W188 Early prediction tools for the selection of reproductive traits on spring born crossbred Angus heifers. R. A. Franco1, G. Scaglia2, W. S. Swecker3, and M. L. Wahlberg4. 1Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, 2Louisiana State University AgCenter-Iberia Station, Jeanerette, 3Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg.

The objective of this study was to evaluate the efficacy of insulin like growth factor-1, leptin, insulin, thyroxin, triiodothyronine, glucose, lactate dehydrogenase, hip height (HH), frame score (FS), and body weight (BW) as predictors of pregnancy, calving and weaning of a live calf on spring born beef cattle. Thirty-six Angus crossbred heifers (BW=280.5±3.8 kg, mean age=308±2 d) were blocked by BW into six groups and randomly assigned to one of six paddocks. The experimental period covered six months from December 21, 2006 to the start of the breeding season in May 2007. Reproductive outcomes were computed (BW=280.5±3.8 kg, mean age=308±2 d) were blocked by BW into six groups and randomly assigned to one of six paddocks. The experimental period covered six months from December 21, 2006 to the start of the breeding season in May 2007. Reproductive outcomes were computed.

Key Words: endometrium, receptors, premature luteolysis

W189 Endometrial gene expression of estradiol, progesterone, and oxytocin receptors in anestrous Bos indicus cows treated with progesterone. O. G. Sa Filho*, D. M. Guerra, and J. L. M. Vasconcelos, FMVZ/UNESP, Botucatu, SP, Brazil.

In anestrous cows, treatment with progesterone (P4) prior to ovulation prevents subsequent premature luteolysis. In Exp. 1 we evaluated the effects of follicular diameter on endometrial mRNA for receptors of estradiol (ER), P4 (PR), and oxytocin (OTR) in anestrous Nelore cows. Cows (30-60 DPP) had ovaries examined by ultrasound daily and were hysterectomised when follicles reached 7 (n=4), 8.5 (n=4), or 10 mm (n=4). An additional group (n=4) of cows had mean follicular diameter of 11.31±0.03 mm at 48 h after temporary weaning (TW). In Exp. 2 we evaluated the effects of treatment with P4 on endometrial mRNA for ER, PR, and OTR in anestrous Nelore cows. Cows (30-60 DPP) were randomly assigned as control (CTL; n=20) or to receive a CIDR containing 1.9 g of P4 (CIDR; n=20) from days -8 to -2 before GnRH injection on Day 0. All cows were submitted to 48 h TW from Day -2 to 0. Hysterectomies were performed at Days -8, -2, 0, and 5 (n=5/treatment/day point). In both experiments, endometrial tissue was dissected and samples were submitted to real-time RT-PCR. The mRNA abundance of target genes are presented relative to CYC-A. Data were analyzed by PROC MIXED of SAS. In Exp. 1, no effects of follicular size were found on PR-mRNA, ER-mRNA, and OTR-mRNA. In Exp. 2, PR-mRNA was not affected by day or treatment, whereas treatments affected (P<0.05) ER-mRNA at Days -2 (CTL: 6.8±1.6; CIDR: 2.5±1.6), 0 (CTL: 5.6±1.0; CIDR: 3.5±1.0), and 5 (CTL: 4.3±0.9; CIDR: 2.4±0.9), and OTR-mRNA at Days -2 (CTL: 14.3±1.2; CIDR: 2.5±1.2), 0 (CTL: 14.7±1.4; CIDR: 7.7±1.4), and 5 (CTL: 6.9±1.2; CIDR: 3.8±1.2). We conclude that in anestrous cows: 1) PR-mRNA, ER-mRNA, and OTR-mRNA were expressed in high concentrations, similar to those observed during the proestrus/estrus, and did not change as the dominant follicle developed; 2) treatment with P4 for 6 d caused a decrease in ER-mRNA and OTR-mRNA with no changes PR-mRNA. These responses may be related to the mechanisms underlying and preventing premature luteolysis by PR treatment in anestrous cattle.

Key Words: endometrium, receptors, premature luteolysis


Ovsynch is most effective when initiated on d 6 or 7 of the estrous cycle. Thus, it is imperative that a pre-Ovsynch strategy be developed to allow for the greatest percent of cows possible to be at this stage of the estrous cycle when Ovsynch is initiated. We hypothesized that inserting a CIDR following PGF2α would create a persistent follicle that could be time ovulated to control the onset of a new estrous cycle and thus control of the start of Ovsynch. The objectives of these studies were to determine: 1) if treatment with a 1.38 g CIDR insert would result in the creation of a persistent follicle with ovulatory capacity and 2) if pre-Ovsynch strategies that utilized the development of a persistent follicle would increase conception rates following Ovsynch. Lactating Holstein dairy cows were assigned randomly to control and treated groups in two studies. In Study 1 (n=496) and 2 (n=435), controls were treated with two injections of PGF2α 14 d apart and 11 d prior to the 1st GnRH of Ovsynch (14/11). Cows in the treated groups received two injections of 25 mg PGF2α 14 d apart with a CIDR inserted either 7 (P7;Study 1) or 5 (P5;Study 2) d respectively, following the 2nd PGF2α. Treated cows in both studies received 100 μg GnRH 7d following CIDR insertion then received the 1st injection of GnRH of Ovsynch 6 d later. All groups completed Ovsynch (G7d-P56h-G16h AI). In Study 1, 97% of treated cows ovulated a follicle (20.37±0.3 mm) at the GnRH time of CIDR removal. Size of ovulatory follicle was greater (17.4mm±0.4 vs 14.5±0.3; P=0.001) at 1st GnRH of Ovsynch but similar at final GnRH of Ovsynch (16.9mm±0.2 vs 16.5±0.2; P=0.09) in 14/11 vs. P7. Percent of cows with corpora lutea on d of 1st GnRH of Ovsynch was greater in P7 vs. 14/11, 98 vs. 89% (P=0.001). Conception rates were similar (41 vs. 38%; P=0.31), in 14/11 vs. P7. In Study 2, conception rates were similar between treatments (39 vs. 36%; P=0.24). In summary, pre-Ovsynch strategies that induced a persistent follicle to control the start of an estrous cycle did not positively impact conception rates in lactating dairy cows following Ovsynch.

