%; 222/298) than for MP (62.6%; 278/444) cows. The interaction between treatment and parity group did not affect ($P > 0.05$) P/AI, PL, or percent pregnant by 150 DIM. We conclude that extending the duration of the VWP from 60 to 88 DIM for cows receiving TAI after synchronization with the Double-Ovsynch protocol increased overall P/AI. Also, the initial differences in P/AI after first service were compensated by earlier re-insemination of cows in the SVWP group. Supported by New York Farm Viability Institute project AOR13006.

Key Words: timed AI, first service, dairy cow

129 Cows under heat stress have increased uterine size, reduced circulating progesterone, and decreased fertility compared with cows in cooler conditions. Giovanni M. Baez*[1,2], Rafael V. Barletta[1], Eduardo Trevisol[1], Jerry N. Guenther[1], João P. Ferreira[3], and Milo C. Wiltbank[1], *[1]University of Wisconsin-Madison, Madison, WI, [2]Universidad Francisco de Paula Santander, Cucuta, NS, Colombia, [3]São Paulo State University, Botucatu, SP, Brazil.*

Seasonal differences in progesterone (P4) concentrations and pregnancies per AI (P/AI) have been demonstrated in lactating dairy cows. Moreover, an association between uterine size and P/AI has been recently reported. Our objective was to evaluate seasonal differences in uterine size, circulating P4, and fertility in primiparous and multiparous dairy cows. Lactating Holstein cows (n = 704) were synchronized to receive timed AI (TAI) on d 81 ± 3 of lactation by using the Double-Ovsynch protocol (GnRH-7d-PGF-3d-GnRH-7d-GnRH-7d-PGF-56h-GnRH-16h-TAI). At the time of the last injection of PGF, uterine diameter was determined at the greater curvature using ultrasound, uterine length was determined by rectal palpation, and uterine volume was calculated. Circulating P4 at final PGF and GnRH were evaluated to assure synchronization of all cows used in the final analysis (n = 616; primiparous (P), n = 289; multiparous (M), n = 327). At the same time, respiratory rate (number of breaths per minute, BPM) and body temperature was determined in all cows. Fischer's exact test and *t*-test were used to analyze categorical and continuous variables respectively, and logistic regression analysis was used to calculate probabilities of P/AI related to uterine volume. There was a clear delineation in average respiratory rate (40 to 28 BPM) indicating a change of season (hot (H) n = 226 (112 P, 114 M), cool (C) n = 390 (177 P, 213 M). Uterine volume was greater in H than C season for all cows (150.3 ± 2.9 vs. 131.2 ± 1.9 mm^3, H vs. C, $P < 0.0001$), and for P (134.6 ± 4.1 vs. 122.0 ± 2.8, $P = 0.009$) or M (165.7 ± 4.2 vs. 138.8 ± 2.6, $P < 0.0001$) cows. Circulating P4 was lower during H than C season in P (7.5 ± 0.3 vs. 8.1 ± 0.2, $P = 0.08$) and M (6.0 ± 0.3 vs. 6.8 ± 0.2, $P = 0.02$) cows. The P/AI was reduced in H vs. C for all cows (38.5% vs. 47.7%, $P = 0.02$), and for P (41.1%

vs. 56.5%, $P = 0.03$) but not for M (36.0% vs. 40.4%, $P = 0.21$) cows. Logistic regression analyses indicated a relationship between uterine volume and P/AI for M cows in H ($P = 0.03$) and C ($P = 0.03$) seasons but for P cows only in C season ($P = 0.02$) with greater uterine volume associated with reduced P/AI. Thus, heat stress is associated with an increase in uterine size. Greater uterine size partially explains differences in fertility within parities and seasons.

