PHYSIOLOGY AND ENDOCRINOLOGY

306 Testis function, carcass traits and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. C. C. Huxsoll*, E. O. Price, and T. E. Adams, *University of California, Davis.*

The efficacy of active immunization against GnRH as a non-invasive alternative to physical castration was assessed in Hereford × Angus bull calves. Calves were randomly assigned to 1 of 4 treatment groups; unimmunized calves (n = 8), bull calves actively immunized against GnRH (n = 30), or calves castrated at 3 months of age and either non-implanted (n = 9) or implanted with Synovex (n = 9). Immunized calves received primary immunization prior to weaning and secondary immunization at feedlot entry. Anti-GnRH titer was evident in all immunized animals (final titer = 31.3 ± 2.4% of total ^{125}I-GnRH bound by 1:1000 serum dilution). Anti-GnRH antibodies were not evident in steers or unimmunized bulls. Final serum concentration of testosterone (.7 ± .2 and 3.0 ± .4 ng/ml, respectively) and testes weight at slaughter (164 ± 23 and 549 ± 35 g, respectively) were reduced ($p < .01$) in immunized, relative to unimmunized, bulls. The final live weight of non-implanted steers (472 ± 13 kg) was significantly lower that the final weight of unimmunized bulls (530 ± 20 kg). In contrast, the final live weight of immunized bulls (493 ± 8 kg) did not differ ($p > .05$) from the value noted in unimmunized bulls. Aggressive characteristics were comparable ($p > .05$) in immunized bulls and non-implanted steers and significantly lower than the level of aggressive behavior noted in unimmunized bulls. Level of intramuscular fat and quality grade were comparable ($p > .05$) in non-implanted steers and immunized bulls and significantly higher than values noted in unimmunized bulls. These data indicate that active immunization against GnRH suppresses testicular function and reduces the aggressive behavioral traits of male cattle. Feedlot performance and carcass traits are comparable in immunized bulls and steers. Taken together these data indicate that immunization against GnRH is an effective alternative to physical castration in the management of beef cattle.

Key Words: Beef bulls, Immunization, GnRH

307 Immune response, testes function, body growth and carcass characteristics of bulls immunized against GnRH at 3 to 11 mo of age. M. Finnerty[1]*, W. J. Enright[1], and J. F. Roche[2], *[1]Teagasc, Grange Res. Ctr., Co. Meath and [2]Faculty of Vet. Med., Univ. College Dublin, Ireland.*

To determine the effect of immunization (I) of bulls against GnRH at different times during pubertal development (3–11 mo of age) on immune response, testes function, ADG and carcass characteristics, 70 3-mo-old Friesian bulls were assigned to seven treatments: control (C) bulls; castrated at 3 mo (d 0; steers); I against 1 mg HSA-GnRH at: 3 and 5 (I3 & 5); 5 and 7 (I5 & 7); 7 and 9 (I7 & 9); 9 and 11 (I9 & 11); and 3, 5, 7, 9 and 11 (I × 5) mo. Animals were blood sampled for testosterone (T) and weighed every 14 d, and testes measured every 28 d, from d 0 to 560 (slaughter). Post final I, the rate of decline in antibody titres (AT) was fastest ($P < .02$) in the I9 & 11 and slowest ($P < .02$) in the I × 5 bulls. The I3 & 5, I5 & 7 and I × 5 bulls had smaller ($P < .05$) testes than the C bulls for 12–19 mo post primary (P) I compared to a maximum of 6 mo in the I7 & 9 and I9 & 11 bulls. In conclusion, testicular function was suppressed for 4–6 mo by early (3–7 mo) I and for 8.5 mo by repeated I (I × 5) during pubertal development; however, overall ADG of bulls immunized at any time from 3 to 11 mo is similar to that of C bulls when slaughtered 12 to 18 mo post final I.

Trt.	Mean AT post P I[+]	Mean ± SE days of low T*	ADG	Carcass wt.	Back fat
			(kg)	(kg)	(mm)
C bulls		43 ± 28[a]	.90[x]	322	6.9
Steers			.78[y]	274[y]	8.1
I3 & 5	20.8[b]	132 ± 45[b]	.86[x]	308[x]	5.1[x]
I5 & 7	21.8[b]	186 ± 84[bc]	.86[x]	309[x]	6.8
I7 & 9	21.9[b]	80 ± 42[a]	.88[x]	321[x]	5.3[x]
I9 & 11	12.7[a]	59 ± 24[a]	.85[x]	312[x]	6.2
I × 5	29.5[c]	238 ± 59[c]	.89[x]	316[x]	8.2
Min SED	2.13		.032	11.4	1.22

[+]% bound @ 1:2560 dilution.
[abc]Differ ($P < .05$) within column.
[x]Differ ($P < .05$) from steers.
[y]Differ ($P < .05$) from C bulls.
*T < .5 ng/ml.

Key Words: GnRH immunization, Bulls, Growth

308 The effect of delaying the pubertal rise in plasma testosterone (T) on aggressive and homosexual behavior. M. Finnerty[1], J. F. Roche[2], D. Harrington[3]*, and W. J. Enright[1], *[1]Teagasc, Grange Res. Ctr., Co., Meath, [2]Faculty of Vet. Med., Univ. College Dublin, and [3]Teagasc HQ, Dublin, Ireland.*

To determine the effect of reduced plasma T at different times relative to pubertal development (3 to 11 mo of age) on subsequent behavior, 70 3-mo-old Friesian bulls were assigned to seven treatments: control (C) bulls; castrated at 3 mo (d 0; steers); immunized (I) with human serum albumin-GnRH conjugate at 3 and 5 (I3 & 5); 5 and 7 (I5 & 7); 7 and 9 (I7 & 9); 9 and 11 (I9 & 11); and 3, 5, 7, 9 and 11 (I × 5) mo. Animals were blood sampled every 14 d from d 0 to 560 (slaughter) and duration of low T (<.5 ng/ml) determined. Behavior was observed in three phases (Ph); Ph1: each group for 1 h once every 2 wk from 9–15 mo (at pasture); Ph2: as for Ph1 from 15–23 mo (but removed from housing for observation) and Ph3: six animals/group for 1 h/d from d 553 to 556. The pubertal rise in T was delayed until 9, 11 and 13 mo of age in the I3 & 5, I5 & 7 and I × 5 bulls; however, only the aggressive scores of I × 5 bulls were less than those of C bulls and not different to those of steers during Ph2 and Ph3, and were less than those of steers during Ph1. In conclusion, delaying the pubertal rise in T until 13 mo of age suppresses subsequent aggressive but not homosexual behavior.

Trt.	Mean ± SE days of low T	Total aggressive activity score Ph1	Ph2	Ph3	Total homosexual activity score Ph1	Ph2	Ph
C bulls	43 ± 28[a]	697[x]	4546[x]	1281[x]	463[x]	424[x]	73[x]
Steers		178[y]	1300[y]	206[y]	90[y]	96[y]	4[y]
I3 & 5	132 ± 45[b]	304[y]	3704[x]	1066[x]	699[x]	390[x]	38[x]
I5 & 7	186 ± 84[bc]	125[y]	3351[xy]	890[xy]	251[y]	744[xy]	170[xy]
I7 & 9	80 ± 42[a]	194[y]	3035[xy]	943[xy]	191[y]	223[x]	40[x]
I9 & 11	59 ± 24[a]	133[y]	2961[xy]	564[xy]	280[y]	277[xy]	52[x]
I × 5	238 ± 59[c]	76[xy]	1396[y]	332[y]	147[y]	286[xy]	38[x]

[abc]Differ ($P < .05$) within column.
[x]Differ ($P < .05$) from steers.
[y]Differ ($P < .05$) from C bulls.

Key Words: GnRH immunization, Behavior, Bulls

309 Effects of transient neonatal hypothyroidism on testicular function, and serum concentration of metabolic and sex hormones in Suffolk Ram Lambs. A. H. Fallah-Rad[1]*, M. W. Connor[1] and R. P. Del Vecchio[2], *[1]University of Manitoba, Winnipeg, Canada and [2]Agriculture and Agri-Food Canada, Brandon Research Centre, Brandon, Manitoba, Canada.*

In an experiment to study the effects of transient neonatal hypothyroidism, twelve 6 wk-old Suffolk ram lambs were divided into three groups. Four control (Ctrl) lambs received placebo. Four lambs in the treatment group 1 (Trt 1) were drenched daily with 15 mg/kg BW of PTU (a potent reversible goitrogen and 5′ deiodinase enzyme suppressant) for 40 d from 42 to 82 d of age to suppress normal thyroid function. Four lambs in treatment group 2 (Trt 2) received the same treatment for 55 d. To measure the concentration of thyroxine (T4), triiodothyronine (T3), FSH and testosterone (T), blood was collected by indwelling jugular catheter at weekly intervals, every 20 minutes from 0900 to 1520 h. Scrotal circumference (SC), ADG and feed consumption (FC) were measured until 36 wks of age. From 16 to 36 wks of age, semen was collected by electroejaculation and evaluated. During the treatment period, only, mean concentration of T3 & T4 were lower in PTU treated lambs than in Ctrl ($P<.05$). Blood T and FSH levels were not different ($P>.1$) in the three groups throughout the experiment. However, the number of T peaks was greater ($P<.01$) in the Ctrl than in the hypothyroid rams. The SC, FC and ADG were similar among the three groups ($P>.1$). Viable sperm were produced by 28 wks of age in all lambs. Semen volume was not different among the three groups at 36 wks of age ($P>.05$) but sperm concentration in Trt 1 & Trt 2 groups were lower than Ctr ($P<.001$). Histological sections showed the diameter of the seminiferous tubules in the Ctrl was larger than in the PTU treated lambs ($P<.05$). In conclusion, transient early hypothyroid lambs produced less sperm compared to contemporary Ctrl lambs but testes size and sex hormones were not different.