Key Words: persistent follicle, Ovsynch, pre-synchrony

W191 Embryo transfer following treatment of cystic ovaries in cattle. C. E. Ferguson*, F. M. LeMieux1, D. J. Kesler2, and R. A. Godke3. 1McNeese State University, Lake Charles, LA, 2University of Illinois, Urbana, 3Louisiana State University, Baton Rouge.

Cystic ovaries occur at a rate of 6 to 19% in dairy and 0-10% in beef cattle. Although this incidence may represent a small part of a herd, it
W193 Immediate and carryover effects of Gram-negative or Gram-positive toxin-induced mastitis on follicular functions in cows. Y. Lavon¹, G. Leitner2, R. Meidan3, U. Mouldem1, E. Klipper1, and D. Wolfenson1, ¹The Hebrew University, Rehovot, Israel, ²The Veterinary Institute, Bet-Dagan, Israel, ³Agricultural Research Org, Bet-Dagan, Israel.

This study compared immediate and carryover effects of clinical mastitis induced by Gram (-) or Gram (+) toxin on follicular functions. Uninfected cyclic lactating Holstein cows were synchronized and monitored by ultrasound. On d 5.5 of the cycle, cows were treated with PGF₂α and 36 h later, a dose of Gram (-) (LPS, n=8) or Gram (+) peptidoglycan (PTG, n=10) toxin or saline (n=9) was injected into the udder. Seven h later, follicular fluids and granulosa cells were aspirated from the preovulatory follicles and GnRH injected. This (cycle 1; immediate effect) was repeated 3 times (excluding injection of toxins) to induce three 7-d cycles (cycles 2, 3 and 4; carryover effect). Gene expression and steroids were determined by RT-PCR and RIA. Data were analyzed by ANOVA, means ±SE presented. LPS and PTG transiently increased body temperatures, TNFα concentrations, and somatic cells count. Follicles size was similar in all groups. PTG decreased the number of follicles developed (P<0.05). Follicular estradiol (949±77 ng/ml) and androstenedione in control cows remained stable during the 4 cycles. LPS reduced in 1/3 of the treated cows in cycle 1, the level of estradiol (149±18 ng/ml) and androstenedione, and mRNA for LHr reached about 30% of controls (P<0.05; immediate effect). Later, the concentrations in all LPS cows were restored to control levels (cycles 2, 3, 4). In contrast, 30 to 50% of PTG cows exhibited low follicular estradiol (123±100 ng/ml) and androstenedione level, low circulating estradiol (2.2 vs. 6.2 pg/ml in controls), and low mRNA for LHr; all lasted 4 cycles (immediate and carryover effects; P<0.05). LPS-induced mastitis caused an immediate/short-term (typical for G- clinical events) impairment of follicular functioning, whereas the G+, PTG toxin exhibited both immediate and carryover disruptive effects on follicular theca and granulosa cells of the preovulatory follicle.

Key Words: mastitis, follicle, estradiol


One hundred and forty-seven first lactation cows were sampled to determine if plasma progesterone differed between breeds and affected subsequent reproductive performance. Thirty-five cows were Holstein-Jerseys (HJ) crosses, 42 were Jersey-Holsteins (JH) crosses, 45 were Holsteins (HH), and 25 were Jerseys (JJ). Blood samples were collected weekly postpartum for a 10 wk period. Statistical models (MIXED) evaluated breed, profile of progesterone, season (cool – November to May; hot – June to October), and breed by season interaction on days open, the slope of change in progesterone plasma prior to 30 d postpartum and number of services per conception (S/C). Days open were significantly affected by profile and breed. The days open for HH were longest (152.6 ± 4.5 d), not different for HJ (132.4 ± 4.5 d) and JH (128.2 ± 4.1 d) and, and JJ (140.5 ± 5.2 d) was different from all except HJ. Cows with a short profile had 33 to 60 fewer days open than the other groups. There was a breed by season interaction for days open (P < 0.05). Slope was affected by breed, profile, and breed by season interaction (P < 0.05). The HH group had a slope of 0.196 ± 0.023, HJ had a 0.160 ± 0.022 slope, JH had a 0.157 ± 0.020 slope, whereas the JJ slope was 0.229 ± 0.026. The JJ slope was steeper than the HJ and

Key Words: progesterone, response, lactation

W192 GnRH affects emergence of a new follicular wave in cows with cystic ovaries. E. Dirandehe, H. Kohram*, T. Saberifar, and A. Zare Shahneh, University of Tehran, Iran.

The objective of this study was to evaluate the effect of GnRH on follicular wave emergence in cows with cystic ovaries. The estrous cycles of 15 cows were synchronized with two intra-muscular injections of prostaglandin F2α given 11 days apart. The cows were randomly assigned to one of three treatments. Control cows (n=5) received no injection, whereas GnRH cows (n=5) received a GnRH injection on day six of the estrous cycle (day of injection=day 0). In cows with cystic ovaries (n=5), diagnosis of a cyst was based on the palpation per rectum of an abnormally large (≥20 mm) follicle and no corpus luteum then these cows received a GnRH injection. The ovaries scanned by daily ultrasonography from four days before (day -4) the day of GnRH treatment (day 0) until four days after GnRH treatment (d 4). Daily ultrasonography confirmed the presence of a cyst for at least 10 days in the ovaries of the cystic cows then these cows received a GnRH injection (day 0). All follicles were classified as small (4-6mm), or large (≥7 mm) follicles. Data were analyzed using the GLM procedure of SAS. Results showed In controls, the number of small follicles tended (P<0.05) until day two in the GnRH and cystic cows. An increase in the number of small follicles two days following GnRH injection showed that GnRH treatment could promote the emergence of a new follicular wave in cystic cattle similar to the normal cyclic cattle.