Key Words: fertility, uterine size, heat stress

130 Effect of heat stress during pregnancy on intact and adrenal de-medullated fetuses: Placental, fetal, and mammary development in ewes. Antoni Macko*, Sean Limesand, and Robert Collier, *University of Arizona, Tucson, AZ.*

It is well established that heat stress during mid- and late-gestation induces intrauterine growth retardation (IUGR) and decreased milk yield in the subsequent lactation in cattle and sheep. We hypothesized that elevated fetal adrenal norepinephrine (NE) secretion contributes to impaired mammary development in heat stressed pregnant ewes. Pregnant ewes were assigned to one of 4 treatment groups that were a combination of control (C) or hyperthermia-induced IUGR (I) and surgical sham (S) or bilateral fetal adrenal demedullation (D) at 98 d gestational age (dGA; term = 148 dGA)(n = 4 CS, 4 CD, 6 IS, and 4 ID fetuses). At 134 dGA, fetal plasma NE was measured, animals were euthanized, and fetal and placental weights obtained. Ewes' mammary glands were collected, weighed and prepared for analyses of DNA content, and histological evaluation to quantify the numbers of alveolar units per microscopic field and mammary epithelial cells per alveolus. Data were analyzed by Proc Mixed ANOVA, SAS 9.3. In the IS group, fetal plasma NE was 4-fold higher ($P < 0.05$) and placental, fetal and maternal mammary weights and mammary DNA were lower ($P < 0.05$) compared with the CS group and these parameters were partially reversed in the ID group. Placental weight: CS 337 ± 34, CD 271 ± 39, IS 160 ± 36, ID 215 ± 50 g; Fetal weight: CS 3495 ± 214, CD 3343 ± 233, IS 1746 ± 330, ID 2574 ± 296 g; Mammary wet weight: CS 3043 ± 445, CD 2496 ± 345, IS 785 ± 300, ID 1326 ± 316 g; Mammary dry weight: CS 343 ± 34, CD 353 ± 59, IS 192 ± 44, ID 278 ± 58 g; Mammary DNA content: CS 3043 ± 456, CD 2946 ± 353, IS 868 ± 353, ID 1327 ± 322 ng/µl. There were no treatment effects on the number of alveolar units per field: CS 33.6 ± 9.6, CD 17.3 ± 4.0, IS 16.5 ± 5.0, ID 28.5 ± 3.9; or number of cells per alveolus: CS 6.5 ± 1.0, CD 7.5 ± 2.0, IS 8.2 ± 0.3, ID 6.4 ± 1.0. We conclude that heat stress during gestation reduces placental, fetal and mammary development and fetal adrenal de-medullation partially reverses these effects. We also propose that an endocrine signal from the placenta regulates maternal mammary growth during gestation and this signal is responsive to fetal catecholamines.

Key Words: heat stress, placenta, mammary gland

131 Timing of GnRH administration based on estrous response in beef heifers following administration of the 14-d CIDR-PG protocol with split-time AI. Brianne E. Bishop*, Jordan M. Thomas, Jillian M. Abel, Mark R. Ellersieck, Scott E. Poock, Michael F. Smith, and David J. Patterson, *University of Missouri, Columbia, MO.*