Key Words: Ram, Neonatal, Hypothyroidism

310 Excitatory amino acids (ExAA) modulate secretion of LH and testosterone in boars. M. J. Estienne[1]*, J. M. Harter-Dennis[1], C. R. Barb[2], and T. G. Hartsock[3], *[1]University of Maryland Eastern Shore, Princess Anne, [2]USDA-ARS, Athens, GA, and [3]University of Maryland, College Park.*

In this investigation we examined serum concentrations of LH and testosterone in boars treated with n-methyl-d,l-aspartate (NMA), a potent agonist of the ExAA aspartate and glutamate, or ketamine hydrochloride (KET), a non-competitive antagonist of ExAA receptors. Blood was sampled at 15-min intervals for 8 h from 12 crossbred boars (125 ± 3 kg BW; mean ± SE) fitted with jugular catheters. At h 4 and 6, boars received i.m. injections of KET (19.9 mg/kg BW; n = 4) or .9% saline (n = 4), or i.v. injections of NMA (2.5 mg/kg BW; n = 4). Prior to injections (i.e., h 0 – 4), mean LH concentrations were similar ($P > .1$) between groups and were .29 ± .03 ng/ml. During the period after the initiation of injections (i.e., h 4 – 8), mean LH concentrations were increased ($P < .01$) in KET-treated boars (.53 ± .04 ng/ml) but were unaltered ($P > .1$) in boars injected with NMA (.29 ± .01 ng/ml) or saline (.33 ± .08 ng/ml). Concentrations of LH reached a peak of .76 ± .10 ng/ml at h 8 in KET-treated boars. Concentrations of testosterone were similar ($P > .1$) between groups prior to injections and were 7.36 ± 2.01 ng/ml. During h 4 – 8, testosterone concentrations were increased ($P < .07$) in KET-treated boars (16.77 ± 3.41 ng/ml). Concentrations of testosterone were unaffected ($P > .1$) by administration of NMA or saline. The results of this experiment are consistent with the concept that ExAA inhibit secretion of LH and testosterone in boars.

Key Words: LH, Testosterone, Boars

311 Calf sex ratio in Holstein bulls by ejaculate. J. E. Chandler*, R. W. Adkinson, and E. B. Moser, *LSU Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge.*

Biomolecular (PCR) results that implied unequal sex ratio between ejaculates was verified by examining the percentage of male calves for ejaculates of Holstein bulls. Calving ease data were collected at Eastern Artificial Insemination Cooperative and contained identification of herd, cow, sire, calving date and calf's gender (n=239,081). Technician breeding receipt data were collected and contained identification of herd, cow, sire, ejaculate and breeding date (n=320,367). Data records were merged on cow and sire and kept if calving date occurred between 280 ± 15 days after the breeding date. This produced 7550 calf records identified by sire, ejaculate, herd and gender. Male calves were scored 1 and females 0. Records were summarized by sire, ejaculate and herd. Percent males were expressed as the sum of scores divided by the total number of calves per sire, ejaculate, herd combination. Records (n=2027) were selected if the sire had at least 2 ejaculates and a total of at least 5 calves per ejaculate. Percent males were converted to logits and weighted by n*p*q, where n=number of calves per subclass, p= percent males, and q=1-p. Transformation and weighting were done to normalize values. Least squares methods were used to analyzed weighted logits. Data represented 704 ejaculates within 178 sires across 195 herds. Sire variation tested with ejaculate within sire was not a significant source ($P > .99$). Ejaculate within sire contributed to variation in %male ($P < .0001$). Overall mean was 49.79% male. The minimum and maximum ejaculate within sire mean and 90% confidence limits of %males was 11.3%<16.1%<22.5% and 63.3%<72.3%<79.8%. Both %males per ejaculate and PCR analysis per ejaculate indicated that differences in proportion of y chromosome bearing sperm between ejaculates within sires is not due to sampling variance alone under assumption that p=.5. The naturally skewed sex ratio in some ejaculates may account for observations about preponderance of single sex calf crops from some sires.

Key Words: Sex ratio, Bovine, Ejaculate

312 The effect of temporary scrotal insulation on semen quality of hair sheep rams in a tropical environment. J. R. Collins*, E. L. Hensley, and R. W. Godfrey, *Agricultural Experiment Station, University of the Virgin Islands, St. Croix.*

St. Croix White rams (312.8 ± 1.2 d of age) were used to determine the effect of acute scrotal insulation on semen quality. Six rams had insulating material (disposable diaper) placed around the scrotum (d 0) for a period of 48 h (INS) and five rams were untreated (CONT). The insulating material was held in place with cloth tape placed loosely around the scrotum above the testes. Semen was collected by electroejaculation on d –1 and 0, and then at 3 or 4-d intervals through d 42. Semen samples were evaluated for percentage of progressively motile (MOT) and live sperm (LIVE), and sperm concentration and morphology. Sperm morphology consisted of percentage of normal cells (NORM), abnormal heads (HEAD), abnormal tails (TAIL) and proximal droplets. On the d of semen collection scrotal circumference (SC) and right and left testis length were measured and used to calculate paired testes volume (PTV). During the period of insulation, the temperature between the insulating material and the scrotum (ST) and ambient temperature (AT) were recorded daily . The mean ST and AT during the 2-d period of insulation were 36.8 ± .3 and 35.4 ± .4°C, respectively. The MOT, LIVE and NORM of INS rams decreased ($P < .05$) between d 10 and 35 compared to CONT rams. In the semen of INS rams HEAD increased ($P < .005$) between d 14 and 28 and TAIL increased ($P < .005$) between d 7 and 35. Percentage of proximal droplets was similar ($P > .10$) between INS and CONT rams. Beginning on d 7 and continuing to d 38, INS rams had lower ($P < .01$) sperm concentration than CONT rams (1.49 ± .14 vs 2.60 ± .16 × 10^9 cells/ml, respectively). There was no difference ($P > .10$) in SC or PTV between the two groups. These results indicate that tropically adapted hair sheep rams are susceptible to elevated scrotal temperatures as evidenced by the decline in semen quality.

Key Words: Sheep, Semen, Temperature

313 A novel nozzle for more efficient flow cytometric analysis and sorting of X & Y-chromosome spermatozoa based on DNA content. W. Rens, G. R. Welch, and L. A. Johnson*, *Germplasm & Gamete Physiology Lab., ARS, USDA, Beltsville, MD.*

Improving the efficiency of viable sperm sexing (Beltsville Sperm Sexing Technology) by flow cytometric sorting of X- and Y-chromosome-bearing sperm based on DNA is dependent on improving the proportion of sperm that are properly orientated to the laser beam during DNA analysis. In an effort to improve the efficiency of the technology we developed a modification to a standard jet in air nozzle to improve the percentage of sperm being orientated to the laser beam. Analyzing bull sperm heads with the new nozzle with a low sample rate (<500/sec) gave the the same performance as the beveled needle. Testing the novel nozzle using rabbit, boar, mouse and human sperm heads show that the nozzle is suitable for many species. Higher sample rate experiments (2000/sec) with the novel nozzle demonstrated orientation performance superior to the regular nozzle using a beveled needle. Generally a twofold increase (from approximately 25% to 60%) in analysis rate is achieved with the nozzle. Experiments were also performed with viable intact bull and boar sperm. The percentage of intact sperm that were orientated was increased and further more was independent of sperm motility and sample flow rate. Sorting performance and accuracy of DNA measurement was not affected negatively by the use of the novel nozzle. The efficiency of sorting an average of 88% pure samples of X or Y sperm (bull and boar) was increased by more than two times using the novel nozzle system. These results substantially enhance the efficiency of the USDA Beltsville Sperm Sexing Technology for use in producing sex preselected offspring.

Key Words: X & Y-chromosome bearing sperm, Flow cytometry, Gender

314 Leptin and growth hormone (GH) receptor gene expression in adipose tissue of young pigs treated with growth hormone. R. L. Matteri[1]*, J. A. Carroll[1], T. L. Veum[2], R. S. MacDonald[2], J. A. Pardalos[2], and L. S. Hillman[2], *[1]Animal Physiology Research Unit, Agricultural Research Service, USDA and [2]University of Missouri, Columbia, MO.*

The objective of this study was to evaluate the effects of recombinant GH treatment and age on adipose tissue leptin and GH receptor gene expression in the early developmental period of pigs. Three groups of intact males were used in this study. Daily i.m. injections of GH (200 μg/kg) or vehicle were administered beginning at either 1, 4, or 9 wk of age (8 treated and 8 control animals/age group). Animals were sacrificed after 2 wk of treatment. Serum IGF-1 concentrations in GH vs control piglets sacrificed at 3, 6, and 11 wk of age were 83.5 vs 67.8, 200.5 vs 157.9, and 331.3 vs 225.1 ng/ml, respectively (LSE = 25.7; GH, P = .008; Age, P < .0001; GH × Age, P = .16). Serum IGF-1 concentrations and weight gains were highly correlated (r =.9, P < .0001), however growth was unaffected by GH treatment (P = .46). Percent body fat (11.3% overall average), evaluated by dual-energy x-ray absorptiometry (DEXA), did not differ with age (P = .29) and was not affected by GH treatment (P = .17). Levels of GH receptor mRNA in adipose tissue were highest at 6 wk of age (P = .051) and were reduced in all age groups by GH treatment (P = .002). GH receptor and leptin mRNA levels were correlated in adipose tissue (r = .69, P = .0007), however the quantity of leptin mRNA was not affected by GH treatment (P = .44). A significant decrease in leptin mRNA values was observed with increasing age (P < .0001), providing the first evidence for developmental regulation of leptin gene expression in the pig.

