1905 Use of ECP in a timed insemination program. S. M. Pancarci*[1], C. A. Risco[1], F. L. Lopes[1], F. Moreira[1], E. R. Jordan[2], and W. W. Thatcher[1], [1]*University of Florida, Gainesville, FL,* [2]*Texas A&M University, College Station, TX.*

Experiment 1 evaluated pregnancy rates (PRs) when estradiol cypionate (ECP) replaced the second GnRH injection of a timed artificial insemination (TAI) protocol for lactating dairy cows. Cows were pre-synchronized with two injections of $PGF_{2\alpha}$ (Lutalyse®, Pharmacia Corp., 25 mg; i.m.) given to multiparous cows (n=170) at 40 ± 3 and 54 ± 3 d postpartum (dpp) and to primiparous cows (n=201) at 62 ± 3 and 76 ± 3 dpp. The TAI protocol consisted of an injection of GnRH (Cystorelin®; Merial Ltd., 100 μg, i.m.) at 68 ± 3 and 90 ± 3 dpp for multiparous and primiparous cows, respectively, followed by $PGF_{2\alpha}$ 7 d later. Cows were injected with GnRH (Treatment I: Ovsynch, n=179) 48 h after $PGF_{2\alpha}$ and inseminated 16 h later. In Treatment 2 (ECPsynch; n=192), ECP® (Pharmacia Corp., 1 mg, i.m.) was injected 24 h after $PGF_{2\alpha}$, and cows were inseminated 48 h later. Pregnancy was determined at 46 ± 3 d after TAI. For primiparous and multiparous cows, PRs for Ovsynch were 43.5 ± 6.9 and 30.6 ± 7.2 % compared to 50.7 ± 6.3 and 19.4 ± 6.5 % for ECPsynch. Effects of parity ($P<0.01$) and parity by treatment ($P<0.10$) were detected on PRs. In Experiment 2, onset of estrus and time of ovulation were determined in lactating dairy cows submitted to the ECPsynch protocol as described in Experiment 1. Estrus was detected by HeatWatch® and ovulation time determined by ultrasound at 8-h intervals beginning 18 h after onset of estrus or at 48 h after ECP injection. Frequencies of detected estrus and ovulation were 75.7 % (28/37) and 89.2 % (33/37), respectively. Mean intervals to ovulation were 58.5 ± 4.0 h (n=33) after ECP and 27.1 ± 1.1 h (n=28) after onset of estrus. Estrus occurred at 33.6 ± 4.4 h (n=27) after ECP. Percent cows ovulating at an optimal interval of ≥42 h to ≤70 h after ECP was 75.8 % (n=25/33). Synchronization of ovulation and fertility results indicated that ECP can be utilized to induce ovulation for a timed insemination.

Key Words: Timed artificial insemination, ECP, Ovulation

1906 Determining the effect of gonadotropin releasing hormone to synchronize returns to estrus in dairy heifer. K.S. Rosenkrans*[1], D.K. Hardin[1], M.C. Lucy[1], J.W. Tyler[1], and R.L. Larson[1], [1]*University of Missouri, Columbia, MO/USA.*

This study evaluated the efficacy of gonadotropin releasing hormone (GnRH) administered on day 21 post breeding to synchronize returns to estrus. One hundred-twenty-two Holstein heifers were initially synchronized using 2 injections of prostaglandin $F_2\alpha$ (PG). Twenty-five mg PG were administered im on days 0 and 11. HeatWatch® transmitters were applied on day 11 and monitored estrus activity for the remainder of the trial. The heifers were bred by fixed-timed artificial insemination on day 14 and randomized into two groups (treatment and control). On day 35, the treatment group received 100 μg GnRH im and the control group received 2 ml saline im. Pregnancy status was determined on day 42 using transrectal ultrasonography. Heifers that were not pregnant (n=85) were given a 25 mg injection of PG im and estrus activity was monitored until day 48 . There was no difference in median time to estrus between treatment groups. Data on heifers that returned to estrus (n=56) were analyzed using a Fischer's exact test. Data cells were collapsed into groups that expressed estrus at <60 hrs post PG and >60 hrs post PG. The GnRH group had significantly greater synchrony ($p<0.05$) than the saline group. More heifers in the GnRH group expressed estrus in the first 60 hrs following PG administration as compared to the saline group. Eighty five percent of the GnRH group expressed estrus between 36-60 hrs after PG administration compared to 55% of the saline group. In this study, GnRH was not efficacious in synchronizing returns to estrus when administered 21 days post breeding because of the large number of heifers that did not return to estrus. However, heifers that did return to estrus had greater synchrony if treated with GnRH compared to saline.

Variable	GnRH	Saline
n	41	44
Observed in estrus	27	29
Median time to estrus (hrs)	45	57
<36 hrs	1 (4%)	2 (7%)
36-60 hrs	23 (85%)	16 (55%)
>60 hrs	3 (11%)	11 (38%)

Key Words: Heifer, Synchronization, GnRH

1907 Efficacy of using Ovsynch to initiate artificial insemination at the onset of the breeding season in lactating dairy cows managed for seasonal calving in a grazing based dairy system. M.C Cordoba* and P.M Fricke, *University of Wisconsin-Madison, Department of Dairy Science.*

Lactating dairy cows (n=226) were subjected to a 21 d artificial insemination (AI) breeding period beginning at the onset of the breeding season (Day 0) followed by introduction of natural service sires. Throughout the AI breeding period, cows received AI based on tail paint removal, which was evaluated twice daily at milking. Beginning 10 d before the breeding season, cows were randomly assigned to receive synchronization of ovulation (50 mg GnRH, Day -10; 25 mg $PGF_{2\alpha}$, Day -3; 50 mg GnRH, Day -1) and fixed-time AI (Day 0) followed by estrus detection and AI for the remainder of the AI breeding period (Ovsynch; n=114), or estrus detection and AI for the duration of the AI breeding period (Control; n=112). Blood samples collected on Day -20, -10, -3, and -1 were classified based on serum progesterone (P) concentrations as High (≥0.5 ng/ml) or Low (<0.5 ng/ml). Overall, the proportion of anestrus cows (Low P on Day -20 and -10) was 14% (31/220) and did not differ between treatment groups. Although days to first AI was greater ($P<0.01$) for Control vs. Ovsynch cows (12 0.6 vs. 0 0, respectively) and the 21-d AI service rate was greater for Ovsynch vs. Control cows (100%, 114/114 vs. 86%, 96/112, respectively), conception rate to first AI service was greater ($P<0.01$) for Control vs. Ovsynch cows (47%, 43/91 vs. 27%, 30/100, respectively). Only 61% (69/114) of Ovsynch cows underwent luteolysis (50% reduction in P from Day -3 to Day -1) after $PGF_{2\alpha}$ administration. No Control cows received a second AI service during the AI breeding period, compared to 47% (51/108) of Ovsynch cows (mean d to second AI = 17 5.8). Conception rate to second AI service was 43% (22/51), which did not differ from that of Control cows at first AI service. Cumulative pregnancy rate for Control and Ovsynch cows was similar at Day 49 (47%, 43/91 vs. 46%, 50/108, respectively) and Day 179 (80%, 66/82 vs. 83%, 80/96, respectively). Ovsynch failed to synchronize lactating dairy cows managed in a grazing based dairy system thereby resulting in lower first service conception rates than AI to spontaneous estrus. Tail paint is an effective reproductive management tool for conducting AI in grazing based dairies. *Supported by Hatch project WIS04222*

Key Words: Ovsynch, TAI, Grazing

1908 Ovulation synchronization protocols affect early postpartum reproductive efficiency in crossbred dairy cows. J.L.M. Vasconcelos*, R.L. Valarelli, R.L.A. Cerri, A.H. Souza, and M. Meneghetti, *FMVZ-UNESP, Botucatu, SP/Brazil.*

Crossbred Holstein-Gir cows have lactation curves with low persistence and high incidence of postpartum anestrus, which negatively impact profitability. Objectives of this study were to evaluate three ovulation synchronization protocols and their impacts on reproductive efficiency. The study was conducted between March and December of 2000 in Brazil. Cows were randomly assigned to one of the four treatments: 1) Control (n=118), which consisted of estrus detection and prostaglandin injections in cows with a palpable corpus luteum (CL) every 14 d; 2) GnRH (Cystorelin®, 50mcg, im)-6d-PGF2a (Lutalyse®, 25mg, im)-2d-GnRH-16 to 18h-AI (n=133); 3) GnRH-5d-GnRH-6d-PGF2a- 2d-GnRH-16 to 18h-AI (n=132); and 4) CIDR + GnRH-6d-PGF2a + CIDR removal-2d-GnRH-16 to 18h-AI (n=129). PGF2a injection on day 6 after the first GnRH aimed to optimize ovulation synchronization after the last GnRH injection. Treatment with two GnRH injections 5d apart intended to increase the proportion of cows with a responsive CL when PGF2a was used. Use of CIDR in conjunction with GnRH aimed to minimize the incidence of short luteal phases in cows that might not

respond to the first GnRH. Interval from the end of the VWP to conception and days open were analyzed by ANOVA. Interval from the end of the VWP and conception were affected by treatment (P<0.01) and averaged 49.1±5.5, 34.8±4.8, 31.9±5.1, and 28.1±5.1 days for treatments 1, 2, 3, and 4, respectively. Similarly, days open were affected by treatment (P<0.01) and averaged 127.7±5.5, 114.0±4.8, 110.6±5.1, and 107.1±5.1 days, respectively. An interaction between parity and treatment was detected (P<0.05) for the interval between the end of the VWP and conception. This interval was reduced in primiparous cows receiving treatments 2, 3, and 4 compared with those on treatment 1, indicating that ovulation synchronization protocols might induce cyclicity. Reproductive protocols that synchronize ovulation reduced the interval from the end of the VWP to conception and days open when compared with regular prostaglandin treatments.

Key Words: lactating dairy cows, synchronization, anestrous

1909 Administration of hCG during estrus and its effect on corpus luteum size and progesterone production. J.A. Bartolome, S.M. Pancarci*, T. Dickerson, L.F. Archbald, and W.W. Thatcher, *University of Florida, Gainesville, FL*.