Key Words: GnRH, follicular wave, cystic ovaries
H slopes. Profile of progesterone affected slope. Cows with a delayed profile had a slope of 0.0024 ± 0.014, suggesting that there was no increase in progesterone during the early postpartum period. The breed by season interaction showed a greater slope for JI in summer and a lower slope in winter than other breed combinations. Number of S/C was affected by breed, season and the breed by season interaction (P < 0.05). Holsteins had 2.3 ± 1 S/C which was higher than JH (2.0 ± 1.1), JI (1.9 ± 0.1) and JJ (2.1 ± 0.1). S/C were higher in the cool season (2.3 ± 0.05) compared to the hot season (1.9 ± 0.06). Cool season had higher S/C in all breeds except JI. Breed differences were evident in measures of reproductive efficiency.

Key Words: reproduction, progesterone, crossbreds

W195 Pregnancy success and luteal function of lactating Holstein cows after hCG on day 5 after insemination. E. Urzua1, C. G. Gutiérrez2, A. Garza2, C. Corona2, G. Mapes3, and J. Hernandez-Ceron4,*
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Low serum progesterone concentration after breeding is being associated with pregnancy losses. History of estrous gonadotropin (hCG) treatment on day 5 after estrus induces ovulation of the first-wave dominant follicle and formation of an accessory corpus luteum. Here, we tested whether an injection of hCG on day 5 after artificial insemination (AI) increases conception rate and plasma progesterone in lactating dairy cows. Nine hundred eighty-nine multiparous Holstein cows were used. Cows were inseminated at fixed timed after an Ov-synch protocol or after natural estrus. Five days AI, cows were randomly assigned to either a treated (hCG; n =482) or a control (n = 507) group; animals in the treated group were given 3,500 UI im of hCG. Pregnancy diagnoses were performed on day 30 and 60 post-AI by ultrasonography and rectal palpation respectively. Blood samples were taken on days 5, 11 and 15 from 100 (41.1 > 31.6%) after AI. Pregnancy losses were similar between groups. The number of previous inseminations affected the response and 60 (41.1 > 31.6%) after AI. Pregnancy losses were similar between groups. The number of previous inseminations affected the response to the hCG treatment. First insemination cows improved conception rate when treated with hCG both on day 30 (55.9% > 41.0%) and 60 (45.2% > 33.1%). However, this effect was not observed in cows of two or more inseminations (P >0.20). Progesterone concentrations were higher (P <0.05) in cows treated with hCG than in control cows. It is concluded that hCG on day 5 after AI increases conception rate and plasma progesterone in first service lactating dairy cows.

Key Words: hCG, fertility, dairy cows

W196 Plasma LH concentrations and CL function in Holstein cows given porcine LH, GnRH, or estradiol benzoate. M. G. Colazo1, T. O. Ree2, A. G. A. Lamont3, J. P. Kastelic4, R. J. Mapleton5, and D. J. Ambrose1,3,*
1Alberta Agriculture and Rural Development, Edmonton, AB, Canada, 2Lakeland College, Vermilion, AB, Canada, 3University of Alberta, Edmonton, AB, Canada, 4Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, 5University of Saskatchewan, Saskatoon, SK, Canada.

We compared LH concentrations and CL function in nonlactating Holstein cows following treatment with estradiol benzoate (EB), porcine LH (pLH), GnRH, or no treatment during proestrus. Cows (n=28) were given a 1.9 g progesterone (P4) intravaginal device (CIDR) for 5 d and 500 μg cloprostenol (PG) at CIDR removal. On d 6 after estrus, cows were treated with 2 PG 12 h apart and allocated randomly to 1 of 5 groups to receive im: 1 mg EB, 20 h after first PG, 12.5 or 25 mg pLH or 100 μg GnRH 36 h after PG, or no treatment (Control). Ovulation (d 0), follicles and CL diameter were assessed by transrectal ultrasonography. Plasma concentrations of LH were determined for 8 h (except in Control cows) from treatment, and plasma P4 was determined once daily from d 0 to 12. Mean (±SEM) plasma LH (ng/mL) was greater (P <0.05) in cows treated with 25 mg pLH (5.0±0.5) than those in those given 12.5 mg pLH (2.7±0.5); it was intermediate in those given GnRH (3.8±0.5) or EB (4.1±0.5). Preovulatory follicle diameter (POFD; mm) was largest (P <0.05) in Control cows (15.6±0.8, 13.7±1.0, 15.2±0.8, 14.3±1.0 and 18.4±0.9, mm, for EB, 12.5 pLH, 25 pLH, GnRH and Control, respectively). On d 12, CL diameter (mm) was largest in Control (25.1±1.2) cows, intermediate in pLH (22.8±1.3 and 22.7±0.9 for 12.5 and 25 mg) and EB (22.5±1.0) cows, and smallest in GnRH cows (20.4±1.2). However, CL area did not differ among treatments. The POFD was correlated to CL diameter (P <0.01, r=0.6) but not to CL area (P =0.15, r=0.33). Nothing was significantly correlated with plasma P4. Mean plasma P4 in cows given either 25 mg pLH or GnRH did not differ from that in Control cows, but 12.5 mg pLH or EB resulted in lower plasma P4 after ovulation. In summary, cows given 25 mg pLH had greater LH concentrations; Control cows had larger POFD and CL diameter, but P4 concentrations did not differ from cows given 25 mg pLH or GnRH.