Key Words: Leptin, Growth Hormone, Pig

315 Melatonin feeding that simulates a short day photoperiod (SDPP) suppresses circulating insulin-like growth factor-1 (IGF-1) in pre-pubertal heifers. J. D. Smith*, L. W. Douglass, J. A. Coyne, and G. E. Dahl, *University of Maryland, College Park.*

There is evidence that photoperiod influences circulating levels of IGF-1 in many species. In ruminants, IGF-1 increases in cattle exposed to a long day photoperiod (LDPP). To test the hypothesis that melatonin feeding to mimic a SDPP would suppress circulating levels of IGF-1, 12 Holstein heifers blocked by BW, received 0mg/100kg BW/d melatonin (Con;n=6) or 4mg/100kg BW/d melatonin (Mel;n=6) for 8 weeks. All animals were exposed to a LDPP (16L:8D), however melatonin was fed at 1200h daily to mimic a short day pattern of circulating melatonin. Blood was sampled on d1 and d56 to measure growth hormone (GH) concentrations, and weekly to quantify circulating levels of IGF-1 and progesterone. Mean GH concentrations did not differ between Mel and Con animals, however an age related decline in circulating GH was observed in both groups (P=.02; Mel d1=12.1 ng/ml, d56=8.0 ng/ml; Con d1=8.9 ng/ml, d56=7.4 ng/ml). Progesterone concentrations were uniformly low in both groups thus confirming that the animals remained pre-pubertal throughout the study. Compared with controls (BW d1=156.0 kg; BW d56=194.0 kg), BW was not affected (P=.89) by melatonin treatment (BW d1=155.7 kg; BW d56=195.1 kg). Analysis of circulating IGF-1 revealed a significant (P=.02) time*treatment interaction between Mel and Con animals, with circulating IGF-1 increasing in Con animals throughout the study (d1=95.8 ng/ml, d56=111.6 ng/ml) but not changing in Mel animals (d1=93.2 ng/ml, d56=98.5 ng/ml). These results suggest that the short day pattern of circulating melatonin prevented the increase in IGF-1 observed in animals on LDPP. We conclude that modulation of circulating IGF-1 may be a mechanism whereby cattle use photoperiodic signals to regulate growth.

Key Words: Melatonin, Photoperiod, Insulin-Like Growth Factor-1

316 Stimulation of dopamine D_1 receptors suppresses basal and growth hormone-releasing factor-induced secretion of growth hormone in meal-fed steers. C. D. McMahon*, L. T. Chapin, K. J. Lookingland, and H. A. Tucker, *Michigan State University, East Lansing.*

Stimulation of dopamine D_1 receptors increases release of somatostatin (SRIF) from perifused bovine hypothalamic slices, and SRIF inhibits in vivo secretion of growth hormone (GH) from the anterior pituitary gland. Our objective was to determine whether the selective dopamine D_1 agonist SKF 38393 (RBI, Natick, MA) reduced basal and growth hormone-releasing factor (GRF)-induced secretion of GH in meal-fed steers. Holstein steers were randomly assigned within a crossover design to receive either SKF 38393 (5 mg/kg, s.c.) or vehicle (sterile water) (n=8 per group). In experiment 1, the time-course for the effect of SKF 38393 on concentrations of GH was assessed. Jugular blood was sampled at 20-min intervals from 40 min before, to 140 min after injection of SKF 38393. Basal concentrations of GH were reduced (P<0.05) between 80 and 140 min in SKF 38393-treated (8.9 ng/ml) compared with vehicle-treated (20.6 ng/ml) steers (SED=4.3). In experiment 2, recombinant Leu^{27}, Hse^{45}-bGRF (1-45) lactone (The Upjohn Company, Kalamazoo, MI; 20 μg/kg, i.v.) was injected 100 min after SKF 38393. The GRF-stimulated area under the GH curve from 100 to 160 min was lower (P<0.05) in SKF 38393-treated (793 ng·min·ml^{-1}) compared with vehicle-treated (1369 ng·min·ml^{-1}) steers (SED=194). We concluded that stimulation of dopamine D_1 receptors suppressed both basal and GRF-induced secretion of GH in meal-fed steers. These data support our hypothesis that stimulation of dopamine D_1 receptors increases release of SRIF, which, in turn, suppresses secretion of GH into blood. We speculate that SKF 38393 acted on SRIF neurons in the hypothalamus because dopamine D_1 receptors are not present in the anterior pituitary gland.

Key Words: Growth Hormone, Dopamine, Cattle

317 Effect of zeranol on transcriptional regulation of pituitary synthesis and secretion of growth hormone in growing wether lambs. M. G. Thomas[1]*, S. R. Raymond[1], J. A. Carroll[2], R. L. Matteri[2], L. Hillman[1], F. Chanetsa[1], K. J. Rozeboom[1], R. J. Lipsey[1], and D. H. Keisler[1], *[1]University of Missouri-Columbia and [2]USDA-ARS, Columbia, MO.*

The DNA binding protein, Pit-1, is a regulator of the growth hormone (GH) and prolactin genes. In rodents, Pit-1 interacts with the estrogen-estrogen receptor complex to transactivate the prolactin gene. Since estrogens increase pituitary secretion of GH in ruminants, we hypothesized that estrogen and Pit-1 would interact to enhance pituitary synthesis and secretion of GH. To test this hypothesis, 66 crossbred wethers (≤ 2 wk of age) were assigned by breed-type to either a control (CT) or one of three groups implanted with the estrogenic growth promotant, zeranol (Z, 12 mg, Ralgro®, Mallinckrodt Vet). Implant groups were: Z0 (implanted d 0, d 45, and d 90), Z45 (implanted d 45, and d 90), and Z90 (implanted d 90). Twenty-eight d after implant, serum was collected every 12 min for 4 h prior to pituitary collection from a subset of wethers from CT and Z groups (n ≥ 4 per group). Pituitary mRNA levels of GH and Pit-1 were determined with RNA slot blot analyses and densitometry. Spearmans correlation of the relationship of these factors to serum concentrations of GH, pituitary weight, and treatment are as follows (* = r P < .05):

Pituitary weight	Treatment	GH mRNA	Pit-1 mRNA	
–.11	.37*	.40*	–.10	Serum GH
	.36*	.57*	.44*	Pituitary Weight
		.08	.10	Treatment
			–.31*	GH mRNA

These data do not provide evidence to support the hypothesis that estrogen stimulates pituitary secretion of GH in ruminants through an interaction involving Pit-1. Work supported in part by USDA Postdoc Grant 95-37206-2119.

Key Words: Zeranol, Growth Hormone, Pit-1

318 Effects of suppressing cortisol following castration of bull calves on ACTH, in vitro interferon-γ production, leukocytes, acute phase proteins, growth, and feed intake. A. D. Fisher[1]*, M. A. Crowe[2], E. M. O'Nuallain[2], M. L. Monaghan[2], D. J. Prendiville[1], P. O'Kiely[1], and W. J. Enright[1], *[1]Teagasc, Grange Research Centre and [2]University College Dublin, Ireland.*

To determine the effects of reducing the plasma cortisol rise following castration, on plasma ACTH concentrations, keyhole limpet hemocyanin (KLH)- and concanavalin A (Con A)-induced in vitro interferon (IFN)-γ production, white blood cell (WBC) numbers, neutrophil:lymphocyte (N:L) ratio, plasma haptoglobin and fibrinogen concentrations, and ADG and ADFI, 40 five-mo-old Friesian bull calves (169 ± 1.7 kg) were assigned to four treatments (given on day 0): 1) control (CON); 2) oral metyrapone administration (MET); 3) surgical castration (SURG); and 4) oral metyrapone administration and surgical castration (MET + SURG). Metyrapone (3 g) was administered every 4 h from –44 to 4 h. Cortisol, ACTH, IFN-γ production, haptoglobin, fibrinogen, ADFI and ADG were not different between CON and MET calves. Compared with SURG, the MET + SURG calves had lower (P < .001) peak and mean cortisol during .25 to 1.5 h, but area under the cortisol vs time curve from 0 to 12 h did not differ (P > .39). Peak ACTH was higher (P < .05) for MET + SURG compared with SURG calves. There were no differences between MET + SURG and SURG calves in IFN-γ production, WBC numbers, and ADFI. On d 1, both MET + SURG and SURG calves had lower (P < .01) KLH- and Con A-induced IFN-γ production and higher (P < .05) neutrophil numbers and N:L ratio compared with CON calves. Haptoglobin on d 1 and 3 and fibrinogen on d 3 and 7 were elevated (P < .05) for MET + SURG and SURG compared with CON calves, while SURG had greater (P < .05) haptoglobin and fibrinogen than MET + SURG calves on d 7. ADG was lower (P < .05) in SURG than in MET + SURG calves during d 0 to 7. Metyrapone treatment partially suppressed cortisol and increased ACTH in castrated calves, but did not alter the castration-induced suppression of IFN-γ and increases in neutrophil numbers and the N:L ratio.