The experiment was designed to evaluate timing of GnRH administration in beef heifers based on estrous status with split-time AI. Estrus was synchronized in 816 heifers across 4 locations by using the 14-d CIDR-PG protocol (CIDR insert [1.38 g progesterone] on d 0 with removal on d 14; 25 mg $PGF_{2\alpha}$ [PG] 16 d after CIDR removal on d 30; and 100 µg GnRH depending on treatment). Estrous detection aids (Estrotect) were applied at PG on d 30, with estrus recorded at 66 and 90 h after PG on d 33 and 34, respectively. Treatments were balanced across locations using tract score and weight. Timing of insemination was based on expression of estrus 66 h after PG. Heifers in each treatment that exhibited estrus by 66 h were inseminated; whereas, AI was delayed 24 h until 90 h after PG for heifers failing to exhibit estrus by 66 h. Heifers in treatment 1 were administered GnRH 66 h after PG irrespective of estrus expression; whereas, in treatment 2, heifers were administered GnRH coincident with delayed insemination only if not detected in estrus at 66 h after PG. Data were analyzed using PROC FREQ in SAS. There was no effect of treatment on overall estrous response (1 = 85%; 2 = 87%; $P = 0.49$) or AI pregnancy rate (1 = 55%; 2 = 58%; $P = 0.54$). There were no differences between treatments in estrous response at 66 h (1 = 70%; 2 = 69%; $P = 0.64$); and pregnancy rate resulting from AI for heifers inseminated at 66 h was not influenced by GnRH (1 = 62%; 2 = 64%; $P = 0.65$). Furthermore, there were no differences between treatments in estrous response during the 24 h delay period (1 = 50%; 2 = 58% $P = 0.22$), or pregnancy rate resulting from AI (1 = 40%; 2 = 44%; $P = 0.55$). In summary, when split-time AI is used in conjunction with the 14 d CIDR-PG protocol, administration of GnRH at AI to heifers that exhibit estrus by 66 h after PG is not warranted. These data suggest, however, that among heifers for which AI is delayed based on failure to exhibit estrus by 66 h after PG, timing of GnRH administration (66 vs. 90 h after PG) may be more flexible.

Key Words: estrous synchronization, split-time artificial insemination, beef heifer

132 Timing of GnRH administration based on estrous response in beef cows following administration of the 7-d CO-Synch + CIDR protocol with split-time AI. Brianne E. Bishop*, Jordan M. Thomas, Jillian M. Abel, Mark R. Ellersieck, Scott E. Poock, Michael F. Smith, and David J. Patterson, *University of Missouri, Columbia, MO.*

The experiment was designed to evaluate timing of GnRH administration in beef cows based on estrous status with split-time AI. Estrus was synchronized in 622 cows across 6 locations by using the 7-d CO-Synch + CIDR protocol (100 µg GnRH + CIDR insert [1.38 g progesterone] on d 0; 25 mg $PGF_{2\alpha}$ [PG] at CIDR removal on d 7; and 100 µg GnRH depending on treatment). Estrous detection aids (Estrotect) were applied at CIDR removal and PG on d 7, with estrus recorded at 66 and 90 h after PG on d 10 and 11, respectively. Treatments were balanced across locations; cows within location were randomly assigned to one of 2 treatments based on age, BCS, and days postpartum. Timing of AI was based on expression of estrus 66 h after PG. Cows in each treatment that exhibited estrus by 66 h were inseminated; whereas AI was delayed 24 h until 90 h after PG for cows failing to exhibit estrus by 66 h. Cows in treatment 1 were administered GnRH 66 h after PG irrespective of estrus expression; whereas in treatment 2, cows were administered GnRH coincident with delayed AI only if not detected in estrus at 66 h after PG. Data were analyzed using PROC FREQ in SAS. Treatment affected overall estrous response (1 = 85%; 2 = 90%; $P = 0.04$) but did not affect total AI pregnancy rate (1 = 58%; 2 = 57%; $P = 0.89$). There were no differences between treatments in estrous response at 66 h (1 = 73%; 2 = 75%; $P = 0.47$); and pregnancy rate resulting from AI for cows inseminated at 66 h was not influenced by GnRH (1 = 63%; 2 = 59%; $P = 0.50$). Estrous response during the 24 h delay period differed between treatments (1 = 45%; 2 = 61% $P = 0.04$), although AI pregnancy rate for cows inseminated at 90 h did not differ (1 = 44%; 2 = 49%; $P = 0.51$). In summary, when split-time AI is used in conjunction with

the 7 d CO-Synch + CIDR protocol, administration of GnRH at AI to cows that exhibit estrus by 66 h after PG is not warranted. These data indicate that delayed administration of GnRH to 90 h coincident with AI among cows failing to exhibit estrus by 66 h after PG results in a greater overall estrous response.