Key Words: LH, CL function, progesterone

W197 Prostaglandin (PG) E1 or E2 (PGE1, PGE2) luteal implants prevent luteolysis in cows. C. W. Weems1, Y. S. Weems2, R. C. Vann3, S. P. Ford1, D. A. Neuendorff4, A. W. Lewis5, T. A. Welsh6, T. M. Nett6, P. J. Bridges1, and R. D. Randel2, 1University of Hawaii, Honolulu, 2Mississippi State University, Raymond, 3University of Wyoming, Laramie, 4Texas AgriLife Res., Overton, 5Texas A&M University, College Station, 6Colorado State University, Fort Collins, 7University of Kentucky, Lexington.

Loss of progesterone secretion at the end of the estrous cycle is via uterine PGF2α secretion; however, uterine PGF2α is not decreased during early pregnancy in ewes to prevent luteolysis as the embryo imparts resistance to PGF2α-induced luteolysis via the two-fold increase in PGF2α during early pregnancy. Infusion of PGE1 or PGE2 every 4 hr intrauterine prevents spontaneous or an estradiol-17β or an IUD-induced premature luteolysis in ewes (C. W. Weems, Y. S. Weems, R. D. Randel, The Vet. J. 171:206-228, 2006). Estradiol-17β or PGE2 infused every 8 hr did not prevent luteolysis, but estradiol-17β or PGE2 inhibited luteolysis in heifers (L. P. Reynolds, D. Robertson, S. P. Ford, J. Reprod. Fertil. 69:703–709, 1983). The objective of this experiment was to determine whether intraluteal implants of PGE1 or PGE2 prevent luteolysis in Brahman or Angus cows. Cows received intraluteal implants of Dow Corning 360 elastomer containing Vehicle, PGE1, or PGE2 from D-13-D-19 post-estrus via a flank laparotomy. Corpora lutea (CL) were recovered on D-19 and weighed. D-19 CL weights of Vehicle-implanted cows were lower (P <0.05) than those treated with PGE1 or PGE2 from D-13-D-19. D-13 Angus CL weights were higher (P <0.05) than Vehicle-treated Angus cows on D-19, but weights of D-13 CL were similar (P >0.05) to D-13-D-19 PGE1 or PGE2-treated...
Angus CL. It is concluded that PGE1 or PGE2 alone prevents luteolysis regardless of breed.

**Key Words:** prostaglandins, cows, corpus luteum

**W198 The effect of a shortened dry period on follicular dynamic in early lactation Holstein cows.** S. Safa1, A. Heravi Mousavi*1, M. Danesh Mesgaran1, and A. Soleimani1,2, 1Department of Animal Science, Ferdowsi University of Mashhad, Iran, 2Islamic Azad University-Kashmar Branch, Iran.

The study was designed to test the effect of dry period length on follicular dynamics in early lactation cows. Cows were randomly assigned in 1 of 2 treatments: 1) traditional 60 d dry period (n=14) and 2) a shortened 20 d dry period (n=13). Holstein cows were blocked in pairs based on their previous 305 d milk, parity and expected calving dates. All cows were fed by routine ration of farm (total mixed diet) twice a day at 0800 and 1400 h and had at all time free access to water. To monitor follicular parameters, ultrasound measurements of follicular activity were made on alternate days from 10-35 days postpartum (PP) to determine the characteristics and fate of the 1st follicular wave, using a 7.5 MHz rectal transducer. Dominant follicle development was characterized by follicular mapping of recorded ultrasound images. A dominant follicle was defined as a follicle that was >10 mm in diameter in the absence of other large (>9 mm) growing follicles. The data were analyzed using the GLM procedure of SAS for a completely randomized design. The number of follicles (5 to 10 mm) present on day 10 (p=0.12; 4.14±0.44 and 3.08±0.48, respectively) and 14 PP (p=0.11; 5.21±0.47 and 4.08±0.51, respectively), number of days until detection of a follicle ≥ 10 mm in diameter (p=0.24; 11.43±0.82 and 12.85±0.85 d, respectively), diameter of the first dominant follicle on d 14 PP (p=0.25; 13.57±0.73 and 12.29±0.79 mm, respectively), maximum diameter of the first dominant follicle (p=0.38; 15.32±0.66 and 14.46±0.69 mm, respectively), and days to first ovulation (p=0.62; 29.31±2.89 and 27.18±3.14 d, respectively) were all similar among the groups. Results of this study showed that the reduced dry period length had no apparent effect on follicular parameters and days postpartum to first ovulation.

**Key Words:** dairy cows, dry period, follicular dynamic

**W199 Characteristic of the largest follicle of the waves emerged after treatment with GnRH during estrous cycle of Iranian Holstein cows.** E. Dirandeh and H. Kohram*, University of Tehran, Karaj, Tehran, Iran.

This study was done to consider the effect of GnRH on largest follicle of the waves in Iranian Holstein cows. The estrous cycles of 10 cows were synchronized with 2 im injections of Prostaglandin F₂₀ given 11 d apart. The cows were randomly assigned to 1 of 2 groups. In control group of animals no injection of GnRH was performed. GnRH administered on Day 6 of the estrous cycle (estrus = Day 0). The diameter of the largest follicle was also recorded. Ovarian follicular development was monitored daily by transrectal ultrasonography with a real-time linear scanner using ultrasound diagnostic system. Ultrasonography was performed once daily from the day that second PGF₂₀ inject until the day of next estrus. The follicular wave during which growth phase the treatment was administered was designated as wave 1. Any follicular wave induced by treatment was designated as wave A. The follicular wave emerging after the induced wave (GnRH treated cows) or the follicular wave emerging after 7–8 days after the emergence of wave 1 (control cows) was designated as wave 2. Comparisons of waves 1, A, and 2 detected in GnRH-treated cows were made by one-way RM ANOVA. Results are reported as least square means ± SEM. There was no significant effect (P>0.05) of wave, group, or a wave*group interaction for the parameter of largest follicles of waves 1 in both GnRH-treated and control cows. There was no significant effect (P>0.05) of wave for the parameters above for waves 1 in GnRH-treated cows (Table 1). The ovulatory follicle in control group grew larger (14.0 ± 1.8 vs. 12.6 ± 1.1 mm, P<0.05), and maintained for a longer period of time (P<0.05) than in GnRH-treated cows (9.50 ± 0.6 vs. 5.8 ± 0.4). The results suggested that administration of GnRH on day 6 of estrous cycle induce ovulation and the ovulatory follicle in GnRH-treated cows was older than that in control cows.