Progesterone (P4) production by the corpus luteum (CL) influences establishment and maintenance of pregnancy in cattle. Human Chorionic Gonadotropin (hCG) has LH-like activity, binds LH-receptors in luteal tissue and stimulates luteinization. The objective of this study was to evaluate the ability of hCG to enhance CL development and increase production of P4 when injected at estrus to supplement the natural LH surge. Twenty-three cows were treated with GnRH (100 ug, im, Cystorelin®) and $PGF_{2\alpha}$ (25 mg, im, 2 doses 8 hs apart, Lutalyse®), 7 d later. Seventeen cows that showed estrus (according to Heat Watch®) between 2 and 3 d after $PGF_{2\alpha}$ were randomly assigned to receive either hCG (3,000 IU, im, Chorulon®, n=9) or saline (n=8) 8 to 10 h after the onset of heat. Blood samples were collected every 12 h and ovarian ultrasound examination was done every three days to evaluate plasma P4 concentration, number of follicular waves and CL size during 23 days after treatment. Data for plasma P4 concentration and CL size were evaluated using repeated measures ANOVA for mixed models, and the number of follicular waves were compared using Fisher exact test. There was no difference in the number of follicular waves between cows treated with hCG (78% of cows with 2 waves) and control cows (75% of cows with 2 waves). Plasma P4 concentration was lower in cows treated with hCG (6.46 ng/ml) than in control cows (7.89 ng/ml; P<0.05), with major differences detected between days 14 and 16. In addition, on day 16, CL tended to be smaller (P<0.08) in cows treated with hCG compared to control cows. It was concluded that the administration of hCG during the spontaneous LH surge may have produced an excess of LH and LH-like activity and negatively affected CL differentiation and development.

Key Words: hCG, corpus luteum, progesterone

1910 Follicular dynamics in postpartum cows after treatment with either GnRH or Estradiol benzoate (EB) at the intiation of a 7 d controlled intravaginal progesterone-releasing device (CIDR). MK. V. Dahms*, C. R. Barthle, E. A. Hiers, G. E. Portillo, and J. V. Yelich, *University of Florida, Gianesville*.

Crossbred (*Bos indicus* X *Bos taurus*) lactating cows averaging 56 d (range 23-77 d) postpartum were used to characterize follicular dynamics after treatment with either EB or GnRH at the initiation of a 7 d CIDR (Eazi-Breed™CIDR®; day of insertion = d 0). Cows were allotted to treatments by cycling status and size of the largest follicle present on the ovaries on d 0 of experiment as determined by ultrasonography (US). At d 0, treatments 1 (n = 7; USGCIDR) and 3 (n = 8; GCIDR) were administered 100 μg GnRH i.m. (Fertagyl) and treatments 2 (n = 7; USEBCIDR) and 4 (n = 5; EBCIDR) were administered 2 mg EB i.m. On d 7 of the experiment, CIDRs were removed and all cows received 25 mg $PGF_{2\alpha}$ i.m.(Lutalyse). In the USGCIDR and USEBCIDR cows, ovaries were examined via US daily from d 0 until observed estrus, and 7 d after estrus or d 14 of experiment if estrus was not observed. Ovaries were examined in the GCIDR and EBCIDR cows on d 0 and 7 of the experiment, and 7 d after estrus or d 14 of experiment if estrus was not observed. Estrus was detected using Heat Watch®. Response to treatment on d 0 in USGCIDR was defined as ovulation to GnRH (71.4%), and as turnover of the largest follicle to EB (100%) in USEBCIDR cows. Of cows responding to treatment, day of emergence of the next follicular wave occurred earlier (P > 0.10) in USGCIDR (3.3 ± 0.6 d) compared to USEBCIDR (4.1 ± 0.6 d). Diameter of the newly emerged follicle(s) on d 7, 8 and 9 of the experiment were similar (P > 0.10) between USGCIDR (10.3, 11.1 and 13.0 mm; respectively) and USEBCIDR (9.9, 11.0 and 11.4 mm; respectively) cows. Estrus expression was influenced by US as fewer (P < 0.05) USGCIDR and USEBCIDR (7/14 = 50%) cows exhibited estrus than GCIDR and EBCIDR (12/13 = 93%) cows. Interval to the onset of estrus was similar (P > 0.05) within the US and non-US treatments, but the US (65.5 ± 13.1 h) cows had a shorter (P < 0.05) interval than non-US (84.7 ± 9.3 h) cows. Diameter of the ovulatory follicle was similar for USGCIDR (15.0 mm) and USEBCIDR (14.7 mm). These results suggest that there is no significant difference in follicular development between the GCIDR and EBCIDR treatments, although daily US significantly affected estrus behavior after CIDR removal.

Key Words: CIDR, Estrogen, GnRH

1911 Resynchonization of Ovulation and Timed Insemination in Beef Cattle. S Lares*[1], G Dominguez[1], N Formia[2], C Scena[3], O Rambeaud[4], and R.L. de la Sota[1], [1]*Instituto de Teriogenologia, Fac. Cs. Veterinarias-UNLP,* [2]*Escuela M.C. y M. Inchausti-UNLP,* [3]*Intervet Argentina SA,* [4]*INTA-Brandsen*.

A field trial was conducted to evaluate the effectiveness of Resynch, a resynchronization protocol for prompt insemination of cows diagnosed open at d 25 post AI. Postpartum suckled beef cows with BCS >3 (scale 1-5) were randomly allocated to one of four treatments: 1) Norgestomet implant d0-9, 400IU PMSG d9, timed AI (TAI) d11 (n=21; NOR-TAI); 2) same as 1 plus heat detection (HD)+AI d9-10, TAI d11 of remaining cows (n=21; NOR-IDH); 3) 8ug GnRH (Buserelin) d0, 25mg of PG (cloprostenol) d7, 8ug GnRH+TAI d9 (n=19; COS-TAI) and 4) same as 3 plus HD+AI d7-8, 8ug GnRH+TAI d9 (n=20, COS-IDH). Calves did not suckle for 48 h (NOR protocols, d9-11; COS protocols, d7-9). The Resynch protocol was: 8ug GnRH d18, pregnancy diagnosis with ultrasonography d25, and open cows were injected with 25mg of PG on d25 and 8ug GnRH+TAI on d27. Calves did not suckle for 48 h (d25-27). Pregnancy diagnosis was repeated at d25 after the 2nd AI to evaluate the PR to the Resynch protocol and the early embryonic death. The CATMOD procedure in SAS was used to analyze the data. First AI pregnancy rate (PR) were not different between the NOR groups (43 [TAI] vs. 43%[IDH]) and between the COS group [47[TAI] vs. 40% [IDH]). The PR for cows diagnosed open and resynchronized were not different between the NOR groups (46[TAI] vs. 58%[IDH]) and between the COS groups (20[TAI] vs. 25%[IDH]).Furthermore, the accumulated PR for both AI were also not different between the NOR (67[TAI] vs. 76%[IDH]) and the COS groups (58[TAI] vs. 55%[IDH]). Hence, data were pooled into two groups (cows synchronized with NOR vs. COS) and were reanalyzed. Although PR were not different in the 1st AI (43 vs. 44%), the PR was higher in the NOR group compared to the COS group in the resynchronized cows (52 vs. 23%; p<0.05)and the accumulated PR for both rounds of AI (71 vs. 56%; p<0.05). The early embryonic death rate was 4% (2/46). In conclusion, the Resynch protocol succesfully resynchronized open cows for TAI but the PR depended on the type of protocol used for the 1st AI. Cows 1st synchronized with a progestin had a 29% higher PR in the 2nd AI compared to those synchronized with GnRH. Also they had a 15% higher accumulated PR. Supported with grants BID AR/OC 08-04360 and UNLP-V11/107 to RLS.

Key Words: Timed insemination, Resynchronization, Early pregnancy diagnosis

1912 Luteolysis after $PGF_{2/alpha}$ on day 6 or 7 of the estrous cycle in Angus and Angus x Brahman heifers. G. E. Portillo*, E. A. Hiers, C. R. Barthle, MK. V. Dahms, W. W. Thatcher, and J.V. Yelich, *University of Florida, Gainesville, Florida.*.

Cycling Angus and crossbred Brahman heifers (5/8 Angus x 3/8 Brahman and 3/8 Angus x 5/8 Brahman) were used to evaluate the effectiveness of a single injection of $PGF_{2/alpha}$ administered during the early estrous cycle to initiate corpus luteum (CL) regression (CLREG) as measured by progesterone concentrations. Estrous response (ESTRES), interval from $PGF_{2/alpha}$ to the onset of estrus (INTEST), duration of estrus (ESTDUR), and total number of mounts received (TMTS) during the duration of estrus were also evaluated. Heifers were pre-synchronized with a modified two-injection $PGF_{2/alpha}$ (Lutalyse) protocol (25 mg

i.m. on d 14 and 12.5 mg i.m. on d 3 and 2 of the experiment). Estrus was designated as d 0 of experiment. On d 6 or 7 of the subsequent estrous cycle, heifers were injected with 25 mg $PGF_{2/alpha}$ i.m. Estrus was monitored using HeatWatch® for the pretreatment synchronization and treatment phase of the experiment. The experiment was replicated twice using the same pre-syncronization and treatment protocols and heifers. There were no replication or replication by breed effects ($P \geq 0.10$) so data were pooled. CLREG was greater ($P \leq 0.05$) for Angus (25/26 = 96.2%) than for crossbred (25/31 = 80.6%) heifers. The ESTRES was similar ($P \geq 0.10$) between Angus (15/26 = 57.7%) and crossbred (21/31 = 67.7%) heifers. INTEST and ESTDUR were similar ($P \geq 0.10$) between Angus (51.5 ± 3.8; 10.6 ± 1.4 d, respectively) and crossbred (52.4 ± 3.1; 12.9 ± 1.1 d, respectively). Crossbred heifers had more ($P \leq 0.05$) TMTS than Angus (51.1 ± 7.1 vs. 28.9 ± 9.2, respectively). In conclusion, breed had a significant effect on the ability of $PGF_{2/alpha}$ to regress the early (d 6 or 7) developing CL as more Angus than crossbred heifers had CL regression.