Key Words: estrous synchronization, split-time artificial insemination, beef cow

133 Influence of estrus expression prior to fixed-time AI on embryo survival to maternal recognition of pregnancy. Emmalee J. Northrop*[1], Olivia L. Amundson[1], Brittany N. Richardson[1], Anthony K. McNeel[2], Robert A. Cushman[2], and George A. Perry[1], *[1]Department of Animal Sciences, South Dakota State University, Brookings, SD, [2]USDA-ARS, US Meat Animal Research Center, Clay Center, NE.*

Estradiol has been reported to play a critical role in pregnancy establishment and embryonic survival. Our objective was to focus on the role of preovulatory estradiol in embryo survival from fertilization to maternal recognition of pregnancy. Estrus was synchronized in beef cows (n = 29) with the CO-Synch protocol and artificially inseminated (d 0). Blood was collected to determine estradiol (d −2 to 0) and progesterone (d 0 to 16) concentrations. Cows were then divided into 2 groups based on expression of estrus (estrus and no estrus). On d 16 uteri were flushed to collect embryos. Total cellular RNA was extracted from blood leukocytes (d 16) to measure the expression of interferon-stimulated genes (ISG): ISG-15, OAS-1, and MX2. Flush media was analyzed for protein and glucose concentrations. Data were analyzed by PROC MIXED. There was an effect of estrus, time, and estrus by time ($P < 0.01$) on circulating concentrations of estradiol, but there was no effect of estrus ($P = 0.85$) or estrus by time ($P = 0.26$) on circulating concentrations of progesterone. There was no difference in embryo recovery rate ($P = 0.97$; 45% vs. 44%). When corrected for volume of flush media, there was no difference between estrus and no estrus for uterine flush protein ($P = 0.51$; 218 ± 94 vs. 124 ± 104 mg/mL); or glucose (1960 ± 274 vs. 2003 ± 304 mg/dL) content between estrus and no estrus. There was no difference ($P > 0.20$) in d 16 expression of ISG-15, OAS-1, or MX2 between estrus and no estrus animals, nor a difference between cows an embryo was or was not recovered from. However, there was a tendency for cows in which an embryo was recovered from to have greater concentrations of protein ($P = 0.056$; 314 ± 104 mg/mL) and glucose ($P = 0.09$; 2347 ± 303 mg/dL) in uterine flushes compared with cows in which an embryo was not recovered (28 ± 94 mg/mL and 1616 ± 274 mg/dL). In summary, there were no differences between cows that did or did not express estrus in ISG expression, or in protein or glucose concentration of uterine flushes. Therefore, the increased embryo survival to d 30 of gestation among cows that express estrus is not associated with embryo survival until maternal recognition.

Key Words: embryo survival, estradiol, fixed-time AI

134 Post insemination interventions: Effect of human chorionic gonadotropin, gonadotropin-releasing hormone, and progesterone on ovulation and conception rates in Nili-Ravi buffaloes. Ali Husnain, Umair Riaz, Muhammad Ilyas Naveed, Mubbashar Hassan, Mian Abdul Sattar, and Nasim Ahmad*, *Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.*