**Key Words:** ultrasonography, GnRH, follicle

**W200 Subclinical mastitis effects on steroid concentrations and gene expression in theca cells of preovulatory follicles in cows.** Y. Lavon*1, G. Leitner2, R. Meidan1, E. Klipper1, and D. Wolfenson1, 1The Hebrew University, Rehovot, Israel, 2The Veterinary Institute, Bet-Dagan, Israel.

We have recently observed that subclinical mastitis (SCM) lowered steroid concentrations and gene expression in granulosa cells of preovulatory follicles in about 1/3 of the infected cows. To complement these studies, we examined the effect of SCM on follicular steroid levels and gene expression in the other follicular steroidogenic cell – the theca cells. Cyclic lactating Holstein cows (n=20) were diagnosed for mastitis by somatic cell counts and bacteriological examinations. On day 6 of the estrous cycle, synchronized cows were treated with PGF₂₀ and 42 h later, the cows were slaughtered and their ovaries were collected. Follicular fluids and theca cells were obtained from preovulatory follicles. Gene expression and steroids were determined by RT-PCR and RIA. Data were analyzed by ANOVA and means ± SE presented. One third of SCM cows (n=4) exhibited low estradiol concentrations in the follicular fluid, whereas the remaining 2/3 (n=8) cows, and uninfected cows (n=8), exhibited normal concentrations (269±71 vs. 815±127 and 870±62 ng/ml, respectively, P<0.01). The SCM cows with low estradiol also exhibited low follicular androstenedione concentrations (32±12 vs. 109±31 and 130±30, respectively, P<0.05), and estradiol to progesterone ratios (6.4±1.3 vs. 14.8±2.4 and 13.3±1.4, respectively, P<0.05). Accordingly, mRNA expression in theca cells, for LH receptor, cytochrome P450 side chain cleavage, and cytochrome P450 17α-hydroxylase were lower in SCM cows with low estradiol than in SCM cows with normal estradiol levels, and uninfected cows (P<0.05). However, 3βHSD and StAR mRNAs were not affected by SCM. Results show that low gene expression in theca cells is associated with low preovulatory steroid concentrations. The resulting low estradiol level in 1/3 of the SCM cows could be associated with delayed preovulatory LH surge and ovulation, as documented in our earlier studies. These mechanisms may explain mastitis-induced low fertility in dairy cows.

**Key Words:** mastitis, estradiol, theca cells

**W201 Effect of dry period lengths on complete blood count in early lactating Holstein cows.** A. Soleimani*1,2, A. Heravi Mousavi1, M. Danesh Mesgaran1, A. Golian1, and S. Safa1, 1Department of Animal Science, Ferdowsi University of Mashhad, Iran, 2Islamic Azad University of Mashhad, Iran.

The effect of a shortened dry period on complete blood count in early lactating Holstein cows. A. Soleimani1,2, A. Heravi Mousavi1, M. Danesh Mesgaran1, A. Golian1, and S. Safa1, 1Department of Animal Science, Ferdowsi University of Mashhad, Iran, 2Islamic Azad University of Mashhad, Iran.

The study was designed to consider the effect of dry period length on estrous cycle induce ovulation and the ovulatory follicle in GnRH-treated cows was older than that in control cows.
University-Kashmar Branch, Iran.

The study was designed to test the effect of reducing dry period length on complete blood count and differential white blood cell count in early lactating cows. Cows were randomly assigned in 1 of 2 treatments: 1) traditional 60 d dry period (n=14) and 2) a shortened 35 d dry period (n=15). Holstein cows were blocked in pairs based on their previous 305 d milk, parity and expected calving dates. All cows were fed by routine ration of farm (total mixed diet). Using vacutainer tubes, blood samples were collected weekly from -7 to 50 day relative to calving via venipuncture of coccygeal vessels before the morning feeding to monitor complete blood count (CBC). The blood samples were kept in room temperature until analyzing for CBC by a hematology analyzer. The data were analyzed using the Mixed procedure of SAS for a completely randomized design with repeated measures. The white blood cells (P=0.64; 12277 ± 1798 and 13468 ± 1733 /μl, respectively), red blood cells (P=0.58; 5283037 ± 104596 and 5363237 ± 100280 /μl, respectively), platelet (P=0.13; 250271 ± 16913 and 286354 ± 16122 /μl, respectively), hemoglobin (P=0.91; 8.09± 0.15 and 8.11± 0.14 g/dl, respectively), hematocrit (P=0.70; 27.97 ± 0.5 and 28.24 ± 0.5%, respectively), and also number of neutrophils (P=0.52; 3445.3 ± 560 and 3622.6 ± 188, respectively), lymphocytes (P=0.59; 7703.9 ± 1609 and 8920.2 ± 1547, respectively), monocytes (P=0.82; 685.6 ± 100 and 716.5 ± 96.4, respectively), and also number of eosinophils (P=0.52; 173.8 ± 23 and 152.8 ± 22, respectively) were all similar among the groups. Red blood cell, hemoglobin and hematocrit were decreased and platelet were increased over the time (P<0.05). Result of this study showed that the reduced dry period length had no effect on cell blood count and differential white blood cell count.