Key Words: *Bos indicus*, $PGF_{2/alpha}$, Corpus luteum Regression

1913 Reproductive performance of beef heifers following administration of an oral progestogen or GnRH. H. E. Blackmon*[1], M. E. Hockett[1], T. M. Towns[1], N. R. Rohrbach[1], R. B. Simpson[1], A. M. Saxton[1], and F. N. Schrick[1], [1]*Department of Animal Science, University of Tennessee, Knoxville.*

Oral progestogen or GnRH was administered to beef heifers 60 days prior to the breeding season to examine effects on reproductive performance. During a 3 year replicated study, 222 beef heifers were allotted by weight (320 ± 3 kg), age (11 to 13 mo), and breed (Angus, n = 198; Hereford, n = 24) to one of three treatments: 1) 2 mL saline (CON, n = 74), 2) 2 mL GnRH (GnRH, n = 74), and 3) 2 mL saline and 0.75 mg/(hd) melengestrol acetate for 14 days (MGA, n = 74). Control and GnRH heifers were fed non-MGA pellets for 14 days. Blood samples were collected twice 10 d prior to the start of the study (Period 1) and before the breeding season (Period 2) to determine cyclicity (progesterone > 1.0 ng/mL). Estrus was synchronized and heifers inseminated (AI) following visual estrus. Heifers not exhibiting estrus were time-inseminated at 48 h after implant removal. Following AI, heifers were exposed to bulls through the breeding season. Pregnancy was determined at 30 and 150 d following AI. A SAS based mixed model and Chi square were utilized for analysis of data. Fifty-two percent of heifers were prepuberal prior to initial treatments (Period 1), and exhibited an increased cyclicity prior to the breeding season (Period 2) after administration of MGA (59%) compared to CON (26%; P = 0.02), with GnRH (37%) intermediate. However, AI pregnancy rates did not differ (MGA, 41; GnRH, 35; and CON, 37%). In heifers cycling prior to the study (48%), AI pregnancy rates were higher in MGA (67%) and GnRH (65%) heifers compared to CON (43%; P = 0.06). Overall AI pregnancy rates were increased in MGA (55%) heifers than CON (40%; P = 0.07); whereas, GnRH (46%) heifers were intermediate. In conclusion, administration of MGA to prepuberal heifers 60 days prior to breeding increased cyclicity at initiation of the breeding season, but did not improve AI pregnancy rates. Treatment with MGA and GnRH increased pregnancy rates in heifers that had attained puberty prior to study.

Key Words: Progestogen, Puberty, Pregnancy

1914 A comparison of three progestin-GnRH-prostaglandin $F_{2\alpha}$ (PG) based protocols for estrus synchronization of beef cows. J. E. Stegner*, J. F. Bader, F. N. Kojima, M. F. Smith, and D. J. Patterson, *University of Missouri, Columbia, MO.*

This experiment was designed to compare three progestin-GnRH-PG based protocols for estrus synchronization in beef cows. Primi- and multiparous cycling crossbred cows were assigned to one of three treatments. Cows assigned to treatment 1 (T1) were fed melengestrol acetate (MGA; .5mg·hd^{-1}·d^{-1}) for 14 d followed by an injection of GnRH (100 μg Cystorelin®) on d 26 and PG (25 mg Lutalyse®) on d 33. Cows assigned to Treatment 2 (T2) were fed MGA for 17 d followed by GnRH on d 19, and PG on d 26. Cows assigned to Treatment 3 (7-11 Synch; T3) received MGA for 7 d followed by PG on d 7 of MGA, GnRH on d 11, and PG on d 18. Transrectal ultrasonography was performed daily on all cows to monitor follicular dynamics during the MGA feeding period and through the synchronized period after PG. Response to GnRH, determined by ovulation of the dominant follicle at the time GnRH was administered, was as follows: 8/9 (88 %), 6/9 (66 %), and 8/8 (100 %) for cows assigned to T1, T 2, and T3, respectively (P > .10). Beginning 48 h after PG, ultrasonography was performed every 12 h until ovulation occurred. Cows were observed for signs of behavioral estrus for 7 d after PG. Estrus response for cows assigned to each treatment was as follows: 9/9 (100 %), 7/9 (77 %), and 8/8 (100 %) for cows assigned to T1, T2, and T3, respectively (P > .10). Two of 9 cows (22 %) in T2 exhibited estrus 24 h before PG. Mean interval to estrus was shorter (P < .01) for cows assigned to T2 (46 ± 4.6 h), and T3 (52 ± 4.2 h), than in cows assigned to T1 (79 ± 7.8 h). The range in estrus response was 48-126, 24-54, and 24-60 h for cows assigned to T1, T2 and T3, respectively. Synchrony of estrus after PG did not differ among treatments. Follicular diameter at GnRH, PG, or 24-72 h after PG did not differ (P > .10) among treatments. Characterization of follicular dynamics and timing of estrus from these data will support development of protocols that facilitate fixed-time AI in beef cattle. (Supported by USDA-NRI grant 2000-02163)

Key Words: Estrus synchronization, Progestin, Beef cows

1915 Evaluation of a fixed-time AI protocol for postpartum beef cows. G. A. Perry*, J. F. Bader, M. F. Smith, and D. J. Patterson, *University of Missouri, Columbia, MO.*

Treatment with the oral progestin, melengestrol acetate (MGA) prior to GnRH and $PGF_{2\alpha}$ effectively synchronizes estrus and maintains high fertility in postpartum beef cows. The objective of this experiment was to determine whether treatment with MGA prior to a GnRH-$PGF_{2\alpha}$-GnRH protocol would improve pregnancy rates resulting from fixed-time AI. Multiparous crossbred cows at two University of Missouri farms (n=90 and n=137) were assigned by age and days postpartum (dpp) to one of two treatments: Cows were fed carrier (1.8-kg·hd^{-1}·d^{-1}) with or without MGA (0.278-mg·kg^{-1}) for 14 d. GnRH (100μg Cystorelin) was administered to all cows 12 d after MGA or carrier withdrawal and 7 d before $PGF_{2\alpha}$ (25 mg Lutalyse) injection. All cows received a second injection of GnRH and AI 72 h after $PGF_{2\alpha}$. Mean dpp for MGA and Control cows at the initiation of treatment were 39.6 and 38.9 for herd 1; and 51.9 and 50.9 for herd 2, respectively (P > 0.05 within herds). Blood samples were collected from all cows 10 d before and the day before MGA or carrier began, and at the time GnRH and $PGF_{2\alpha}$ were administered. Concentrations of P_4 in serum at the initiation of treatment were elevated (>1ng/ml) in 0% of MGA and 7% of Control cows in herd 1, and 54% of MGA and 49% of Control cows in herd 2 (P > 0.05 within herds). Pregnancy rates to fixed-time AI were determined by ultrasound 50 d after AI. Pregnancy rates in herd 1 were 58% (26/45) and 51% (23/45) for MGA-treated and Control cows, respectively (P = 0.52); and 63% (44/70) and 45% (30/67) for MGA-treated and Control cows in herd 2, respectively (P < 0.03). Differences in pregnancy rates to fixed-time AI remained significant (P < 0.05) when data from the two herds were combined [MGA=70/115 (61%); Control=53/112 (47%)]. There was no difference (P > 0.2) in final pregnancy rate between treatments, within herds, or combined. In summary, pregnancy rates resulting from fixed-time AI may be improved with treatment of MGA prior to a GnRH-$PGF_{2\alpha}$GnRH protocol. (Supported by grants from Select Sires, Inc. and USDA-NRI 2000-02163).

Key Words: Estrus synchronization, Fixed-time AI, Beef cows

1916 Stage of cycle effects on response to different GnRH + prostaglandin $F_{2\alpha}$ (PG)treatments in *Bos indicus* x *Bos taurus* cows. E. A. Hiers*, C. R. Barthle, J. K. Fullenwider, G. E. Portillo, MK. V. Dahms, J. M. Kramer, and J. V. Yelich, *University of Florida.*

Synchronization rate was evaluated using three different GnRH/PG protocols initiated on different days of the estrous cycle in *Bos indicus* x *Bos taurus* cows. Cows were presynchronized with a modified two-injection PG protocol (Lutalyse; 25 mg on d -14 and 12.5 mg on d -3 and -2 of experiment). Estrus was detected with HeatWatch®. After expression of estrus, cows were allotted to four groups based on day of the estrous cycle, d 2 (n = 15), 5 (n = 13), 12 (n = 12) and 18 (n = 13), when injected with GnRH (100 μg i.m.; Fertagyl). At GnRH injection, cows within each day were randomly subdivided into one of three PG treatments 7 d later: 1) PG (25 mg i.m.; Lutalyse; n = 16); 2) split-PG (12.5 mg i.m. on d 7 and 8; Lutalyse; n = 18); and 3) cloprostenol (500 μg i.m.; Estrumate; n = 19). At PG injection and every 48 h until

estrus and/or ovulation, a blood sample was collected and ovaries were examined by ultrasound. Cows treated with GnRH on d 18 tended (P = 0.10) to have a decreased 5-d estrous response (54%) compared with d 2, 5 or 12 (80, 77, and 92%, respectively). Interval from PG injection to onset of estrus was greater (P $\leq$ 0.05) for d 18 (101 $\pm$ 15 h) compared with d 2, 5 or 12 (87 $\pm$ 12, 82 $\pm$ 13, 62 $\pm$ 12 h, respectively). Percentage of cows ovulating after PG was decreased (P $\leq$ 0.05) for d 18 (38%) compared with d 2, 5 and 12 (100, 100, and 100%, respectively). The PG, split-PG, and cloprostenol treatments had similar (P $\geq$ 0.10) 5-d estrous response (63, 89, and 74%, respectively) and percentage of cows with corpus luteum (CL) regression (81, 88, and 95%, respectively). Interval from PG injection to onset of estrus was similar for PG, split-PG and cloprostenol (90 $\pm$ 13, 81 $\pm$ 11, and 77 $\pm$ 11 h; respectively). In conclusion, stage of the estrous cycle when GnRH was administered affected response to PG. Although not significant, split-PG and cloprostenol treatments resulted in increased CL regression and estrous response in *Bos indicus* x *Bos taurus* cows.

Key Words: Bos indicus, estrus, GnRH

1917 Effects of exogenous GnRH infusion and steroid replacement on gonadotropins in ovariectomized nutritionally anovulatory cows. J.A. Vizcarra*[1] and R.P. Wettemann[1], [1]*Animal Science Department, Oklahoma Agricultural Experiment Station.*

Twenty-four nutritionally induced anovulatory ovariectomized cows were used to determine the effect of estradiol, progesterone, and exogenous GnRH pulses on LH and FSH in serum. Cows were randomly assigned to four 6 x 6 Latin Squares (LS). Control cows in LS 1 received a sham progesterone and estradiol vaginal insert. Cows in LS 2 were treated with a vaginal estradiol insert (E2), while cows in LS 3 received a progesterone insert (P4). Cows in LS 4 received an estradiol and progesterone vaginal insert (E2P4). Within each LS, cows were randomly assigned to six treatments in a 2 x 3 factorial. Doses of GnRH (0, 2 or 4 g) in 2 mL were infused at frequencies of once every h or once every fourth h for 2 d. Blood samples obtained every 10 min for 8 h on d 1 and d 2 and were analyzed for LH and FSH by RIA. In addition, blood samples were taken daily to evaluate progesterone and estradiol concentrations. Estradiol was increased from 0.1 0.1 pg/mL in control cows to 1.6 .1 pg/mL in the E2 insert treatment, and progesterone was increased from 0.1 0.1ng/mL to 2.3 0.1 ng/mL with the P4 insert. In the absence of exogenous GnRH, concentrations of LH were increased (39% greater than control; P < 0.001) while concentrations of FSH were reduced (34% less than control; P < 0.01) when ovariectomized nutritionally anovulatory cows were treated with E2, P4 or E2P4 inserts. Cows treated with a E2 and E2P4 insert had increased (P < 0.03) LH concentrations when either 2 or 4 g of GnRH was infused, compared with control cows. The GnRH-induced LH secretion was not increased when pulses were given every fourth h. Cows treated with a E2, P4, and E2P4 insert had decreased (P < 0.04) FSH concentrations when GnRH (2 g) was infused compared with control cows. However, only cows treated with a E2 and E2P4 insert had decreased (P < 0.1) FSH concentrations when GnRH (4 g) was infused compared with control cows. We concluded that estradiol, progesterone and GnRH pulses differentially regulate secretion of LH and FSH from the pituitary of beef cows.