We hypothesized that enhancing luteal tissue (or progesterone) would improve conception rate in Nili-Ravi buffaloes. The objective of the present studies were to standardized the dose of human chorionic gonadotropin (hCG) for ovulation induction on 7 d post insemination (experiment 1) and to see the effect of standardized dose of hCG, GnRH and exogenous progesterone (CIDR insert) on ovulation and conception rates in Nili Ravi buffaloes (experiment 2). In experiment 1, ovaries of the adult Nili Ravi buffaloes were scanned and mapped 7 d post synchronized estrus to confirm the presence of corpus luteum (CL) and dominant follicle (>8 mm) and assigned randomly to treatments of saline, or 500, 1000, 1500, 2000, 2500, or 3000 IU hCG (IVF-C, LG, Korea; i.m., n = 47) and ovulation induction or accessory CL, was confirmed a week later with ultrasound. Ovulation incidence in response to ≥2500 IU hCG was more (100%) than that of other doses or saline. The minimum effective dose was determined through dose response curve. In experiment 2, ovarian responses and conception rates were assessed by minimal effective dose of hCG (2500 IU, n = 58), GnRH (n = 28), CIDR insert for a week (1.38 g, n = 26) and control (n = 44) on 7 d post AI in previously CIDR synchronized in Nili-Ravi buffaloes. Overall significance was determined with Chi-squared and association between groups was checked using Z test in SAS software. More buffaloes were induced to ovulate with hCG (95%) and GnRH (80%) when compared with control and CIDR insert ($P < 0.05$). Conception rates were significantly higher ($P < 0.05$) in hCG (62%; 36/58) treated buffaloes compared with control (32%; 14/44). In contrast, these were non-significant in GnRH (50%; 14/28) and CIDR (42%; 12/26) treated buffaloes when compared with control. In conclusion 2500 IU of hCG is sufficient to induce the accessory CL and this treatment enhance the conception rate when given 7 d post insemination in Nili-Ravi buffaloes.

Key Words: progesterone, conception rate, Nili-Ravi buffaloes

135 Relationships between hair coat shedding and hair cortisol concentrations and age at first calving in crossbred beef heifers. Rhonda C. Vann*[1], Michael Robinson[2], Scott T. Willard[2], Thomas H. Welsh, Jr.[4], and Ronald D. Randel[3], *[1]MAFES-E.G. (Gene) Morrison Brown Loam Branch Experiment Station, Raymond, MS, [2]Department Animal & Dairy Science, Mississippi State University, Mississippi State, MS, [3]Texas A&M AgriLife Research Center, Overton, TX, [4]Texas A&M Department of Animal Science, College Station, TX.*

The objective was to evaluate the relationships among hair coat shedding, hair length and hair cortisol concentrations to age at first calving. Spring born (n = 101) crossbred beef heifers (7 to 8 mo. of age) were evaluated for hair length (HL; 1 = short and 5 = long), hair luster (HST; 1 = glossy, healthy appearance and 5 = dull and unthrifty), hair shedding (HS; 1 = short, slick hair, sheds early and 5 = long, late shedding, or full winter coat) at weaning. Calves were weighed, blood samples collected for evaluation of serum cortisol concentrations pre-weaning and at weaning by RIA. At weaning, hair samples were collected over the shoulder, rib and hip area (7.6 cm x 12.7 cm clipped area) for evaluation of hair cortisol concentrations. Hair samples were washed and dried overnight, samples ground using a Retsch mixer miller (200 mm for 10 min), sonicated (at 25 Hz) and incubated 8 h at room temperature. The supernatant was pipetted off and dried under a stream of atmospheric air. Samples were reconstituted before quantification of cortisol using Salimetrics cortisol EIA assay. Data were analyzed using PROC CORR and PROC Mixed of SAS. Hair cortisol concentrations at the rib, hip and shoulder were ($P < 0.001$) greater in cattle with a red or brown coat color compared with cattle with black coat color. Hair length, HST or HS were not influenced by hair coat color ($P > 0.10$). Hair length, HST

and HS were negatively correlated with adjusted 205-d weaning weight ($P < 0.006$) and serum cortisol ($P < 0.001$). Hair length, HST, HS or hair cortisol concentrations were not correlated with age at first calving ($P > 0.10$). Calving day of the year was negatively correlated with hair coat luster ($P < 0.04$) and tended to be negatively correlated with hair length ($P < 0.07$). Pregnancy status was not influenced by hair cortisol concentrations or HL, HST or HS ($P > 0.10$). In summary, hair cortisol concentrations were influenced by hair coat color. However, hair cortisol concentrations and hair length, luster or hair coat shedding were not adversely associated with age at first calving.

Key Words: hair coat shedding, hair cortisol concentrations, beef heifers