Key Words: dairy cow, dry period, complete blood count


The objective was to compare sperm motility during storage for semen from boars fed diets supplemented with organic or inorganic sources of selenium. At weaning, boars were assigned to one of three treatments: I. basal diets with no supplemental selenium (controls), II. basal diets supplemented with 0.3 ppm organic selenium (Sel-Plex; Alltech, Inc., Nicholasville, KY), and III. basal diets supplemented with 0.3 ppm sodium selenite (n = 10 boars/treatment). At sexual maturity, ejaculates were collected, processed and stored at 18°C in Beltsville Thawing Solution and Androhep-Lite (Minitube of America, Inc., Verona, WI) (3 x 10^9 sperm/85 mL semen and extender) and sperm motility assessed daily for 10 d using a computer-assisted sperm analysis system (Hamilton Thorne Research, Beverly, MA). Data were analyzed using repeated measures ANOVA and individual ejaculate was the experimental unit. There were no effects of day x extender or day x treatment x extender (P > 0.1), thus data were pooled between extenders. Effects of treatment x day were detected for percent motile spermatozoa (P < 0.01), path velocity (smoothed cell path; VAP) (P=0.06), amplitude of lateral head displacement corresponding to the mean width of the head oscillation as the sperm swim (ALH; P = 0.02), frequency with which the sperm track crossed the sperm path (BCF; P = 0.04), straightness (P = 0.01) and percent static spermatozoa (P = 0.009). In general, values were indicative of an enhanced ability of sperm cells from Sel-Plex-fed boars to maintain good motion characteristics during storage. For example, VAP (μm/s) was greater (P < 0.03; SE = 2.5) for sperm from boars fed Sel-Plex (77.5) after 3 d of storage compared to control (66.5) or selenite (65.5) boars. After 10 d of storage, VAP was greater (P < 0.01; SE = 2.5) for sperm from boars fed Sel-Plex (77.2) compared to selenite-fed boars (57.2). Sperm VAP from control boars (65.2) was not different (P > 0.16) from either the Sel-Plex- or the selenite-fed boars. Results indicate that dietary organic selenium supplementation may help ameliorate the negative effects of semen storage on sperm motility.

Key Words: boar, selenium, semen

W203 Effect of melatonin on in vitro manipulated rat oocytes and embryos. S. Nandi1,2, V. Girish Kumar2, and F. C . Gwazdauskas3, 1National Institute of Animal Nutrition and Physiology, Bangalore, India, 2Karnataka Veterinary Animal and Fishery Sciences University, Bangalore, India, 3Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg.

Melatonin, N-acetyl-5 methoxytryptamine, acts as a powerful agent against reactive oxygen species (ROS) and a potent apoptosis blocker. The aim of the present study was to investigate the effect of different concentrations of melatonin on the development of rat oocytes and embryos in vitro. In experiment 1, control (38.5°C) and heat stressed (39°C during maturation) and chemically stimulated (glycolytic stimulator dinitrophenoil, DNP: 10 μM and glycolytic inhibitor hexametaphosphate, HMP: 100 μM) oocytes were matured in vitro in 9 different concentrations (0, 1, 5, 10, 25, 50, 100, 500 and 1,000 μM) of melatonin. The maturation rates were recorded after 24 hrs of culture. The oocytes were fertilized in vitro and the resultant embryos were further cultured for the production of morulae/blastocysts. Supplementation of melatonin at 10 μM concentration in the oocyte culture medium resulted in a significantly higher (P < 0.05) maturation rate (control: 90.3%, heat stressed: 84%; DNP: 86% and HMP: 78%) and morula/blastocyst yield (control: 25.3%, heat stressed: 16%; DNP: 20% and HMP: 18%) compared to control (without melatonin). Based on result of experiment 1, in vivo produced embryos were divided into 2 groups: control and heat stressed (39°C during first 2 d of culture). The heat stressed embryos were cultured in medium supplemented with 10 μM melatonin. Supplementation of melatonin in the embryo culture medium resulted in comparable morula/blastocyst yield in control and heat stressed embryo groups. Melatonin also decreased the death (assessed by trypan blue staining) of oocytes and surrounding cumulus cells and also decreased the developmental block, asynchronous development and degeneration of embryos from 5 to 100 μM concentrations. However, melatonin decreased the oocyte development at the 1,000 μM concentration. In conclusion, enriching the culture medium with 10 μM melatonin improved the development of in vitro manipulated rat oocytes.

Key Words: melatonin, oocyte, rat

W204 17β-estradiol and spontaneous myometrial contractions in ovariec-tomized rats. O. Yildiz-Gulay*, A. Bulbul2, M. S. Gulay1, K. Altbunbas3, and O. Ozden-Akayya3, 1Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Physiology, Burdur, Turkey, 2Afyonkarahisars Kocatepe University, Faculty of Veterinary Medicine, Department of Physiology, Afyonkarahisars, Turkey, 3Afyonkarahisars Kocatepe University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Afyonkarahisars, Turkey.

The aim of this study was to evaluate the effect of injecting different doses and time intervals of 17β-estradiol on spontaneous myometrial contractions of ovariec-tomized rats. Three to 6 months old 71 female
Sprague Dawley rats, weighing 270±20 g, were used in the current study. The ovarietomized rats were randomly assigned to one control (Ov) and three experimental (17β-estradiol injected) groups of 18 rats each. Rats in the Ov group received daily sesame oil (0.2 ml, IM), whereas each rat in the three experimental groups was treated with daily IM injections of 25, 50 and 100 µg estradiol in sesame oil, respectively. Each group was further divided in 3 subgroups: 6 rats in each group were sacrificed by cervical dislocation at 18, 90 and 162 hr. In order to determine endogenous nitric oxide (NO) activity, L-arginine solution was used. Sodium nitroprusside was used for evaluation of the exogenous NO pathway. In addition, L-NNA (nitro-L-arginine) treatment was applied in order to determine the effect of endogenous NO at receptor level. Immunohistochemical evaluation was performed to determine cGMP-PK1 expression from the uterus samples. In the current study, 17ß-estradiol treatments increased spontaneous myometrial contraction in dose and time dependent manner. Treatments also inhibited L-Arginine-NOS-NO-cGMP-PK1 pathway. However, our results indicated that 17ß-estradiol did not show its effect through cGMP-PK1.