Key Words: Bovine, GnRH, LH and FSH

1918 Vaginal electrical conductance for determining the timing of ovulation is also effective for monitoring rates of uterine involution in the postpartum dairy cow. S.D. Bowers*[1], B.S. Gandy[1], J. Spencer[1], K.B. Graves[1], A.B. Moore[1], and S.T. Willard[1], [1]*Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS.*

The principle behind vaginal electrical conductance measurements (VEC) is that a change in the ionic balance of vaginal and cervical mucus occurs in response to cyclical changes in reproductive hormones. The objective of this study was to determine the efficacy of using VEC for monitoring not only follicular development and ovulation, but also uterine involution (UI) in dairy cattle postpartum (PP). In Experiment I, the applications of VEC for monitoring follicular development and ovulation using the Ovatest meter (Animark Inc.) were verified. Jersey cows (n = 16) were administered prostaglandin (PG; d 0) and sampled at 12 h intervals for 7 d post-PG. At each sampling period VEC measurements (relative units; RU) were obtained, ultrasonography was used to measure follicular diameters, and serum samples collected for quantification of estradiol (E2) by RIA. Nine of the 16 cows that responded to PG ovulated within 7 d (56%) and were retained for further analysis. Ovulatory follicle size did not differ (P $\geq$ .10) from PG to ovulation but increased (on average) 5.9 mm in size during this interval. While 2 of the 9 cows that ovulated exhibited variable E2 profiles post-PG, serum E2 decreased (P $\leq$ .01) 3.1 $\pm$ .84 pg/ml in relation to ovulation (n = 7). Moreover, VEC measurements decreased (P $\leq$ .001) 20.5 $\pm$ 4.5 RU between PG and ovulation. In summary for Experiment I, VEC decreased prior to ovulation and patterned, though not significantly, follicular growth and serum E2 through ovulation. In Experiment II, Jersey cows (n = 11) were sampled 2X/week from 1 to 60 d PP to assess the use of VEC for monitoring UI. On test days, VEC was recorded (Ovatest), follicular sizes and cross-sectional area of the pregnant vs. non-pregnant uterine horns captured (ultrasonography), and uterine tone scores assigned (UTS; rectal palpation). Uterine horn size differences were negatively correlated with UTS (r = -.52; P $\leq$ .001) and VEC (r = -.52; P $\leq$ .001); as uterine horn differences decreased over time PP, UTS and VEC measurements increased. Finally, UTS was also positively correlated (r = .44; P $\leq$ .01) with VEC. These data suggest that VEC may be used as an objective tool for quantifying UI in dairy cattle.

Key Words: Vaginal electrical conductance, Uterine involution, Dairy cattle

1919 Pregnancy rates of lactating beef cows losing body weight during the breeding season. T. M. Towns*[1], M. D. Davis[1], M. E. Hockett[1], N. R. Rohrbach[1], and F. N. Schrick[1], [1]*Department of Animal Science, University of Tennessee, Knoxville.*

Effect of weight loss during the breeding season on reproductive performance was assessed in 150 beef cows. Cows were sorted by calving date, body condition (BCS; mean = 5.5), BW (mean = 510 $\pm$ 8 kg), age, and breed. Restricted (RES) cows were fed to lose 5% BW from d–30 to d 0 (start of breeding season), and an additional 10% BW from d 0 to d 60 (end of breeding season). Control (CON) cows were fed to maintain BW. All cows reinitiated cyclicity (progesterone $\geq$ 1 ng/mL) prior to start of the breeding season. Estrus was synchronized and cows were inseminated at estrus and 12 h later. Bulls were placed with each group after the synchronization period through the breeding season. Pregnancy was determined at d–30, 60, and 150. Measurements for BW, BCS, and blood samples for progesterone, insulin, nonesterified fatty acids (NEFA), and urea nitrogen (BUN) were collected weekly from d 30 to d 60. Body weight of RES cows decreased 56. 9 kg; whereas, CON cows increased 10.8 kg from d–30 to d 60. Insulin decreased throughout the experimental period in RES cows (d–30, 0.33 $\pm$ 0.02 ng/mL; d 60, 0.24 $\pm$ 0.02 ng/mL; P < 0.05); however, NEFA (d -30, 0.77 $\pm$ 0.12 mEq/dL; d 60, 0.95 $\pm$ 0.12 mEq/dL; P < 0.05) and BUN (d -30, 20.4 $\pm$ 0.9 mg/dL; d 60, 29.1 $\pm$ 0.9 mg/dL; P < 0.05) increased. Neither estrous response (86.3 vs. 88.3%) nor conception rates (68.3 vs. 76.5%) differed between RES and CON cows, respectively. Pregnancy rates did not differ on d 30 (58.9 vs. 67.5%), d 60 (89.0 vs. 93.5%), or d 150 (91.8 vs. 96.1%) between RES and CON cows, respectively. Calves from RES cows had lower BW than CON at the end of the breeding season (145.4 $\pm$ 2.9 vs. 176.8 $\pm$ 2.9 kg; P < 0.05). In conclusion, cows which had reinitiated estrous cyclicity but were losing weight during the breeding season did not have reduced reproductive performance. These results suggest lower pregnancy rates associated with poor nutrition may be more related to failure of animals to reinitiate postpartum cyclicity rather than inability to establish and maintain pregnancy.

Key Words: Nutrition, Body weight, Pregnancy

1920 Use of doppler ultrasonography to estimate fetal age and monitor fetal heart rate and uterine artery pulse rate in dairy cattle. S. Willard[1], A. Webb*[1], S. Bowers[1], and B. Gandy[1], [1]*Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS 39762.*

The objective of the present study was to evaluate the use of Doppler ultrasonography (US) to monitor both uterine blood flow and fetal heart rate (FHR) during pregnancy in dairy cattle. Possible applications of Doppler US include the monitoring of fetal distress when a pregnancy becomes compromised, or as a method for estimating fetal age through changes in FHR during gestation. In this study, 30 pregnant Holstein

heifers were sampled repeatedly between 54 to 262 d post-breeding. On test days, transrectal Doppler US (Medata Systems) was performed to quantify FHR and dam uterine artery pulse rates (UAP). Following Doppler US, B-mode US was performed to obtain placentome diameter measurements as an additional measure of pregnancy stage. For the 104 total individual tests, 48 FHR, 101 UAP and 78 placentome measurements were obtained. Doppler US was also linked to a PowerLab Chart Recorder (ADI Instruments) to record FHR and UAP wave-forms for analysis. Data were analyzed using correlation and regression analysis relative to stage of gestation. Fetal heart rate was negatively correlated ($r = -.61$, $P \leq .01$) with d of gestation. Fetuses that were $\leq$ 140 d of age had higher ($P \leq .01$) FHR (155.8 ± 3.2 beats per minute; BPM) than fetuses $\geq$ 140 d of age (142.8 ± 2.4 BPM). As expected, average placentome size was positively correlated ($r = .83$; $P \leq .01$) with d of gestation. However, UAP of the dam did not change ($r = .16$; $P \geq .10$) relative to d of gestation (mean: 74.1 ± 1.0 pulses per minute). Of the 104 attempts at Doppler US, FHR were determined 46% of the time and UAP found 97.1% of the time. Using the PowerLab, FHR waveforms (gestation d 140 to 145) varied 808.0 ± 13.9 mV in amplitude from baseline (62.9 ± 5.9 mV), at a frequency of 150 BPM. In contrast, UAP signatures were not different ($P \geq .10$) from background levels (81.2 ± 3.2 MAX mV; 44.8 ± 3.1 MIN mV). In summary, transrectal Doppler US is effective for estimating fetal age in the bovine and for monitoring FHR characteristics. However, assessments of uterine blood flow using standard transrectal Doppler US will require additional investigation to detect changes in uterine blood flow under varied conditions. [This project was part of an undergraduate directed individual study program].

Key Words: Doppler ultrasonography, Fetal heart rate, Dairy cattle

1921 Factors affecting temporal relationships between estrus, ovulation and insemination in a commercial sow herd.

B. A. Belstra*, W. L. Flowers, K. J. Rozeboom, and M. T. See, *North Carolina State University, Raleigh, NC.*

Our objective was to examine the effect of genotype (A, B, C), lactation length (11 to 20 d), parity (1, 2, $\geq$ 3), weaning-to-estrus interval (WEI; 3, 4, 5, $\geq$ 6 d) and season (spring, summer) on the estrus, ovulation and subsequent fertility characteristics of sows. Duration of estrus and time of ovulation were estimated via boar exposure and transabdominal real-time ultrasonography, respectively, every 6 h from 2 to 10 d postweaning in 174 sows (87/season). The mean and range of duration of estrus (DE) and onset of estrus-to-ovulation interval (EOI) was 58.1 h, 24 to 84 h and 44.1 h, 12 to 90 h, respectively. Genotype, lactation length and parity did not affect DE or EOI ($P > .18$). There was a stepwise decrease in DE as WEI increased from 3 to 5 d ($P < .03$) but not 5 to $\geq$ 6 d ($P > .3$). Sows weaned in the spring compared to the summer had a shorter DE (53.1 vs. 60.8 h, $P < .001$) and a shorter EOI (38.1 vs. 48.5 h, $P < .001$). Sows were inseminated on an 8 am d 1 of estrus, 8 am and 3 pm d 2 of estrus schedule. Variation in WEI, DE and EOI resulted in insemination-to-ovulation intervals with a mean and range of 35.9 h, 87 to - 3 h; 12.0 h, 63 to - 27 h and 4.8 h, 56 to - 34 h, for services 1, 2 and 3, respectively. Only season affected insemination-to-ovulation intervals ($P < .001$) but there was no difference in spring vs. summer number of inseminations within 24 h prior to ovulation (1.3 vs. 1.3, $P > .9$), conception rate (94.6 vs. 90.5%, $P > .3$), farrowing rate (91.9 vs. 86.5%, $P > .3$) or pigs born alive (11.0 vs. 10.9, $P > .9$). Sows that received 0 vs. 1 or 2 inseminations within 24 h prior to ovulation had a similar conception and farrowing rate ($P > .7$) but tended to have fewer pigs born alive (9.5 vs. 11.3, $P < .09$). In this particular herd, season and weaning-to-estrus interval altered duration of estrus and time of ovulation. However, these changes were not large enough to affect sow fertility because the multiple insemination schedule in place ensured that at least one insemination occurred near ovulation.