Key Words: Cattle, Castration, Cortisol

319 Differences in adrenal integrity between Angora and Spanish goats. C. A. Toerien[1]*, R. Puchala[1], J. P. McCann[2], and T. Sahlu[1], *[1]E (Kika) de la Garza Institute for Goat Research, Langston University, OK and [2]Oklahoma State University, Stillwater.*

Previous studies showed that Angora goats were inadequate in coping with metabolic stress. Angora goats may have sub-clinical hypoadrenocorticism relative to other goat breeds. We tested this hypothesis by measuring the adrenocortical response (plasma cortisol) in stress-tolerant (Spanish goats) and suspected stress intolerant Angora goats under conditions of simulated acute and chronic stress. Angora (n = 6; 37 ± 2 kg and Spanish (n = 6; 36 ± 2 kg) wethers were injected iv with low (.4 IU/kg) and high 2.5 IU/kg) doses of ACTH on d 1 and 4 of Exp 1 (acute stress). ACTH (15 mU/kg·min or saline 15 mL/h) was infused iv for 6 h in Exp 2 (d 15 or 18) to stimulate chronic stress in Angora and Spanish goats (n = 3/group). Basal plasma cortisol concentrations (ng/mL) before ACTH stimulation in Exp 1 and 2 were similar in Angora (4.2 ± .3) and Spanish (5.0 ± .4) goats. Response areas (ng/mL·min × 10^3) above basal during low (7.6 ± .5 vs 9.0 ± 1.7) and high (12.7 ± 1.1 vs 14.3 ± .8) levels of acute stress (Exp 1) and during chronic stress (45 ± 6 vs 35 ± 2) in Exp 2 were similar (*P* > .05) in both breeds. We conclude that suspected stress intolerance in Angora goats is not due to deficiency in adrenocortical responsiveness to ACTH.

Key Words: Angora Goat, Adrenal Function, Spanish Goat

320 Body weight, condition and postprandial energy metabolites in aged mares following daily injection with equine somatotropin. S. L. Ralston*, K. Malinowski, and R. A. Christensen, *Rutgers University, New Brunswick, NJ.*

To evaluate the effect of daily injection with equine somatotropin *eST* on BW, body condition score *BCS* and energy metabolism in aged mares, mares *20-26 yrs old* were assigned to one of two treatments: Vehicle *VEH, n=9, 0 mg eST/d* or eST *n=9, 12.5mg eST/d* for 89d. Body weights *kg*, BCS and plasma glucose *GLU*, insulin *INS*, and non-esterified fatty acids *NEFA*, μ*Eq/L* responses to feeding were measured at ±2, 43, 85 and 127d. Mares were initially fed hay and grain rations at 110% NRC*1989* Mcal. Rations were adjusted at day 60 due to weight loss in both groups. Final Mcal intakes were 133%*VEH* and 139%*eST* NRC maintenance recommendations. Weight and BCS changes did not differ between groups during treatment *P > .5* but eST mares tended *P < .1* to have greater weight loss and decreased BCS 38d after eST treatment was ended *127d*. At 85 d relative to VEH, eST treated mares had increased *P < .05* prefeeding INS *13±3.2 vs 23.0±3.0* μ*IU/mL, respectively* and NEFA *93±8 vs 320±32* μ*Eq/L, respectively*. Relative to VEH, postprandial INS was increased *p<.001* in eST treated mares at 43d *89±16 vs 183±17* μ*IU/mL, respectively* and 85d *79±14 vs 243±21* μ*IU/mL, respectively* as was postprandial NEFA at 43d *81±5 vs 100±5* μ*Eq/L, P < .05*. GLU was not affected *P > .1* by eST. Daily eST caused insulin resistance and NEFA mobilization in aged mares. Aged mares may require higher Mcal intakes for adult maintenance than currently recommended by NRC.

Key Words: Equine, Energy metabolism, Somatotropin

321 Chronic effects of equine somatotropin (eST) on plasma somatotropin and insulin-like growth factor (IGF-I) concentrations and ultrasonic muscle thickness in aged mares. K. Malinowski*, R. A. Christensen, S. H. Bokman, and H. D. Hafs, *Rutgers, The State University of New Jersey, New Brunswick.*

Mares aged 20-26 yr were given daily sc injections of eST (vehicle or 12.5 mg; n = 9 and 9, respectively) for 12 wk. Blood samples were taken weekly immediately before feeding and eST injection. Hormone concentrations were analyzed by radioimmunoassays validated for horses. Linear scan (Aloka 500V) ultrasound measurements of lumbar, thigh, and proximal and distal gluteal muscle thickness (mm) were taken on d 46 and 87 of eST treatment, and on d 40 post-treatment. Data were analyzed by ANOVA for repeated measures. Weekly plasma somatotropin sampled in blood immediately prior to daily injection was elevated (P < .001) in mares given eST compared to vehicle treated mares; (range 3.5-10 ng/mL, and 1.8-3.4 ng/mL, respectively). This rise was 4- to 5-fold in eST treated mares from wk 3-12. Plasma somatotropin remained elevated about 2.5 fold (P < .001) 6 wk after cessation of treatment. Plasma IGF-I in the same samples increased nearly 3-fold (P < .001) in eST- compared to vehicle-treated mares; (range 323-428 ng/mL, and 138-158 ng/mL, respectively). IGF-I remained elevated (P < .01) for 1 wk after eST treatment ended. Muscle thickness was not affected (P > .05) by 89 d of eST treatment. We conclude that daily injection of eST leads to sustained increases in both plasma somatotropin and IGF-I, but no change in muscle thickness. The cause of the sustained increase in plasma somatotropin for 6 wk after cessation of eST treatment remains to be elucidated.

Key Words: Horse, Somatotropin, Muscle

322 Chronic recombinant equine somatotropin (eST) administration does not affect aerobic capacity or exercise performance in geriatric mares. K. H. McKeever*, K. Malinowski, R. A. Christensen, and H. D. Hafs, *Rutgers, The State University of New Jersey, New Brunswick.*

This experiment tested the hypothesis that chronic eST administration would increase aerobic capacity and improve exercise performance in old mares. Fifteen, healthy, unfit, aged (21-26 yrs) mares were randomly assigned to a daily treatment with eST (12.5 mg/d, IM, n=7) or vehicle (3 ml/d, IM, n=8) for 85 d. Aerobic capacity and exercise performance were measured using a standardized exercise test (SET) performed on a high speed treadmill. Tests were conducted before (–21d), during (+43 d and +85 d) and after (+127 d) treatment. During the SET, resting data were collected and then the horses ran up a fixed 6% grade, starting at 4 m/s, with a 1 m/s increase every 60 s (omitting 5 m/s) until fatigue. Oxygen uptake (VO_2) was measured using an open flow calorimeter and blood lactate concentration (LA) via a lactate analyzer. Venous blood samples (10 mL) were collected at rest, during the last 10 s of each step, and after exercise, and used to measure LA, plasma protein concentration (PP), hematocrit (HCT), and the plasma concentrations of creatine kinase (CK) and aspartamine transferase (AST). There were no differences ($P > .05$) in resting VO2, LA, TPP, or HCT due to treatment or test time. Furthermore, there were no differences ($P > .05$) in maximal oxygen uptake(VO_{2max}), top run velocity, run time, watts at VO_{2max}, velocity to produce a lactate of 4 mMol/L (VLA4), watts at VLA4, peak HCT or peak LA. Finally, there were no differences ($P > .05$)in resting or post- exercise CK or AST. These data indicate that chronic eST administration does not affect aerobic capacity or indices of exercise performance in aged mares.

Key Words: Horse, Somatotropin, Exercise

323 Effect of day of the estrous cycle on timed insemination in dairy heifers. F. Moreira*, R. L. de la Sota, T. Diaz, and W. W. Thatcher, *University of Florida, Gainesville.*

Dairy heifers submitted to a timed insemination (TI) protocol, involving use of gonadotropin releasing hormone agonist (GnRHa) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), have a greater incidence of short return intervals (< 16 days) and lower conception rates. Present objective was to identify stages of the estrous cycle in which a TI program is initiated that may compromise fertility to TI. Dairy heifers (n=25) were assigned randomly to five groups that received a GnRHa injection (Buserelin Acetate, Receptal® Hoechst-Roussel Agri-Vet, Somerville, NJ; 8μg, im) at either d2, d5, d10, d15, or d18. Day 0 was day of ovulation as determined by ultrasound. Heifers were injected 7 days later with $PGF_{2\alpha}$ (Lutalyse®, Pharmacia-Upjohn Co., MI; 25mg, im). At 36 hours after $PGF_{2\alpha}$, GnRHa was injected and heifers inseminated 16 hours later. Plasma progesterone (P_4) concentrations were measured daily until 16 days after TI. Ultrasound of ovarian structures was performed daily throughout the TI protocol and then every other day until 16 days after TI. Ultrasound diagnosis of pregnancy was made at 28 days after TI. Heifers injected at day 15 regressed the original and/or induced corpus luteum (CL) 1 day prior to $PGF_{2\alpha}$ injection, and this was reflected by differences in P_4 profiles ($P<0.001$). Rate of growth of the preovulatory dominant follicle for the day 15 group was greater than the other groups ($P<0.025$). Heifers injected at day 15 ovulated before TI (3/5), and this did not occur in other groups ($P<0.025$). Pregnancy rates for heifers initiating the TI protocol at day 15 (0/5) differed compared to the other groups (9/19; $P<0.05$). Thus, in heifers, initiation of TI on day 15 of the estrous cycle results in failure to synchronize ovulation for TI.