**Key Words:** 17ß-estradiol, nitric oxide, myometrium


Real-time quantitative PCR (qPCR) is the technique of choice for detection and quantification of mRNA expression in cells or tissues. Suitable reference genes are used for normalization of mRNA across different samples for an exact comparison of mRNA transcription level. The purpose of the present study was to evaluate the expression stability of six reference genes B2M, ACTB, GAPDH, SDHA, HPRT1 and ARBP using qPCR in mouse liver after immunity stimulation. Twenty four Kunming white mice were divided into four groups. They were injected with different vaccines as following isotonic Na chloride, Lipase + isotonic Na chloride, Immunostimulating Complexes (ISCOM), Lipase + ISCOM at 4 days after parturition. It was found that the specific antibody titer in serum increased significantly (P<0.05) at 8 days after parturition. The stability of these reference genes was investigated using geNorm application. The range of expression stability in the analyzed genes was (from the most stable to the least stable): ACTB/GAPDH, SDHA, ARBP, HPRT1 and B2M. Results from the present study suggest that ACTB and GAPDH genes were relatively more stable than other tested genes. Based on our study we recommend that ACTB and GAPDH are better suited reference genes for normalization of mRNA across different samples while studying genes expression in mouse liver after specific treatments.

**Key Words:** quantitative real-time PCR, immunity stimulation, geNorm program

W206 Detection of alternative splicing form of PRL mRNA in the chicken anterior pituitary gland. N. Kansaku*1, T. Sasanami 2, T. Ohkubo3, G. Hiyama1,4, and D. Zadworny4, 1Hokkaido University, Sapporo, Japan, 2Shizuoka University, Shizuoka, Japan, 3Kagawa University, Miki-cho, Japan, 4McGill University, Montreal, QC, Canada.

Prolactin (PRL) and Growth hormone (GH) belong to the same hormone gene family and are mainly produced in the anterior pituitary gland. The expression and presence of alternative splicing forms of GH mRNA were reported in several mammalian species including humans, where an exon skip splicing form of GH mRNA was detected using RT-PCR. However, it is unknown whether similar alternative splicing forms of PRL mRNA exist in mammals or birds. Accordingly, this study aimed to examine the possibility of presence of alternative splicing forms of PRL in the anterior pituitary gland of chickens. Total RNA was extracted from anterior pituitary gland and One micro gram of RNA was reverse transcribed using random hexamers. One-tenth of the reaction mixes were used for subsequent thermocycling. Amplification was conducted for 35 cycles using antisense primers which contained 2 bases of skipped exon sequence at the 3’-end (E3S, E4S, E3-4S) and sense primer (complimentary to 5’ untranslated region) and polymerase with and without exonuclease activity individually. After PCR, products were separated by electrophoresis and the size of products was identified. Polymerase with exonuclease activity produced the expected size of amplicon for normal PRL cDNA, whereas exon3, exon4, exon3-4 skipped PCR products were amplified from the PCR using non-exonuclease activity polymerase. Although the relative amounts of normal PRL mRNA to the exon skipping forms requires further investigation, the results of this study may indicate the presence of small molecular weight isoforms of PRL in the anterior pituitary gland. In conclusion, expression of alternative splicing forms of PRL mRNA may result in truncated proteins which may provide novel roles and/or functions to PRL. Since exon 3 of chicken PRL gene contains the region encoding the putative glycosylation site and Cys which play important roles in formation of disulphide bond structure and globular structure, PRL isoforms lacking exon 3 or exon 4 coding region may have different physiological activity or function in the chicken.

**Key Words:** prolactin, splicing, exon

W207 Culture of chicken germline stem cells. J. N. Petitte*, J. Angerman-Stewart, R. Wysocki, and P. E. Mozdziaik, Department of Poultry Science, North Carolina State University, Raleigh.

Germline stem cells are those cells that give rise to haploid gametes. In the unincubated chick embryo, 25 to 35 germline stem cells or primordial germ cells (PGCs) can be detected before gastrulation. Upon gastrulation, PGCs are found in the anterior germinal crescent. At 55 h of incubation, the PGCs enter the blood and circulate throughout the embryo. From 55 to 72 h of incubation, the PGCs leave the circulation and migrate to the gonadal ridge. In previous work, when FACS-purified PGCs from blood or the gonadal ridge are injected into the unincubated embryo, they retain their ability to migrate to the gonadal ridge, and they can develop into functional gametes in the adult bird. Further studies have been hampered by the low number of PGCs/embryo. The purpose of this study was to establish cultures of chicken germline stem cells from PGCs. Individual embryonic blood samples were obtained from stage 15 embryos, and they were seeded onto inactivated STO feeder layers. PGCs were cultured in KO-DMEM, 7.5% FBS, 2.5% chick serum, 20% BRL-conditioned media, essential and nonessential amino acids, and nucleosides. In some cases, hSCF, mLIF, and bFGF were added. Six lines of germ line stem cells were established from White Leghorn and Barred Plymouth Rock embryos. The ability of each line to migrate to the gonadal ridge was tested by labeling the cells with PKH-26 before injection into the subgerminal cavity of the unincubated embryo or into the vasculature of a stage 17 to 18 embryo. After 3.5 days of incubation the embryonic gonad was removed and the presence of PKH-labeled germline stem cells was examined. Germ line stem cells were also injected into embryos, incubated to hatch, and raised to sexual maturity. No somatic cell chimeraism was observed. Roosters were test
mated to determine germ line transmission. The frequency of germ line chimeras ranged from 13 to 100%. Three lines always gave rise to germ line chimeras. The frequency of germ line transmission varied between lines and ranged from 0 to 41%. The results of this study confirm that cell lines of germ line stem cells can be established from chicken PGCs and can only give rise to fully functional gametes.