Key Words: Estrus, Ovulation, Sow

1922 Hormonal Changes After Manual Rupture of Follicular Cysts.

Ahmet Gumen* and Milo C. Wiltbank, *Department of Dairy Science, University of Wisconsin-Madison.*

Follicular cysts are commonly treated by manual rupture. In this study, we induced follicular cysts by causing a GnRH/LH surge with 5 mg estradiol benzoate (EB; produces circulating estradiol = 67 pg/ml) at a time when a dominant follicle was not present on the ovary (Day 0). After treatment 8 of 20 (40%) cows developed a large follicle anovulatory condition similar to follicular cysts. On Day 10, cows were again challenged with EB. Six of 8 cows had no detectable LH surge but 2 cows had some LH increase (8 and 3 ng/ml) after EB. At Day 50, cows were randomized into 2 groups: Manual Rupture (MR; n=4), Control (n=4). MR cows had all follicles $\geq$ 15 mm removed by exerting pressure through the rectum similar to what is done routinely in the field. Prior to treatment all cows had periodic (every 15-25 d) development of large anovulatory follicles and corresponding increases in circulating estradiol. At MR, 2 cows had elevated estradiol (12 and 29 pg/ml) that decreased to basal concentrations after MR. There was a subsequent surge in FSH and emergence of a new follicular wave. Two MR cows had basal estradiol and a follicular wave was emerging at the time of MR. There was no FSH surge in these cows following MR. Two cows (one control and one MR) had a spontaneous double ovulation at Days 56 and 57. These ovulating cows were the same 2 cows that had an LH increase in response to EB at Day 10. The remaining 6 cows were challenged with EB on Day 71. Two of the cows (one control and one MR) had an LH surge and ovulation in response to EB; while 4 had no LH surge and no ovulation. The non-ovulating cows were treated for 7 d with progesterone (CIDR on Days 78-85) followed by EB on Day 86. All four cows had an LH surge and ovulation in response to EB. Thus, MR can remove functional large anovulatory follicles and start a new follicular wave. However, MR does not appear to resolve the underlying physiological problem that cystic cows do not have an LH surge following high circulating estradiol. Progesterone treatment can resolve this physiological problem.

Key Words: Follicular Cysts, Manual Rupture, Estradiol

1923 Pregnancy rates in lactating dairy cows following timed embryo transfer under heat stress conditions.

Y.M. Al-Katanani*[1], M. Drost[1], R.L. Monson[2], J.J. Rutledge[2], C.E. Krininger III[1], J. Block[1], and P.J. Hansen, [1]*University of Florida, Gainesville, FL/USA,* [2]*University of Wisconsin, Madison, WI/USA.*

Timed embryo transfer (TET) using in vitro-produced (IVP) embryos without estrus detection can be used to reduce adverse effects of heat stress on fertility. One limitation is the poor survival of IVP embryos to cryopreservation. Objectives were to 1) confirm beneficial effects of TET on pregnancy rate during heat stress as compared to timed artificial insemination (TAI) and 2) determine the efficacy of vitrification as a method for cryopreservation. For vitrified embryos (TETV), vitrification and thawing procedures, using excellent and good quality d 7 Holstein blastocysts, were as described (Theriogenology 50:129). For fresh embryos (TETF), Holstein oocytes were shipped from Wisconsin to Florida overnight, fertilized and embryos cultured in modified KSOM for 7 d using the method for production of vitrified embryos. Excellent and good quality blastocysts on d 7 were transported to the cooperating dairy in a portable incubator. Non-pregnant, lactating Holsteins (n=155) were injected with GnRH (100 μg, im), followed 7 d later by $PGF_{2\alpha}$ (25 mg, im) and GnRH (100 μg) on d 9. Cows in the TAI group (n=68) were inseminated the next day with semen from a single bull that was also used to produce embryos. Cows in the other groups (n=33 for TETF and 54 for TETV) received an embryo on d 7 after anticipated ovulation (d 8 after second GnRH). The proportion of cows that responded to synchronization (plasma progesterone $\leq$ 1.5 ng/ml on d 0 and $\leq$ 1.5 ng/ml on d 8 after GnRH) was 67.7%. Pregnancy rate for all cows at d 45 was higher ($p<0.05$) in the TETF group than for the TAI and TETV groups (19.0 ± 5.0%, 6.2 ± 3.6%, and 6.5 ± 4.1%). For cows responding to synchronization, pregnancy rate was higher ($p<0.05$) for TETF than for other groups (26.7 ± 6.4%, 5.0 ± 4.3%, and 7.4 ± 4.7%). In conclusion, ET of fresh IVP embryos can improve pregnancy rate under heat stress conditions but pregnancy rate following transfer of vitrified embryos was no better than TAI.

Key Words: Heat stress, Embryo transfer, Vitrification

1924 Factors affecting the time intervals between estrus, LH surge and ovulation in high-yield dairy cows.

A. Bloch*[1], D. Wolfenson[1], M. Kaim[2], Z. Roth[1], R. Braw-Tal[2], and Y. Folman[2], [1]*Faculty of Agriculture, Hebrew University, Rehovot, Israel,* [2]*Agricultural Research Organization, Bet-Dagan, Israel.*

The short fertile lives of the male and female gametes in the female tract necessitate accurate timing of insemination. We examined the effects of season, parity, milk yield, body condition, and concentrations of steroids

and LH surge prior to estrus, on the intervals: estrus-ovulation (E-O), estrus-LH surge (E-LH), and LH surge- ovulation (LH-O), and on post-O progesterone curves. Holstein cows (n=74) in first-fifth lactation, yielding 35-57 kg milk/day (d), were examined at 60-80 d postpartum. After synchronization, cows were examined around subsequent spontaneous E. Blood samples were taken every 2 d in cycles before and after E, and every 3 to 8 h around E and expected O. Cows were watched for E continuously for 5 d from the expected time of E. From 20 h after standing E, time of O was checked by ultrasound every 4 h. For most cows (73%), with E-O<30 h, E-O, E-LH and LH-O intervals were 27±0.3, 2.3±0.4 and 24±0.4 h, respectively (normal interval; meansem); 17% of cows had a 31-35 h E-O (long interval) and 10% (n=7) ovulated 35 to >50 h after E (43±2.6 h; very long interval). E-O, E-LH and LH-O intervals were not affected by season, parity, milk yield, milk composition or body condition. LH surge amplitude in very-long interval group reached 40% of those in other groups (P<0.01). Concentrations of estradiol prior to LH surge tended to be lower (P<0.08), and those of progesterone prior to E were lower (P<0.03) in the very-long than in the other two groups. Post-O progesterone curves were lower in long and very-long than in normal interval group (P<0.03). The data show a subgroup of cows with extended E-O interval, associated with low plasma steroid concentrations and low LH surge prior to O, and with low post-O progesterone, which may result in non-optimal timing of insemination, lowering fertilization rate and leading to low fertility.

Key Words: Estrus, Ovulation, LH surge

1925 Hormonal induction of enhanced removal of impaired follicles improved oocyte quality in the autumn in previously heat-stressed cows. Z. Roth*[1], A. Arav[2], A. Bor[2], R. Braw-Tal[2], and D. Wolfenson[1], [1]*Faculty of Agriculture, Hebrew University, Rehovot, Israel,* [2]*Agricultural Research Organization, Bet Dagan, Israel.*

The autumn conception rate of dairy cattle is low although cows are relieved from summer heat stress. Previously, frequent follicular aspirations in the autumn improved oocyte quality, therefore, the present study examined different hormonal approaches to oocyte quality improvement in autumn by enhanced removal of follicles damaged in the previous summer. In two experiments intended to examine different hormonal treatments, follicles (3-8 mm) were aspirated in the autumn from lactating Holstein cows on day (d) 4 of each cycle by the ovum pick up procedure. Oocytes were morphologically examined, matured in vitro, activated and cultured for 8 d. In the first experiment, recombinant bovine somatotropin (500 mg; bST, Sometribove) was injected into cows (n=8) every 14 d, during 4 estrous cycles. Before bST treatment (cycle 1) percentages of grade-I and cleaved oocytes did not differ between control (n=8) and bST groups, and parthenogenetic blastocysts were nil. Treatment with bST increased grade-I oocyte percentages, compared with control cows (P<0.05), particularly in cycle 2 (72 vs. 26%), less so in cycles 3 and 4 (55 vs. 42%). Cleavage and blastocyst rates gradually rose in cycles 3 and 4, but did not differ between groups. In the second autumn experiment, two doses of FSH (2x200 mg; Follotropin V) were injected at 12-h interval (FSH; n=3) on d 5 and on d 11 of cycle 1. In cycle 1 (before FSH injection), grade-I oocyte (52%) and cleavage (24%) rates did not differ between control (n=4) and FSH groups. In the next cycle, grade-I oocyte and cleavage rates were higher in FSH than in control group (89 vs. 51% and 85 vs. 31%, respectively; P<0.05). Results indicate that a short treatment with bST or FSH in the autumn improved oocyte quality; perhaps recruitment of more growing follicles into follicular waves enhanced the removal of follicles damaged in the previous summer.