Key Words: Timed insemination, Dairy heifers

324 Ultrasonic evaluation for the time of ovulation in ewes treated with norgestomet and pregnant mare serum gonadotropin during the spring and fall breeding seasons. B. E. Cardwell*, G. Q. Fitch, and R. D. Geisert, *Oklahoma State University, Stillwater, OK and USDA-ARS, Ft. Reno, OK.*

Utilization of progestins and follicular stimulants have provided reasonable techniques for estrous synchronization, but the time of ovulation relative to removal of the progestin is unclear. The objective of this study was to monitor follicular development and time of ovulation in ewes during natural and synchronized estrous cycles. Ovaries of sixty Dorset and Rambouillet × Dorset ewes were evaluated during Spring and Fall breeding seasons (n = 30 per season). Ewes were randomly assigned to one of three treatment groups (n = 20/group): control (C) given 5 mg i.m. prostaglandin $F_{2\alpha}$ (Lutalyse, Upjohn Company, Kalamazoo, MI) 9 days apart, 10 day norgestomet implant only (I), and 10 day norgestomet implant + 500 IU i.m. pregnant mare serum gonadotropin (PMSG) at implant removal (PI). Onset of estrus was detected with the HeatWatch Estrus Detection System (DDX Inc., Boulder, CO). Ovaries were monitored via rectal ultrasonography every six hours to determine time intervals from implant removal to onset of estrus (EST), implant removal to ovulation (OVUL), and onset of estrus to ovulation (INT). Only 7 out of 20 (35%) C ewes responded to the synchronized estrus, compared to 13 out of 20 (65%) I and 14 out of 20 (70%) PI ewes ($P < 0.10$). EST and INT were not effected by breeding season ($P > 0.10$), however OVUL was longer ($P < 0.10$) during Fall breeding (79.3h) compared to Spring breeding (70.8h). INT was reduced ($P < 0.05$) in the C ewes (20.9h) in comparison with I (35.9h) and PI (38.0h) ewes. Treatment mean OVUL was prolonged ($P < 0.10$) in the I group (79.4h) compared to the PI group (70.6h), and mean EST was also lengthened ($P < 0.10$) for I ewes (43.5h) compared to PI ewes (34.9h). Present data indicates that ovulation occurs on average 70 to 80 hours after implant removal in ewes treated with norgestomet, and PMSG advances time to ovulation from implant removal.

Key Words: Ewe, Estrus synchronization, Ultrasound

325 The effect of duration of nutritional anestrus and GnRH treatment on pituitary LH, FSH and GnRH receptors in cows. J. A. Vizcarra[1]*, R. P. Wettemann[1], and T. D. Braden[2], *[1]Oklahoma Agricultural Experiment Station, [2]Auburn University, Auburn, AL.*

Twenty-four nutritionally anestrous cows were used in a 2 × 2 factorial to determine the effect of the duration of the suppression of endogenous GnRH stimulation and the response to GnRH treatment on pituitary LH, FSH and GnRH receptors. Cows were used 3 wk after they became nutritionally anestrus (SHORT-NA) or 12 wk after they became nutritionally anestrus (LONG-NA) and were infused during 2.5 min once every hour with GnRH (2 μg i.v. in .9 mL of saline) or Saline. After 5 d of infusion with GnRH, pituitary glands were removed and analyzed for concentrations of LH, FSH and GnRH receptors. Duration of the suppression of endogenous GnRH stimulation influenced ($P < .02$) FSH concentrations (.038 and .057 μg/mg of pituitary for SHORT-NA and LONG-NA respectively; $P < .02$), however, concentrations of LH and GnRH receptors were not affected. There was a significant effect of GnRH treatment on LH and FSH concentrations but not on GnRH-receptors in the pituitary. Concentrations of LH were decreased ($P < .1$) when animals were infused with GnRH (.53 and .35 μg/mg of pituitary for Saline and GnRH, respectively). Similarly, FSH concentrations were decreased ($P < .003$) when cows were infused with GnRH (.060 and .035 μg/mg of pituitary for Saline and GnRH, respectively). Concentrations of GnRH receptors were similar (10.4 ± 1.2 fmoles/mg of protein) for all treatments. We concluded that the duration of the suppression of endogenous GnRH stimulation in nutritionally anestrous cows differentially regulates LH and FSH concentrations in the pituitary, and pulsatile GnRH treatment decreases LH and FSH concentrations in the pituitary.

Key Words: GnRH, LH, FSH

326 Serum FSH, plasma estradiol and IGF-I before the onset of nutritionally induced anovulation in beef heifers. I. Bossis*, S. D. Welty, R. P. Wettemann, J. A. Vizcarra, and L. J. Spicer, *Oklahoma Agricultural Experiment Station, Stillwater.*

To evaluate secretion of FSH and concentrations of estradiol (E_2) and IGF-I preceding nutritionally induced cessation of ovarian cycles, eighteen cyclic Angus × Hereford heifers were used. During each of two replicates, three heifers were fed to maintain BCS (M), while six heifers were fed a restricted diet (R) to lose 1 % of their BW/wk. At the initiation of the study, estrous cycles of all heifers were synchronized to a length of 16 d. Ovaries of M and R were monitored daily by ultrasonography to determine the cycle of anovulation. FSH concentrations were determined in blood samples collected every 10 min for 8 h on d 2 and d 15 (d 0 = estrus) and E_2 and IGF-I concentrations were quantified in daily samples collected from d 8 until ovulation during the last two cycles before anovulation (cycle –2 and –1). During cycle –2, FSH concentrations were not influenced by nutritional treatment. During cycle –1, mean concentrations, pulse frequency and amplitude of FSH, were greater ($P < .05$) in R heifers (.54 ng/mL, 5 pulses/8 h and .26 ng/mL respectively) compared with M heifers (.23 ng/mL, 1.7 pulses/8 h and .09 ng/mL, respectively), on d 15. On d 2 of cycle –1, R heifers had greater ($P < .05$) FSH pulse frequency (4.2 pulses/8 h), compared with M heifers (2.3 pulses/8 h). A treatment × cycle × day interaction was observed for E_2 concentrations ($P < .0001$). M heifers had greater E_2 concentrations in both cycles –2 and –1, however the preovulatory surge in R heifers occurred only in cycle –2. There was a treatment × cycle interaction ($P < .01$) for IGF-I concentrations. M heifers had greater IGF-I concentrations (91.4 and 96.8 ng/ml for cycles –2 and –1, respectively) compared with R heifers (19.5 and 11.2 ng/ml for cycles –2 and –1, respectively). We conclude that FSH is increased and E_2 and IGF-I are decreased in the two cycles before nutritional induced anovulation in beef heifers.

Key Words: FSH, IGF-I, Nutritional Anestrus

327 Serum concentrations of LH increase in feed restricted ewes in response to chronic intracerebral infusion of insulin (± glucose). J. A. Daniel*, M. G. Thomas, C. S. Hale, and D. H. Keisler, *University of Missouri, Columbia.*

Nutritional status of an animal influences its reproductive efficiency. Glucose is known to be the preferred energy substrate of brain tissue, and insulin is a regulator of cellular glucose uptake. The objective of this study was to evaluate the effect of an 8 d lateral cerebroventricular (LCV) infusion of insulin and(or) glucose on pulsatile secretion of LH in ewes in which LH secretion was suppressed due to nutrient restriction. Twenty-two ewe lambs (32 wk of age) were ovariectomized, fitted with two LCV cannulae, and feed restricted for 6 wk (fed 33% of NRC requirement). Artificial cerebrospinal fluid (aCSF) or aCSF containing physiological concentrations of insulin (INS; 40 mU · ml^{-1}), glucose (GLU; 5 mg · ml^{-1}), or INS+GLU was infused into the LCV. INS, GLU or the combination (INS 0 to 80 mU · h^{-1} and GLU 0 to 10 mg · h^{-1}) was delivered with the mass increasing linearly from d 1 to d 8 ($n \geq 5$ ewes per group). Jugular serum was collected every 12 min for 4 h on d 0, 2, 4, 6 and 8. Serum concentrations of LH were determined by RIA. Mean serum concentration of LH per 4 h was compared using orthogonal polynomial contrasts. Chronic LCV infusion of INS and INS+GLU resulted in increased ($P < .06$) mean LH when compared to aCSF treated ewes on d 2 (13.8 ± 1.8 and 12.5 ± 1.3 > 8.0 ± 3.3 ng · ml^{-1}). Mean LH was also greater ($P < .07$) in INS treated ewes relative to aCSF on d 4 (14.8 ± 2.0 > 7.4 ± 3.0 ng · ml^{-1}. Patterns of LH secretion in aCSF treated ewes were similar on d 0 through 8 ($P = .4$). In summary, serum concentrations of LH increased in feed restricted ewes LCV-infused with INS and remained unchanged in aCSF-infused controls. These data provide evidence that insulin may be an important component of hypothalamic mechanisms regulating the secretion of LH in undernourished ruminants. Work supported in part by USDA Grants 96-35203-3209, 94-38420-0985 and Postdoc Grant 95-37206-2119.