Key Words: stem cells, avian, primordial germ cells

Production, Management and the Environment: General

W208 Biodegradation of genetically modified seeds and plant tissues during composting with manure. T. Reuter*, 1, T. W. Alexander1, K. Stanford2, and T. A. McAllister1, 1Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, 2Alberta Agriculture and Rural Development, Lethbridge, AB, Canada.

The increasing global market volume of genetically modified (GM) crops amplifies the potential for environmental impact and/or non-conformance with legislation. Methods proposed for disposal of crop residues should be assessed to prevent unintended distribution of GM materials. Composting is an inexpensive and location-independent option. To determine the effectiveness of composting for disposal of GM plant material, seed viability tests and molecular techniques were used to assess degradation during the composting process. Replicate samples of corn seeds, alfalfa leaves, and GM canola seeds, meal and pellets in sealed nylon bags were implanted into duplicate feedlot manure compost piles and collected periodically during 230 days of composting. The compost piles (25 m × 5 m × 2.4 m; L × W × H) each had initial weight of approximately 85,000 kg. Canola and corn seeds lost germination capacity after 7 days of composting at temperatures exceeding 50°C. Using PCR, plant-specific DNA fragments (882 bp) could be detected in meal and pellets, and in manure samples from random sites in the pile for up to 56 days, whereas those fragments in seeds and leaves were detectable for up to 230 days. Real-time PCR revealed small (<200 bp) plant- and GM-specific fragments in decreasing quantities up to 230 days in all samples. Southern blotting assessment of genomic DNA extracted from canola seeds verified differences in the persistence of intact Rubisco-, 16S- and GM-specific genes during composting. Composting GM and non-GM plant materials with manure resulted in inactivation of germination, and efficient, though not complete, DNA degradation. This study indicates that dissemination of viable GM seed or functional transgenes would be highly unlikely following composting of GM plant material.

Key Words: compost, genetically modified, viability

W209 Arrangements of Acacia decurrens, Acacia melanoxylon and Alnus acuminata as silvopasture systems in a high tropic ecosystem. A. Conde*, 1, L. L. Betancourt1, C. J. Jaramillo1, A. Umaña1, D. Barrera1, and D. R. Chamorro2, 1Universidad de La Salle, Bogotá, Colombia, 2Corpoica, Bogotá, Colombia.

The objective of this study was to evaluate dasometric measures during establishment period and initial effects on soil and the availability and quality of grass in silvopasture arrangements: live fences and tree dispersed in pasture in high tropic in Colombia. Three species of trees were planted in a hill pasture with Pennisetum clandestinum near to Sopo, Colombia (73° 57′ O, 4° 54′ N), 2580 meters mean altitude, 14 °C, mean rainfall 693 mm/yr, in two arrangements: A) Single row of two meters spaced trees like live fence and B) Dispersed Trees in pasture 10 x 5 m each line with one specie. Dasometric measures were: average height, basal diameter and diameter at 40 cm height, Three treatment were imposed: 1) Acacia decurrens (Ad) 2) Acacia melanoxylon (Am) and 3) Alnus acuminata (Aa) in a randomized complete block design with 6 replicates. Changes on soil and availability of forage and its quality was measured. Best growth were achievement for Ad and Am compared with Aa (p<0.05). In all dasometric measures were not differences between Ad and Am but Alnus acuminata showed lower means values than acacias (p<0.05). Pastures with trees did not show differences in chemical characteristic in soil compared with pastures without trees. Availability of forage was higher in pastures with trees dispersed 10x5 (720 g of dry matter/m²) compared with pastures without trees (649 g of dry matter/m²). Carbohydrates and protein fractions in grass did not report differences with or without tree into the pastures (p<0.05). Based in dasometric measures Acacia decurrens and Acacia melanoxylon had faster growth and best performance than the native Alnus acuminata in the reported ecosystem. The long term effects of planting trees in nutrient quality of grass (protein a carbohydrate fractions) and soil characteristics may become cumulative and need further research.

Key Words: silvopastoral systems, carbohydrate fractions, protein fractions

W210 Influence of Acacia mangium on soil chemical characteristics in a silvopastoral system in northwestern Venezuela. T. Clavero* and R. Razz, Centro de Transferencia de Tecnología en Pastos y Forrajes, Universidad del Zulia, Maracaibo, Estado Zulia, Venezuela.

Acacia mangium (L.) Willd is an important multipurpose tree of traditional agroforestry system in tropical semi-arid regions. Understanding the changes in soil properties in silvopastoral system is important in regulating the interactions between trees and understory pastures. In this study, the influence of Acacia mangium on soil chemical characteristics under Brachiaria humidicola pastures in northwestern Venezuela was studied. The trees were seven years old at the time of the study. Transects extending from the tree trunk to open grass areas were established, and soil depths (0-15 and 15-30 cm) samples were taken at 25 and 150% of the average canopy radius (4.5 ± 0.30 m) at five sites. A randomized block design was used with five replications. Soil analyses showed no significant differences (p≥ 0.05) in Na, Ca, K and pH under tree canopy compared to open pastures. Higher levels of soil C, N, P, Mg (p<0.05) were found under Acacia mangium canopies as compared to open grass areas. Soil organic carbon content was higher by 38% in silvopastures than in adjacent open pastures. Soil organic carbon and N were maximum in 0-15 cm (0.88% and 150 mg/kg, respectively) and declined with the depth of soil. Total and mineral P contents were nearly uniform across the depths. Net mineralization rates were higher in silvopastoral system due to greater input of soil organic matter associated with higher soil biological activity from decomposition of litter and dead tree-roots. It was concluded that the incorporation of Acacia mangium in Brachiaria humidicola pastures improved soil chemical conditions.

Key Words: Acacia mangium, Brachiaria humidicola, silvopastoral