Key Words: bST , FSH, Oocyte

1926 Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. G. Inbar[1], D. Wolfenson*[1], Z. Roth[1], M. Kaim[2], A. Bloch[1], and R. Braw-Tal[2], [1]*Faculty of Agriculture, Hebrew University, Rehovot, Israel,* [2]*Agricultural Research Organization, Bet Dagan, Israel.*

Differences between lactating cows (C) and nulliparous heifers (H) in follicular development and circulating hormone concentrations, that may relate to differences in fertility between the groups, were examined. Multiparous Holstein C (n=19) at 77±7 (meansem) days (d) post-partum, yielding 49±2.4 kg milk/d and H (n=20), 13.3±.1 months old, were examined in 2 replicates during 1 estrous cycle, by ultrasound monitoring every 2 d and daily blood samples. Fifteen C (79%) and 14 H (70%) had 2-follicular-wave cycles, 4 C and 5 H had 3-wave cycles and 1 H had a 4-wave cycle. Cycle length was shorter in H than in C, and in 2- than in 3- and 4-wave cycles, both by 2.5 d (P<0.02). Size of ovulatory follicle (F) and number of large Fs in the follicular phase were smaller in H than in C (13.1±.3 vs. 16.5±.5 mm; P<.001; and 1.0.1 vs. 1.9.1 Fs; P<.01), in both wave cycles. Emergence of preovulatory F was earlier in H than in C (10.2±.7 vs. 12.5±.5 d; P<.01), in 2-wave cycles, and similar (17.1±.8) in 3-wave cycles. Dominance duration of ovulatory F was longer in 2-wave than in 3-wave cycles (7.0±.5 vs. 4.6±.8 d; P<.01), similar in C and H in replicate 1, but shorter in H than in C in replicate 2 (5.1±1.0 vs. 9.1±1.5 d; P<.05). Estradiol concentrations were higher around estrus in H than in C, peaking at 10.1±.7 vs. 7.1±.8 pg/ml (P<.01). Progesterone concentrations were higher in H than in C from d 2 to d 16 of cycle (P<.01). LH surges were higher and wider in H than in C, peaking at 19.0±4.2 vs. 8.6±1.8 ng/ml (P<.01). The timing of FSH surge concentrations corresponded well to the emergence of follicular waves in both H and C. Basal and peak surge values of FSH were 15 to 20% lower in H than in C (P<0.05). Results show differences in ovarian functions and gonadotropins secretion that may account for the differences in fertility between nulliparous heifers and multiparous lactating cows.

Key Words: Follicular dynamics , Cows, Heifers

1927 Effects of fertilizing bovine oocytes with sperm aged post-thaw. J.A. Miller*, F.N. Schrick, A.M. Saxton, and J.L. Edwards, *The University of Tennessee, Knoxville, TN, USA.*

The present study examined development of bovine embryos derived from oocytes fertilized with aged sperm. Cumulus oocyte complexes were matured 22.5 h and fertilized with percoll-purified sperm (750,000 sperm/mL). Before percoll-purification, sperm was treated in the following manner: prepared within 1 h post-thaw (control), aged for 8 or 14 h post-thaw at 34.4°C in a H_20 bath, or aged 23 h post-thaw at 4°C in a refrigerator. Spermatozoa from nine bulls, representing various breeds, were evaluated. Proportion of motile sperm was assessed after aging and following percoll-purification. Number of putative zygotes (PZ) that cleaved and developed to blastocyst was evaluated on d 3 and 8 post-fertilization, respectively. Data were arranged in a randomized block design and analyzed using mixed models of SAS. Aging sperm in a H_20 bath for 14 h at 34.4°C or in a refrigerator for 23 h at 4°C reduced proportion of motile sperm when compared to control or sperm aged in a H_20 bath for 8 h post-thaw at 34.4°C (42.1 and 56.8% vs 69.2 and 64.9%, respectively; $P < 0.0001$; SEM = 4.1). Overall, proportion of motile sperm increased after percoll-purification (53.0 vs 64.6%; $P < 0.03$; SEM = 5.1) but remained lower for sperm aged at 34.4°C for 14 h (77.3 and 69.3% for control and refrigerated sperm, respectively vs 48.9%; $P < 0.07$; SEM = 8.8). Fertilization of oocytes with sperm aged for 14 h at 34.4°C reduced proportion of PZ that cleaved, but did not alter ability of embryos to develop to 8-16-cell or blastocyst (Table). Although reducing number of motile sperm is a negative effect of aging post-thaw, results demonstrate that developmentally competent embryos can be obtained. Aging is of concern but inevitable when attempting to manipulate sperm post-thaw after fertilization.

Treatments	Reps	# PZ	Cleaved	8-16-Cell∗	Blastocyst∗
Control	9	310	76.8^{a}	89.7	30.1
34.4°C, 8 h	4	106	74.8^{ab}	85.2	30.6
34.4°C, 14 h	8	317	63.7^{b}	81.1	41.1
4°C, 23 h	5	375	76.8^{ab}	87.8	30.9
P-value	—	—	0.0592	0.2000	0.2994
SEM	—	—	6.4	3.5	6.6

∗Proportion of cleaved embryos developing to 8-16-cell and blastocyst

Key Words: Sperm, Aging, Bovine

1928 Evaluation of the fertility potential of extended cooled equine spermatozoa using the resazurin reduction test and $NADH_2$. W. T. Campbell*, S. A. Ericsson, J. S. Pendergraft, K. K. Korth, and J. A. Pitchford, *Sul Ross State University, Alpine, Texas*.

The objective of this study was to determine if the fertility potential of extended cooled equine semen samples could be assessed using the resazurin reduction test supplemented with $NADH_2$. Semen samples (n=30) from 7 mature stallions were collected using a Missouri style artificial vagina with the gel-free fraction of each sample being evaluated microscopically (250X) with a Makler counting chamber. Samples that contained at least the minimal concentration (20 X 10^6/mL), total number motile (10 X 10^6/mL), and number of progressively motile (5 X 10^6/mL) spermatozoa recommended for a 50 mL insemination dose were extended (1:4 ratio) in Kenney's skim milk with Gentamicin Sulfate to 50 mL and cooled to 5°C. At 32, 56, and 80 h post-extension, 7 (1 mL) aliquots were removed and allowed to warm at 23°C for 30 min. One aliquot was analyzed microscopically to determine if the sample retained the recommended sperm numbers. The remaining aliquots were divided equally into control aliquots (no $NADH_2$) and treatment aliqouts (0.5 mg $NADH_2$/aliqout). Resazurin (50 μL of a 6.77 μM solution) was added to each of the aliquots and the time of reduction of resazurin (blue) to resorufin (pink) was recorded up to a maximum of 30 min. Diagnostic statistics were utilized to assess the accuracies of the two groups. None of the control aliquots reduced resazurin (30 min) at 32, 56, and 80 h. The treatment aliquots at 32 h reduced resazurin at 20.5 min with a 75% sensitivity (Sen), 96% specificity (Spec), 75% positive (PPV) and 96% negative (NPV) predictive values, and 93% overall accuracy (ACC). Resazurin was reduced by the 56 h treatment aliquots at 17.5 min with a 46% Sen, 94% Spec, 86% PPV, 70% NPV, and 73% ACC. The 80 h treatment aliquots reduced resazurin at 16.5 min with a 69% Sen, 100% Spec, 100% PPV, 81% NPV, and 87% ACC. These results suggest that the resazurin reduction test supplemented with $NADH_2$ can accurately assess the fertility potential of extended cooled equine semen.

Key Words: Spermatozoa, Resazurin, $NADH_2$

1929 Motility of frozen-thawed bovine sperm after aging for extended time periods. M.N. Malone*, J.A. Miller, A.M. Saxton, and J.L. Edwards, *The University of Tennessee, Knoxville, TN, USA*.

The objective of the present study was to evaluate effects of aging frozen-thawed bovine sperm. Specifically, sperm motility was evaluated after aging frozen-thawed sperm for an extended time period in nine bulls representing Charolais, Holstein, and Senepol breeds (n = 3 bulls/breed). Straws of frozen sperm were thawed and maintained in a water bath at 34.5°C. Sperm motility was assessed at 0, 5, 7, 9, 11, 13, and 15 h post-thaw. Spermatozoa were diluted 1:20 in Sperm-TALP containing 6.0 mg/mL BSA, 1.0 mM sodium pyruvate, 50 U/mL of penicillin, and 50 μg/mL of streptomycin. Motility of sperm was assessed independently by two individuals. The experiment was replicated three times. Statistical models were fit to include effects of breed, individual bulls, and time as either a class or regression variable. Motility of frozen-thawed sperm decreased from 68.3% immediately post-thaw to 34.7% 15 h later (SEM = 2.5; $P < 0.0001$). Averaged over time, sperm motility was lowest in Charolais bulls (41.1%) compared to Holstein (51.9%) and Senepol (55.3%; SEM = 2.1; $P < 0.0001$). Ability of sperm to withstand stress of aging post-thaw differed according to breed. Regressing over time, motility of frozen-thawed sperm from Charolais and Holstein bulls decreased more rapidly (slope = -2.8 and -2.5% per hour, respectively) than for Senepol bulls (slope = -1.4% per hour; SEM = 0.4; $P < 0.0002$). The rate of decline in motility of aged sperm was very similar for three Senepol bulls examined (slope = -1.7, -1.3 and -1.2% per hour; SEM = 0.4; $P > 0.7$). In contrast, large amounts of variation among individual bulls were noted in Holsteins (slope = -4.2 and -3.0% versus -0.6% per hour; SEM = 0.4; $P < 0.0001$) and Charolais (-3.8% versus -2.6 and -2.1% per hour; SEM = 0.4; $P < 0.02$). Results demonstrated motility of sperm of individual bulls within a breed differed in ability to handle stress of aging. Future efforts will focus on determining if ability of sperm to withstand stress can be used as an indicator of fertility.

Key Words: Sperm, Motility, Aging

1930 Effects of growth hormone (GH) and IGF-I on development of in vitro derived bovine embryos. F. Moreira, F. F. Paula-Lopes, P. J. Hansen, L. Badinga, and W. W. Thatcher, *University of Florida*.

Objectives were to determine if addition of GH to maturation medium and GH or IGF-I during embryo culture affects development of bovine embryos. Cumulus oocyte complexes (COCs) from slaughterhouse ovaries were matured in 50 μl drops of serum-free TCM-199 medium containing FSH (20 μg/ml) and estradiol (2 μg/ml) in groups of 10 oocytes per drop. During maturation, COCs were treated with 100 or 0 ng/ml of GH. COCs were inseminated, denuded, and transferred to 25 μl drops of serum-free KSOM medium containing essentially fatty acid-free bovine serum albumin (2 mg/ml) in groups of 10 presumptive embryos per drop for culture (d 0). Beginning at d 0, the following treatments were added to medium: 1) non-specific IgG (raised to heat shock protein 65 of *Mycobacterium bovis*; 10 μg/ml), 2) GH (100 ng/ml) plus IgG (10 μg/ml; GH/IgG), 3) IGF-I (100 ng/ml) plus IgG (10 μg/ml; IGF/IgG), 4) antibody to IGF-I (10 μg/ml; anti-IGF), 5) GH (100 ng/ml) plus anti-IGF (10 μg/ml; GH/anti-IGF), 6) IGF-I (100 ng/ml) plus anti-IGF (10 μg/ml; IGF/anti-IGF), or 7) no further additions (control). Embryos were observed at d 3 for cleavage and at d 7 and 8 for blastocyst development. The experiment was replicated on 6 occasions. Addition of GH to the maturation medium increased cleavage rates at d 3 compared to control (87.3 ± 1.2 % > 83.9 ± 1.2 %; $P < 0.05$) but had no effects on blastocyst development at d 7 and 8. Blastocyst development was greater ($P < 0.1$) for GH/IgG (16.2 ± 1.8 %) and IGF/IgG (16.5 ± 1.8 %) than for IgG (8.7 ± 1.5 %) at d 7. At d 8, blastocyst development was greater ($P < 0.01$) for GH/IgG (24.8 ± 2.5 %) and IGF/IgG (33.7 ± 2.5 %) than for IgG (16.1 ± 2.1 %) and greater for IGF/IgG than for GH/IgG ($P < 0.02$). Blastocyst development at d 8 did not differ between anti-IGF (20.4 ± 1.8 %) compared to GH/anti-IGF (24.1 ± 1.9 %) and IGF/anti-IGF (17.7 ± 1.9 %), but it was greater for GH/anti-IGF than for IGF/anti-IGF ($P < 0.05$). Both GH and IGF-I stimulated embryonic development and GH effects seem to be independent from IGF-I.