Key Words: Insulin, Glucose, Luteinizing Hormone

328 Cryopreservation of swine embryos: In vitro and in vivo developmental competence. J. R. Dobrinsky, V. G. Pursel, C. R. Long, and L. A. Johnson, *Germplasm & Gamete Physiology Laboratory, U. S. Department of Agriculture, Beltsville, MD.*

Pig embryos are highly sensitive to chilling injury, making them difficult to cryopreserve. This is less apparent in embryos of most mammalian species and is linked to the high lipid content and fragile cytoskeleton of pig embryos. Many cryoprotective agents act to depolymerize cytoskeletal components prior to cooling although this may be toxic to cells. We documented microfilament (MF) disruption during vitrification and utilized a MF inhibitor, cytochalasin-b (cyto-b) to stabilize MF. Laser scanning confocal microscopy revealed reduced fluorescence intensity of MF in cyto-b treated embryos, indicating successful MF depolymerization prior to cryopreservation. Morulae and early blastocysts (MB; n=34), expanded blastocysts (XB; n=52) and hatched blastocysts (HB; n=120) were vitrified with or without cyto-b. While MB did not survive cryopreservation (0%), treatment with cyto-b did not improve their viability (6%, $p>0.05$). However, cyto-b significantly improved XB (22 vs 60%; $p<0.01$) and HB (28 vs 90%; $p<0.01$) development in vitro. Although cyto-b-treated XB (60%) have improved viability after vitrification, their development is still lower than cyto-b treated HB (90%; $p<0.05$). Cyto-b treated and vitrified HB were transferred into two recipient females after warming. Four live and developmentally normal fetuses were recovered at 25 days of gestation from one recipient. Additional transfers of vitrified embryos are ongoing to determine the efficiency of in vivo development after cryopreservation. These experiments show that the cytoskeleton is affected during vitrification, and that MF depolymerization prior to cryopreservation improves embryonic development.

Key Words: Pig, Embryo, Cryopreservation

329 Modified methods for in vitro production of swine embryos. C. R. Long*, J. R. Dobrinsky, and L. A. Johnson, *Germplasm & Gamete Physiology Laboratory, ARS, USDA, Beltsville, MD.*

The utilization of in vitro produced pig embryos for research is dependent upon the development of an optimized and effective methodology. Our objective was to establish a consistent in vitro embryo production system to compliment sperm sexing and cryopreservation experiments. Ovaries from prepuberal gilts were transported in saline at 25°C within 6 hours post mortem. Cumulus oocyte complexes (COC) were aspirated from 2-8 mm follicles, washed in Hepes-PVA and selected for in vitro maturation by modified methods of Funahashi *et al*. (JRF 1994;101:159). Maturation media (MM) consisted of NCSU37 without BSA supplemented with 5 mg/ml insulin, 10.0% porcine follicular fluid, 50 μM β-mercaptoethanol, 0.6 mM cysteine, 10 ng/ml EGF, 10 U/ml PMSG, 10 U/ml hCG and 1.0 mM db-cAMP. COC were cultured 22 h before washing and further culture in MM without PMSG, hCG and db-cAMP for 22 h. Fifty denuded oocytes were transferred to 100 μl drops of fertilization medium (FM; Yoshida *et al*. Therio. 1993; 39:1303). Fresh semen was diluted in FM to a final sperm concentration of 2×10^4 motile sperm/ml. Sperm and oocytes were coincubated at 38.7°C for 6 h in 5.0% CO_2 and air. Zygotes were cultured in 500 μl NCSU23. Penetration, cleavage and blastocyst development rates were determined at 18, 44 and 144h, respectively. A total of 646 zygotes were cultured in 8 replicates. Monospermic penetration averaged(%±SEM) 37.3±7.9 and mean polyspermy was 36.0±12.0. Cleavage rate was 50.4±13.7 resulting in 52.5±8.52 blastocysts. Blastocyst development from total zygotes 26.1±7.1 and mean cell number of resulting embryos was 60.3±25.5. These procedures consistently produced blastocysts utilizing low sperm numbers and will be used with the Beltsville Sperm Sexing Technology to produce gender preselected embryos in vitro.

Key Words: Swine, In vitro, Embryo

330 Elements of a transcervical embryo transfer procedure for sheep. M. C. Wulster* and G. S. Lewis, *Virginia Polytechnic Institute and State University, Blacksburg.*

We initiated experiments to determine whether exogenous estradiol (E2) and oxytocin (OT) can be used to dilate the cervix and improve transcervical embryo transfer procedures for sheep; but, embryo quality decreased as the superovulatory response to FSH increased, and we were concerned that E2–OT treatment may alter luteal function. Thus, an experiment was conducted to determine the dose of oFSH needed to induce approximately six corpora lutea (CL). Norgestomet implants were used to synchronize estrus in Hampshire and Hampshire × Dorset ewes (n = 23). Ewes received a total of either 0, 18, 27, or 36 mg of oFSH, which was injected i.m. at –24, –12, 0, 12, 24, and 36 h relative to implant removal. The dose at each respective time was 19.4, 19.4, 16.7, 16.7, 13.9, and 13.9% of the total. Ewes received 400 IU of PMSG i.m. at –24 h. The CL were counted laparoscopically on d 6 (d 0 = estrus). Number of CL increased linearly ($P < .01$) with dose of oFSH; 1.8, 3.6, 6.3, and 11.2 CL/ewe, respectively. In another experiment, 32 ewes were assigned to a 2 × 2 factorial array of treatments. On d 7, ewes received i.v. injections of either 100 αg of E2 in 5 mL of 1:1 saline:ethanol or 5 mL of 1:1 saline: ethanol; 12 h later, ewes received i.v. injections of either 400 USP units of OT or saline. Jugular blood was collected on d 7, 8, 9, 10, 12, 14, 16, and 18. The main effects of E2 and OT did not affect progesterone (P4) concentrations or duration of the estrous cycle, and the E2 × OT interaction was not significant. The P4 concentrations changed ($P < .01$) with day after estrus. We conclude that a 400 IU priming dose of PMSG and a total dose of 27 mg of oFSH can be used to induce our target number of CL, and presumably embryos. Also, because the E2–OT treatment did not affect luteal function, it can be used to dilate the cervix and permit us to transfer embryos transcervically.

Key Words: Sheep, Oxytocin, FSH

331 Chromosomal abnormalities in murine oocytes matured *in vivo* or *in vitro*. S. Y. A'arabi*, J. E. Chandler, and J. D. Roussel, *Louisiana State University Agricultural Center and Louisiana Agricultural Experiment Station, Baton Rouge.*

Chromosomal abnormalities of oocytes matured *in vivo* and *in vitro* were compared using 10 virgin CD-1 mice with 2 replications for in vivo or in 4 different culture media. The antral follicles of virgin CD-1 mice were primed with 7.5 IU PMSG for 48 h prior to treatment with 5.0 IU hCG (*i.p.*) 16 h before collecting oocytes (*in vivo*) or isolating and culturing cumulus-oocyte complexes for 16 h at 37 °C (*in vitro*) in minimal essential medium (MEM) with Earle's salt, Waymouth MB 752/1 (MB 752/1), BGjb medium (BGjb), and Tissue Culture Medium-199 (TCM-199). The chromosomes were C-banded to enable an objective analysis of chromosomal number. The data were analyzed by Chi Square. The results showed that none of the oocytes matured *in vivo* were at metaphase I (M I) while a proportion of oocytes matured in MEM (12.7%), TCM-199 (21.0%), BGjb (27.4%), and MB 752/1 (29.1%) were found to be at M I. All oocytes matured *in vivo* (100%) developed to metaphase II (M II) compared to MB 752/1 (70.9%), BGjb (72.6%), TCM-199 (79.0%), and MEM (87.3%). There were no hyperploid oocytes matured in TCM-199 (0.0%) and *in vivo* (0.0%) but there were in MEM (1.1%), BGjb (2.6%), and MB 752/1 (2.8%). The frequencies of polyploid M II oocytes were low *in vivo* (.56%), BGjb (.64%), MEM (.70%), MB 752/1 (1.90%), and TCM-199 (2.0%). The frequency of hypoploid M II oocytes matured in TCM-199 (4.0%) was lower ($P < .01$) compared to MEM (11.3%) and MB 752/1 (14.0%) but it was not different ($P > .05$) from *in vivo* (6.8%), and BGjb (7.1%). The frequency of premature centromere separation (PCS) in M II oocytes *in vivo* (9.6%) was lower ($P < .05$) than in BGjb (20.5%), TCM-199 (24.5%), MEM (26.9%), and MB 752/1 (38.3%). The results suggest that the media used for oocyte maturation caused more maturation delay (being blocked at M I), premature centromere separation, polyploidy, and aneuploidy (such as, hyperploid, hypoploid) than oocytes matured *in vivo*.

Key Words: Oocytes maturation, Chromosomal abnormalities

332 Ovine blastocoele formation in vitro is influenced by human sera. M. D. Fanning*, H. R. Sawyer, and R. V. Anthony, *Colorado State University, Fort Collins.*

Sheep embryos cultured in synthetic oviduct fluid (SOF) supplemented with 20% heat-inactivated human sera are known to form blastocysts earlier, and result in lambs born with heavier birth weights. Embryos cultured in SOF supplemented with amino acids and bovine serum albumin (SOF+aaBSA) result in lambs born with birth weights similar to in vivo controls. It was hypothesized that embryos cultured in SOF supplemented with 10% heat-inactivated human sera (SOF+HS) would develop similar to embryos cultured in SOF+aaBSA. A total of 18 ewes, in 3 replicates, were estrous synchronized, superovulated, and mated to fertile rams. Embryos were surgically collected 24h after mating, washed in synthetic oviductal fluid and randomly assigned to one of two culture treatments: 1) SOF+HS (n=57 embryos); or 2) SOF+aaBSA (n=47 embryos). Four embryos were placed into a 20μl drop of medium under paraffin oil in an atmosphere of 5% CO_2 in air. Embryos were transferred into fresh medium every 48h. Stage of development was evaluated every 24h for 6d. Additionally, 2 ewes, serving as controls, were estrous synchronized, superovulated, and mated to fertile rams. Embryos were surgically collected 7d after mating and number of blastocysts formed were determined (n=16 embryos). Percent blastocoele formation by day was compared using Chi-square analysis. No blastocoele formation occurred prior to d4 in vitro (d5 in vivo). Twenty-two percent of embryos cultured in SOF+HS developed into blastocysts on d4 in vitro compared to zero for SOF+aaBSA ($P<.01$). After d4, there was no difference ($P>.10$) observed in the number of embryos reaching the blastocyst stage with all embryos reaching blastocoele formation by d6 in vitro (d7 in vivo; $P>.10$). In conclusion, our data confirmed that culturing embryos in the presence of human sera, even at a reduced concentration, resulted in early blastocoele formation. It will be of interest to determine which component(s) of human sera stimulate early blastocoele formation and the postulated enhancement of fetal growth.