Key Words: Bovine IVF, GH, IGF-I

1931 Nuclear progression of bovine oocytes maintained at germinal vesicle stage up to 66 hours using roscovitine. A.M. Clarke*, L.M. McCann, and J.L. Edwards, *The University of Tennessee, Knoxville, TN, USA*.

The objective of this study was to determine effectiveness of roscovitine (inhibitor of $p34^{cdc2}$/cyclin B kinase) to maintain bovine oocytes at germinal vesicle (GV) stage for 21, 42 or 66 h after removal from follicles without compromising subsequent progression to metaphase II (MII). Cumulus oocyte complexes were cultured with 50 μM roscovitine for 21, 42, or 66 h in 5.5% CO_2 at 38.5°C in M199 containing 10% fetal bovine serum, 1 X nucleosides, 2 mM L-glutamine, 1 X nonessential amino acids, 0.1 mM β-mercaptoethanol, 50 U/mL penicillin, and 50 μg/mL streptomycin. After 21, 42, or 66 h, oocytes were divided within treatment: nuclear status was determined in approximately half while those remaining were washed of roscovitine and cultured for an additional 24 h in medium containing 0.3 μg/mL LH and 5.0 μg/mL FSH. Nuclear status of oocytes was evaluated using Hoechst staining. Number of oocytes with a GV, metaphase I (MI), anaphase I, telophase I, or MII configuration was recorded. Data were arranged in a randomized block design and analyzed using mixed models of SAS. Culture of oocytes with roscovitine, up to 66 h after removal from follicles, maintained >90% at GV stage (Table). Moreover, culture of oocytes with roscovitine for 21 or 42 h after removal from follicles, did not alter proportion progressing to metaphase II after placement in gonadotropins (Table). Inhibiting resumption of meiosis for extended time periods in oocytes after removal from follicles will be important for development of culture systems allowing for the study of immature oocytes.

Time in Roscovitine (h)	Reps	Oocytes*	GV (%)	Matured Oocytes**	Reps	GV (%)	MI (%)	MII (%)
21	4	155	94.8	109	3	17.4	11.7	69.2[a]
42	4	167	96.2	148	4	11.9	10.9	70.4[a]
66	4	185	90.7	167	4	21.6	14.8	52.1[b]
SEM	—	—	1.8	—	—	3.8	4.5	5.5
P-Value	—	—	0.1	—	—	0.2	0.8	0.04

**Number of oocytes placed in medium containing gonadotropins after culture with roscovitine. *Number of oocytes cultured with roscovitine and evaluated for presence of GV.

Key Words: bovine, oocyte, roscovitine

1932 Postweaning growth and puberty of Angus and Romosinuano bulls in Florida. C. C. Chase, Jr.*[1], R. E. Larsen[2], P. C. Sheerin[2], M. J. Williams[1], A. C. Hammond[3], T. A. Olson[2], and S. W. Coleman[1], [1]*USDA, ARS, Brooksville, FL,* [2]*University of Florida, Gainesville,* [3]*USDA, ARS, Athens, GA.*

Postweaning growth and puberty were determined from two consecutive calf crops using Angus (A; yr 1: n = 17, 254±15.9 d, 214±6.6 kg; yr 2: n = 12, 264±7.3 d, 221±11.4 kg) and Romosinuano (R; yr 1: n = 18, 259±15.4 d, 222±6.4 kg; yr 2: n = 24, 232±5.1 d, 182±8.0 kg) bull calves. Following weaning and preconditioning, bulls were managed as a single group on mixed bahiagrass and perennial peanut pastures (and hay) and fed 4.5 kg/d concentrate for 357 to 358 d. At the start of the study and at 28-d intervals, BW, hip height (HH), and scrotal circumference (SC) were measured. Concurrently, when the SC of a bull reached 23 cm, semen collection was attempted by electroejaculation. Ejaculates were evaluated for presence of first spermatozoa (FS), 50 million sperm with at least 10% motility (PU), and 500 million sperm with at least 50% motility (PP). In yr 1, R gained more BW ($P < 0.05$; 308±7.8 vs 281±8.1 kg), HH ($P < 0.05$; 26±0.9 vs 23±0.9 cm), and SC ($P < 0.10$; 13.0±0.6 vs 11.5±0.6 cm) than A bulls. In yr 2 when R were ($P < 0.05$) younger, lighter, and had smaller SC at the start of the study than A bulls, gains in BW and HH did not differ between R vs A (264±7.5 vs 283±10.7 kg and 22±1.0 vs 21±1.4 cm); however, R had greater ($P < 0.001$) gains in SC (14.5±0.4 vs 10.9±0.6 cm). In yr 1, breed (R vs A) did not affect ($P > 0.10$) age at FS (336±18.6 vs 306±19.2 d), PU (357±19.6 vs 339±20.2 d), or PP (397±19.4 vs 362±19.9 d); however, R were heavier (313±10.6 vs 267±11.0, 336±11.2 vs 297±11.6, and 378±11.4 vs 320±11.7 kg) and taller than A at FS, PU, and PP, respectively. Breed did not affect SC at FS and PU, but R tended ($P < 0.10$) to have a larger SC at PP (30.2±0.5 vs 28.9±0.5 cm). In yr 2, R tended ($P < 0.10$) to be older than A at FS (356±10.2 vs 325±14.6 d), but not ($P > 0.10$) at PU (378±10.5 vs 352±15.0 d) and PP (408±11.8 vs 408±16.7 d). In yr 2, breed did not affect BW at FS (250±7.4 vs 258±10.5 kg) or PU (266±9.4 vs 283±13.3 kg), but R tended ($P < 0.10$) to be lighter than A at PP (291±11.0 vs 328±15.7 kg), and R were taller ($P < 0.001$) than A at FS, PU, and PP. Romosinuano had smaller ($P < 0.05$) SC than A at FS and PP, but similar SC at PU (25.7±0.4 vs 26.7±0.6 cm). In conclusion, the tropically adapted R bulls reached puberty at similar ages as A bulls when reared in the subtropics.

Key Words: Bulls, Tropics, Puberty

1933 Concentrations of LH and testosterone in serum of sexually mature boars treated with naloxone. M.J. Estienne*[1], A.F. Harper[1], J.W. Knight[1], G.B. Rampacek[2], and C.R. Barb[3], [1]*Virginia Polytechnic Institute and State University, Blacksburg,* [2]*University of Georgia, Athens,* [3]*USDA-ARS, Athens, GA.*

Treatment of gilts and sows with naloxone, an endogenous opioid receptor antagonist, increases LH secretion, presumably as a consequence of increased GnRH release (Barb et al., 1991; Domest. Anim. Endocrinol. 8:15-27). The objective of the current study was to determine the effects of naloxone on circulating concentrations of LH and testosterone in sexually mature boars. Five littermate boars (Landrace x Yorkshire; 186.9 ± 8.3 kg BW and 289 d of age), fitted with indwelling jugular vein catheters, were used. Beginning 24 h after catheterization, blood samples were collected at 15-min intervals for three hours. Two h after initiation of blood sampling, boars received an i.v. challenge of naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO; 1 mg/kg BW; n = 2) or .9% saline (n = 3). Twenty-four h later the experiment was repeated, but boars that previously received naloxone received .9% saline and vice versa. Serum concentrations of LH and testosterone were not affected by day of experiment ($P > .1$). Prior to i.v. injections, LH concentrations in serum were similar ($P > .1$) between groups and averaged 0.56 ± 0.03 ng/mL. Naloxone increased ($P < 0.01$) LH concentrations, with peak values of 1.75 ± 0.22 ng/mL occurring 45 min after treatment. Injection of .9% saline had no effect ($P > 0.1$) on LH secretion. Serum testosterone concentrations averaged 1.94 ± 0.23 ng/mL prior to naloxone and increased ($P < 0.01$) to 3.06 ± 0.23 ng/mL by 1 h after treatment. Testosterone levels averaged 3.24 ± .23 ng/mL before .9% saline and decreased ($P < .04$) to 2.52 ± 0.23 ng/mL by 1 h after injection. In summary, naloxone increased serum LH and testosterone concentrations. Our results are consistent with the theory that endogenous opioid peptides suppress LH secretion in sexually mature boars.

Key Words: LH, testosterone, boars

1934 Early postnatal concentrations of plasma testicular steroid hormones as indicators of boar taint in market weight pigs. P.A. Sinclair*, E.J. Squires, and J.I. Raeside, *University of Guelph, Guelph , Ontario, Canada.*

If a relationship existed between levels of testicular steroids present in early postnatal pigs, and those present at market weight, then plasma steroid concentrations in young pigs could be used as an indicator of boar taint at market weight. Blood samples were taken from 75 Yorkshire boars at days 14, 21, and again at a market weight of 110 kg. Positive correlations were found between the concentrations of fat androstenone at market weight and the concentrations of plasma androstenone ($r = 0.46$; $P < 0.01$), estrone sulfate ($r = 0.42$; $P < 0.01$), and testosterone ($r = 0.26$; $P < 0.05$) at market weight. Significant correlations were observed between plasma testosterone concentrations at market weight and plasma concentrations of androstenone ($r = 0.57$; $P < 0.05$), and estrone sulfate ($r = 0.49$; $P < 0.05$) in early postnatal animals. However, concentrations of androstenone in fat of market weight animals were not correlated with plasma concentrations of estrone sulfate, androstenone or testosterone in early postnatal animals. This suggests that early postnatal plasma steroid production has no relationship with levels of market weight testicular steroids and cannot be used to predict the potential for boar taint. Additional findings of this study revealed that in market weight animals, a negative correlation ($r = - 0.57$; $P < 0.01$) between backfat thickness and concentrations of androstenone in fat was present. Animals were subsequently sorted according to backfat thickness into lean and fat groups of animals. There was a strong, negative correlation between backfat thickness and androstenone concentrations in fat ($r = - 0.80$; $P < 0.01$), as well as a positive correlation between plasma androstenone and concentrations of androstenone in fat ($r = 0.42$; $P < 0.05$) among the lean group of animals. This suggests that the accumulation of androstenone from plasma into fat may be affected by other factors than the hydrophobicity of androstenone, such as carcass leanness.

Key Words: Boar taint, Androstenone, Postnatal

1935 Vitamin supplements and reproductive performance in boars. I. Audet*[1], J. -P. Laforest[2], G. -P. Martineau[3], and J. J. Matte[1], [1]*Agriculture and Agri-Food Canada, Lennoxville, QC, Canada,* [2]*Laval University, QC, Canada,* [3]*cole Vtrinaire de Toulouse, France.*

The aim of the present study was to determine the effects of dietary supplements of vitamins, on vitamin status, libido and semen characteristics in young boars under normal and intensive semen collection. Sixty boars were randomly allocated from 8 to 15 months of age to one of the following diets: (B, n=15) basal diet (commercial formulation for boars) for minerals and vitamins; (B+C, n=15) B diet supplemented with vitamin C (1 g daily); (B+F, n=15) B diet supplemented with fat-soluble vitamins (3 times for D and 5 times for A, E, K higher than B); (B+W, n=15) B diet supplemented with water-soluble vitamins (10 times higher than B). After puberty (10 months of age), semen was collected at a regular frequency (3 times every 2 weeks) during 2 months. Thereafter, all boars were intensively collected (daily during 2 weeks). A resting period of 10 weeks followed. Sperm quality and quantity were recorded as well as boar libido, on all animals. Blood and seminal plasma samples were taken during this experiment to monitor vitamin status. Semen production was higher during the intensive collection period, for boars supplemented in the B+W group ($P \leq .06$) and B+F group ($P \leq .1$). During the resting period, the percentage of motile sperm was higher in B+W boars ($P \leq .03$) and, in a lesser extent, in B+F boars ($P \leq .1$), as compared to control boars. Sperm morphology and libido were not affected by treatments ($P \geq .15$). High concentrations of B6 ($P \leq .05$) and folic acid ($P \leq .01$) were observed in blood plasma of B+W boars while higher concentrations of vitamin E ($P \leq .01$) were obtained in B+F boars. In seminal plasma, an increase in folic acid concentrations was noted in B+W boars ($P \leq .01$). In conclusion, dietary supplements of water-soluble and fat-soluble vitamins appear to increase semen production in response to stress conditions such as an intensive semen collection.

The transfer of vitamins from the blood plasma into the seminal plasma seems rather limited.

Key Words: Boars, Vitamins, Semen

PSA Physiology: Reproduction and Endocrinology

1936 Laying hen response to molt induction by either pelleted alfalfa or alfalfa meal. K Medvedev*[1], C Woodward[1], X Li[1], L Berghman[1], L Kubena[2], D Nisbet[2], and S Ricke[1], [1]*Texas A&M University, Department of Poultry Science,* [2]*USDA-ARS, Food and Feed Safety Unit.*

Molting is a process commonly utilized by commercial laying facilities to extend and improve the productivity of a flock. This practice usually involves the deprivation of feed for a period of several days in order to rejuvenate the reproductive system. Due to food safety and comsumer awareness factors, alternatives to this approach are being investigated. Feeding alfalfa as an insoluble fiber source with the purpose of inducing molt is one such alternative. In this study, 118 hens were fed one of four diets: Layer Ration, Alfalfa Meal, Alfalfa Pellets, and Feed Deprivation. Hen weights were monitored 5 times during the trial to assess weight loss throughout the molting phase. During the trial, 8 hens per treatment were assessed for stress level using a tonic immobility technique. At the end of the trial, 58 hens from respective diet groups were sacrificed, and ovary weights were obtained to monitor regression of the reproductive system after a completed molting cycle. The diets correlated to the final hen weight with a p value ≤ 0.05 where feed deprivation birds lost 22.55%, alfalfa meal birds lost 16.85%, alfalfa pellet birds lost 13.03%, while birds kept on layer ration gained 2.31%. Ovary weights correlated to diet with a p value ≤ 0.05 where feed deprivation birds had a mean ovary weight of 6.37g, alfalfa meal birds had 5.08g, alfalfa pellet birds had 5.73g, and layer ration birds had 35.72g. No significant differences in stress response were noted in birds from these studies. Based on layer hen response, it appears that alfalfa can induce molt with the same efficiency as feed deprivation.

Key Words: Alfalfa, Hen Response, Molt

1937 Interleukin-1β (IL-1β) reduces the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) in granulosa cells of laying hens. M. A. Alodan*[1] and M. M. Beck[1], [1]*University of Nebraska.*

It is known that high environmental temperature (heat stress, HS) significantly reduces egg production, in part at least through disruption of reproductive hormones, progesterone (P_4), luteinizing hormone (LH), and estrogen (E_2). There are many well documented systemic effects of HS that may affect these hormones, but very little is known about local mechanisms through which HS acts. Interleukin-1 is increased by stress, including HS, and recently, in mammals, it has been shown that cytokines interleukin-1α and IL-1β play a role in ovarian function. The role(s) of cytokines in the bird remain unclear. The aim of this study was to determine the effect of HS and IL-1β on the activity of 3β-HSD, one of the major enzymes involved in steroidogenesis, in the granulosa cells (GC) of the laying hen. Two groups of hens were used in this study; one group was subjected to 24C, 30%RH (control), the other group to 36C, 60%RH (HS treatment). At the end of HS, the F1 follicles were collected from both groups and the GC dispersed enzymatically. The GC preparation from the control group was divided into three aliquots. One served as a control for the HS treatment; the other two were incubated with 100ng/ml IL-1β or without IL-1β (IL control) for 5h at 39C. Approximately 10,000 viable GC from each treatment were incubated in 1.5 ml of medium (PBS, pregnenolone, NAD, and nitroblue tetrazolium, pH 7.4) using 24-well flat-bottom plates at 39C for 90 min. After the incubation period, wells were examined with inverted microscope for dark blue formazan precipitate. A total of at least 100 cells per sample were examined to determine the percentage of cells that stained positive for 3β-HSD (dark blue cells). Both HS and IL-1β significantly reduced the percentage of 3β-HSD positive cells (P=0.000, 0.053, respectively). We hypothesize that HS reduces the activity of 3β-HSD in the granulosa, and that reduction may be in part, at least, mediated through IL-1β.

Key Words: Heat stress, Progesterone, 3β-HSD

1938 Expression of the activin type II receptors and the inhibin/activin subunits during follicular development in broiler breeder hens. A. J. Davis* and S. N. Slappey, *University of Georgia.*

There are two known activin type II receptors (ActRII and ActRIIB). An activin type II receptor must bind with an activin type I receptor to form a complex that can bind activin and then elicit downstream signaling by phosphorylation of proteins. The expression of mRNA for ActRII, ActRIIB, follistatin and inhibin/activin subunits was investigated in the follicles of broiler breeder hens. Total RNA was isolated from individual granulosa and theca layers of the F_1 through F_5 follicles, a pool of the F_6-F_7 follicles, the small yellow follicles (5-12 mm) and from the combined granulosa and theca layers of the large white follicles (2-5 mm) from 6 birds. 40 μg of total RNA per sample was run on a 1.5% agarose/formaldehyde gel and then transferred to nylon membrane for Northern analysis with chicken cDNA probes for the activin type II receptors, follistatin, the inhibin/activin subunits and GAPDH (control). Two ActRII mRNA transcripts of 6.5 and 3.7 kb were detected in all of the theca and granulosa samples. Both transcripts were equally expressed in granulosa samples, but in theca samples expression of the 3.7 kb transcript was significantly greater than the 6.5 kb transcript. ActRIIB was not detected by Northern analysis in any of the samples. Expression of the mRNA for the activin/inhibin binding protein, follistatin was detected in both theca and granulosa samples with the greatest expression found in small yellow follicle samples for both cell layers. Expression of the inhibin α-subunit was detected in the granulosa layer of all the follicles. Expression of the inhibin α-subunit was highest in the F_6-F_7 granulosa layer. Granulosa layers from large hierarchical follicles expressed the most inhibin/activin β-A-subunit, while expression of the inhibin/activin β-B-subunit was highest in nonhierarchical follicles. This is the first report, to our knowledge, of the detection of activin type II receptor mRNA in the hen ovary, and characterization of the expression pattern of the inhibin family in both the theca and granulosa layers throughout follicular development. The presence of activin receptor and follistatin mRNA in both the theca and granulosa layers of the small developing follicles suggests that locally produced activin might have a vital role in early follicular development.

Key Words: Activin receptors, Chicken

1939 Immunization of male broiler breeders against mammalian Gonadotropin Releasing Hormone. J.A. Vizcarra*[1], M.L. Rhoads[1], C.C. Hsu[1], J. Washington[1], J.L.M. Morgan[1], J. Yang[1], H. Tang[1], K. Shaffer[1], and J.D. Kirby[1], [1]*Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701.*

Twenty broiler breeder males were used to evaluate the effect of immunization against mGnRH on the development of antibody titers, adult testis weight, pulsatile LH and FSH secretion, and expression of mRNA for FSH-beta in the pituitary and LH receptors in testis. A mGnRH-fimbriae antigen (Intervet International) was emulsified in Freund's incomplete adjuvant and DEAE. At 10 wk of age (WOA), males were randomly assigned to two treatments, and received a primary immunization against mGnRH (50 g), or were not immunized. Booster immunizations (total of 60 g) were given at 3, 6 and 14 wk after the primary immunization. Weekly plasma samples were obtained from 10 WOA until the end of the experiment to evaluate titers against mGnRH and concentrations of testosterone by RIA. At 28 WOA, a jugular cannula was inserted, and blood samples (1 ml) were collected at 10-min intervals for 8 h. Plasma was stored at -20 C and analyzed for LH and FSH concentrations. At the end of the 8 h acute sampling period, males were killed. Pituitaries were removed to evaluate the expression of mRNA for FSH-beta subunit, and testes were obtained to evaluate daily sperm production (DSP), and expression of mRNA for LH receptors. There was a remarkable variation in titers between birds immunized against mGnRH. Only 2 of 10 birds had titers greater than 50% after the second booster immunization, and 4 of 10 birds had titers greater than 20%. Titers of 4 of 10 birds were