Key Words: Human sera, Embryo, Sheep

333 Identification of sites of production of a seminal fFertility associated antigen (FAA) from rams. J. N. Oyarzo* and R. L. Ax, *University of Arizona, Tucson.*

Sperm bind and are functionally modified by protein secretions of the accessory sex glands. Binding of accessory gland heparin-binding proteins to sperm modifies heparin-sperm interactions, altering the ability of sperm to undergo capacitation. A monoclonal antibody has been produced which recognizes a seminal 31 kilodalton protein in sperm membranes from bulls, rams, boars, and stallions. Presence of that protein on bovine sperm corresponds to an 18 percentage point increase in fertility compared to bulls with sperm lacking that peptide. The protein is referred to as fertility-associated antigen (FAA). The objective of this study was to determine whether FAA was produced in seminal vesicles, prostates, or bulbourethral glands from rams. Accessory gland tissues were obtained immediately after slaughter of four yearling rams. Glands were homogenized in 10% SDS, then frozen. Thawed samples were diluted to a concentration of .5% SDS with PBS, and centrifuged (35,000 g) for 45 min. Supernatants were applied to an Extracti-Gel D column to remove SDS, then extracts were applied to a reverse phase (RP)-HPLC C4 column. The mobile phase was a 40 min linear gradient from 5% to 70% acetonitrile in 0.1% (w:v) trifluoroacetic acid. Proteins collected in each 5 min fraction were separated using SDS-PAGE. Western blots with the monoclonal antibody for FAA were detected using enhanced chemiluminescence (ECL) and autoradiography. Results indicated that protein patterns eluting from RP-HPLC differed among tissue source. FAA eluted when the mobile phase reached a concentration of 42% acetonitrile, which is the same conditions where bovine FAA is detected. FAA was present in protein extracts from the vesicular and bulbourethral glands. However, FAA was not detected in the proteins extracted from prostate glands. In conclusion, the 31 kilodalton protein associated with greater fertility (FAA) is produced in the seminal vesicles and bulbourethral glands of rams.

Key Words: Ram, Fertility protein, Accessory glands

334 Fertility differences between rams corresponding to HPLC profiles of spermatozoal proteins. B. B. Carpenter[1]*, J. N. Oyarzo[2], C. A. Taylor[3], W. S. Ramsey[4], and R. L. Ax[2], *[1]Texas Agricultural Extension Service, Ft. Stockton, [2]University of Arizona, Tucson, [3]Texas Agricultural Experiment Station, Sonora, and [4]Texas A&M University, College Station.*

Identification of a hydrophobic 31 kDa protein in spermatozoal extracts from beef bulls corresponds to an 18 percentage point increase in fertility compared to bulls lacking that protein. That protein can be purified by reverse phase HPLC (RP–HPLC). Objectives of this study were 1) to quantify hydrophobic proteins present in ram seminal fluid and on sperm membranes and 2) to determine if those proteins were associated with individual ram fertility. Semen from five rams (1.5 to 3 yrs-of-age) was collected by electroejaculation. Rams were mated to mature ewes in single-sire pastures. Three consecutive breeding periods (BP; 24, 22, and 21d; respectively) with a 5d non-breeding period between each BP were used to identify individual sires. Native range breeding pastures (21.5 ± 3.1 ha) were located near Sonora, TX. All rams scored satisfactory on breeding soundness examinations. Pregnancy rate (PR) and fecundity for the first BP was determined by ultrasound at 43 to 66d. Differences in PR among sires were detected by Chi-square ($p<.01$). High fertility (HF) rams (n=3) were 41 percentage points more fertile ($p<.01$) than low fertility (LF) rams (n=2). PR was 87, 80, and 70% vs. 30 and 45% for HF vs. LF rams, respectively. Twins detected among pregnant ewes ranged from 33 to 87.5% by sire ($p<.05$). Hydrophilic and hydrophobic protein profiles in both seminal fluid and sperm membranes from five rams were characterized by RP–HPLC. Seminal fluid and SDS detergent extracts of sperm cells were injected onto a RP C4 column and separated with a linear gradient from 5% to 70% acetonitrile in .1% (wt:vol) trifluoroacetic acid over 40 min. For comparison purposes, profiles obtained from RP–HPLC were divided into 5 min windows starting at 5 min, and percentages of total protein in each consecutive time period were compared. Differences in sperm membrane hydrophobic protein profiles were detectable between 30 to 35 min (42% to 56% acetonitrile). Protein concentrations in that window were lower ($p<.05$) in LF rams compared to HF rams (O% and 9.9%, respectively). These results indicate that increases in percentage of hydrophobic proteins in ram spermatozoal extracts may correspond to differences in fertility.

Key Words: Rams, Fertility, Sperm proteins

335 Relationships among motility, morphology and fertility estimates for boar semen. W. L. Flowers* and Z. A. Turner, *North Carolina State Univ., Raleigh.*

A study was conducted to characterize relationships among motility, morphology and fertility estimates of boar semen. Semen was obtained from 12 mature, crossbred boars once per week for 26 weeks. Evaluations for motility (MOT), morphology (MOR) and acrosome integrity (ACR) were conducted for each ejaculate. A portion of each collection was processed for *in vitro* fertilization procedures, while the remainder was extended and used to inseminate sows(n=8/boar/week). All insemination doses were used within 36 hours of collection. Sperm penetration rates (SPR),farrowing rates and litter size were used as estimates of fertility, respectively. Semen parameters were divided into 10 groups, 0-9%, 10-19%, etc., which were treated as an independent variable in the statistical model along with boar and week. Farrowing rates, litter size and SPR were considered dependent variables. In general, farrowing rates, litter size and SPR were not different($P > .2$) among ejaculates exhibiting more than 60% MOT. However, sows bred with semen with less than 60% motile had lower ($P < .05$) reproductive performance than females inseminated with semen of 60% MOT or greater. Similar response patterns ($P < .05$) were observed for fertility estimates for MOR and ACR with 70% and 50% being the levels where performance began to decline, respectively. However, ejaculates with greater than 90% MOR or 70% ACR tended to result in larger litters ($P < .1$) and higher SPR ($P < .08$) than those with 70 - 89% MOR or 50 - 69% ACR, respectively. Finally, significant boar by MOT, MOR and ACR scores were present ($P < .05$). This interaction was attributed to 4 boars, whose fertility tended to be low, when semen parameters were high or vice versa. In summary, these data indicate that common microscopic estimates of semen quality are good qualitative, but poor quantitative indicators of semen fertility.

Key Words: Swine, Spermatozoa, Fertility

336 Assessment of spermatozoal quality in extended cooled equine samples using the reducible dye resazurin. J. A. Dean[1]*, S. A. Ericsson[1], A. R. Comstock[1], and J. E. Bruemmer[2], *[1]Sul Ross State University, Division of Range Animal Science, Alpine, TX and [2]New Mexico State University, Las Cruces.*

The objective of this study was to develop an assay for assessing concentration, total number of motile and progressively motile spermatozoa in extended cooled equine samples. This assay could be beneficial to horse producers for evaluating extended cooled samples prior to artificially inseminating a mare. Resazurin dye (blue color) is reduced by metabolically active spermatozoa to resorufin (pink color). A total of 28 samples from 15 stallions were collected using Missouri (n=21) and Colorado (n=7) artificial vaginas. Resazurin (0.5 μL from a 6.77 μM solution) was added to 1 mL aliquots of Kenney's extended cooled samples, incubated at 37°C until visual reduction to resorufin or a maximum of 160 minutes had elapsed. Samples were assessed microscopically at 250× to determine if they contained at least the minimal number of spermatozoa (20×10^6/mL), total number motile spermatozoa (10×10^6/mL) and number of progressively motile spermatozoa (5×10^6/mL) recommended for a 50 mL artificial insemination dose. Reduction times of resazurin from blue to pink were negatively correlated with concentration ($r=–0.81$; $P=0.0001$), total number of motile spermatozoa ($r=–0.79$; $P=0.0001$) and number of progressively motile spermatozoa ($r=–0.68$; $P=0.0001$). Reduction of resazurin (100 minutes) identified 100% of samples known to contain below recommended spermatozoal values (sensitivity) and 88% of samples which were known to contain at least the recommended spermatozoal values (specificity). Positive predictive value (probability of an unknown sample having below the recommended spermatozoal values when dye is not reduced) was 86% and negative predictive values (probability of an unknown sample having at least the recommended spermatozoal values if dye is reduced) was 100%. Overall accuracy of this test was 92%. These results suggest that the resazurin assay can provide valuable information about quality of an extended cooled equine sample.

Key Words: Fertility, Sperm

337 Effect of progesterone on synthesis and secretion of retinol binding protein by caprine endometrium. K. H. Liu[1]*, J. C. Huang[2], J. H. Lin[3], S. H. MacKenzie[4], and J. D. Godkin[4], *[1]National Chiayi Institute of Agriculture, Taiwan, [2]Taiwan Livestock Research Institute, Pingtung, [3]National Taiwan University, Taipei, R.O.C., and [4]University of Tennessee, Knoxville.*

Retinol binding protein (RBP) is a major secretory product of caprine endometrium. Objectives of this study were to examine the effect of progesterone (P) on production of endometrial RBP and expression of endometrial RBP mRNA in caprine. Ovariectomized (ovx) does (n=4) were treated with exogenous P via the controlled internal drug release dispenser (CIDR) for 23 days. The CIDR devices were replaced every three days. Plasma P levels in ovx does implanted with CIDR were determined and found present in physiological level. Uterine flushings and explants were collected from ovx does treated with or without exogenous P. Uterine explants were either cultured for 24 h in modified minimum essential medium in the presence of [^{35}S]methionine to characterize in vitro production of proteins or subjected to isolate total RNA. Elevated levels of immunoreactive RBP in medium conditioned uterine explants from ovx does after P treatment were detected by immunoprecipitation with antiserum against bovine conceptus RBP. Through use of the antiserum, Western blot analysis showed the amount of RBP in uterine secretions increased in ovx does in response to P treatment. Using a cDNA clone to bovine conceptus RBP, a single RBP transcript of 1.4 kb was detected in caprine endometrium by Northern blotting. Quantitative expression of the endometrial RBP transcript was enhanced dramatically in ovx does after the administration of P compared to that in control does. The findings suggest that P may stimulate the synthesis and secretion of RBP by the uterus of the goats during pregnancy.

Key Words: Goat, Vitamin A, Progesterone

338 Differential responsiveness of the anterior pituitary to GnRH in beef cows is repeatable and related to length of the postpartum anovulatory interval. P. Fajersson*, R. L. Stanko, and G. L. Williams, *Texas A & M University Agricultural Research Station, Beeville.*

It was hypothesized that phenotypic variation in adenohypophyseal responsiveness to GnRH in cattle can serve as a physiological marker for reproductive efficiency. Objectives were to identify subpopulations of beef cows that differ markedly in their response to a maximal stimulatory dose of GnRH, confirm within-animal repeatability of the response, and examine the relationship between this characteristic and length of the postpartum anovulatory interval (PPI). A postpartum cow model, which exploits the phenomenon of pituitary depletion of LH by estradiol during late gestation and provides standardized physiological conditions, was used. Seventy Brahman × Hereford (F_1) cows, maintained in good body condition on pasture, were screened. In 30 of these, the relationship of pituitary response to length of PPI was examined. To confirm repeatability, 19 cows were re-challenged with GnRH at 150-170 days of their subsequent gestation. Suckled cows were administered a pharmacological (100 μg, iv) dose of GnRH between days 5 and 8 postpartum. Blood samples were collected by jugular venipuncture prior to first injection (time 0), and at 10, 30, 60, 120 and 240 minutes thereafter. To confirm onset of first normal luteal phase, twice-weekly blood samples were collected until serum progesterone concentrations were >1 ng/mL for 2 consecutive samples or 14 weeks postpartum. Responses to GnRH resulted in a normal distribution, with 11 high-, 48 intermediate- and 11 low-responding cows identified. Both mean peak LH concentrations and areas under the curve differed ($P < 0.001$) between groups. The PPI was 20-27 days shorter ($P < 0.05$) in high (n=6) and intermediate (n=18) than in low (n=6) cows. Among re-challenged cows, high responders (n=9) released greater quantities of LH, at first ($P < 0.001$) and second ($P < 0.02$) challenges, than low responders (n=10). These data provide evidence that adenohypophyseal responsiveness to GnRH exhibits within-animal repeatability, is related to a reproductive end point, and may serve as a sensitive, physiologically-based selection tool for reproductive merit in cattle.

Key Words: Postpartum cows, Pituitary, GnRH

339 Systemic vs local embryotoxic effect of early luteal regression in cattle. H. J. Hernandez-Fonseca*, B. L. Sayre, R. L. Butcher, and E. K. Inskeep, *West Virginia University, Morgantown.*

In early postpartum beef cows with short estrous cycles, a premature increase of prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$) on d 4 to 9 after estrus is associated with very low fertility, despite progesterone-replacement therapy. Inhibition of PG $F_{2\alpha}$ and removal of the regressing corpus luteum were necessary to achieve normal embryonic survival in these animals. In contrast, lutectomy alone effectively restored fertility in nonlactating cows treated with PG $F_{2\alpha}$ (3 times daily on d 4-7 or 5-8) and given supplemental progestogen. PG $F_{2\alpha}$ and/or products of the subsequent luteal regression could compromise embryonic viability through either a systemic or a local pathway. To test these alternatives, a d 6 embryo (morula or early blastocyst) flushed from a donor cow was transferred into each uterine horn (one ipsilateral and one contralateral to the corpus luteum) of 18 recipient beef cows. Recipient cows were treated with either 15 mg of PGF (n = 9) or saline (n = 9) 3 times per day on d 5 through 8 of the estrous cycle. Flurogestone acetate (FGA; 12 mg, in 2 or 3 portions/d, s.c.) was administered to all recipients from d 4 until diagnosis of pregnancy on d 36. As expected, treatment with PG $F_{2\alpha}$ decreased the embryo-survival rate compared with the saline treatment (33%, 6/18 vs 67%, 12/18 respectively, $P < 0.10$). Survival of embryos did not differ with location by treatment interaction. The proportions of surviving embryos in the uterine horns adjacent and opposite to the corpus luteum were 3/9 and 3/9 in the PG $F_{2\alpha}$-treated cows compared to 7/9 and 5/9 in the saline-treated animals. There was no evidence that the embryotoxic effect of treatment with PG $F_{2\alpha}$ was exerted in a local manner. It is concluded that embryonic mortality associated with luteolytic doses of PG $F_{2\alpha}$ involves compounds that either act systemically or are transported via the uterine lumen to the uterine horn contralateral to the corpus luteum.

Key Words: Embryonic survival, Prostaglandin $F_{2\alpha}$, Corpus luteum

340 Steroidal regulation of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) during the ovine estrous cycle. L. J. Craig* and G. E. Dahl, *University of Maryland, College Park.*

Recent evidence suggests that GH and IGF-1 play a role in reproductive function in the ewe. For example, both GH and IGF-1 increase coincident with the luteinizing hormone (LH) surge in the follicular phase of the estrous cycle. Estradiol (E_2) influences secretion of IGF-1 and GH, but the influence of progesterone (P_4) has not been fully characterized. To investigate the steroidal regulation of GH and IGF-1 during the ovine estrous cycle, 6 post-pubertal ewes were ovariectomized (OVX) during the midluteal phase and subjected to a series of 3 artificial cycles using a physiologic model of the estrous cycle in a latin square design. The 3 treatments imposed on each ewe were 1) normal follicular phase (OVX,–P,+E), 2) the maintenance of P_4 with a rise in E_2 (OVX,+P,+E), or 3) withdrawal of P_4 without an increase in E_2 (OVX,–P). Serum GH concentrations were quantified in blood samples obtained over a 28 hour period beginning 12 hours after the addition of E_2, whereas IGF-1 concentrations were determined in blood samples taken every other day throughout the 3 cycles. The mean follicular phase GH concentration in the OVX,–P,+E treatment (2.7ng/ml) was significantly higher ($P<0.02$) than the OVX,+P,+E (1.5ng/ml) and the OVX,–P (0.9ng/ml) treatments. The intermediate nature of the OVX,+P,+E suggests that unlike LH which is completely blocked by the presence of P_4, the rise in GH is dependent on the increase in E_2 rather than the withdrawal of P_4. Serum IGF-1 increased significantly ($P<.01$)in the OVX,–P,+E and the OVX,+P+E relative to the OVX,–P suggesting that IGF-1 is regulated by GH and is not dependent on the steroid milieu. We conclude that the increase in GH observed during the ovine estrous cycle is dependent on the follicular phase increase in E_2, and that the cyclic increase in IGF-1 is dependent on an increase in GH.

Key Words: Growth Hormone, Insulin-like Growth Factor-1, Estrous cycle

341 Superovulatory response of one ovary is related to the micro- and macroscopic population of follicles in the contralateral ovary of the bovine. R. A. Cushman*, J. C. DeSouza, V. S. Hedgpeth, and J. H. Britt, *North Carolina State University, Raleigh.*

The hypothesis was that superovulatory response (SR) in cattle is related to number of primordial and growing follicles in the ovary. One ovary was removed from each of 26 non-lactating Holstein or Jersey cows, and 2 d later cows were superovulated by a 4-d treatment with FSH (FSH-P; 26 mg). The SR was determined 7 d after estrus by counting CL. Superovulatory response was classified as low (< 5 CL), medium (5 to 14 CL), or high (> 14 CL). At surgery, surface follicles were counted and classified as small (1 to 3 mm), medium (3 to 7 mm) or large (> 7 mm). Then tissue was immediately fixed for histological examination. The tissue was serially sectioned and 170 ± 9 randomly selected fields (2 × 2 mm) were examined for each cow. Follicles < 1 mm in diameter within field were classified as primordial, primary, secondary or tertiary. There was a strong correlation ($r = .71$; $P < .001$) between the total number of tertiary follicles and the SR. The SR was also correlated with total number of primordial follicles ($r = .42$; $P < .05$) and with medium surface follicles ($r = .54$; $P < .01$). Empty fields were negatively correlated with SR ($r = – .44$; $P < .05$) and with number of primordial follicles ($r = – .80$; $P < .001$). Number of primordial, tertiary, small surface follicles and total surface follicles was greater in the medium and high SR groups than the low group ($P \leq .01$). Total number of empty fields was greater in the low SR group than in the medium or high groups ($P < .01$). From these data we conclude that SR in cattle is related to the pools of primordial and growing follicles in the bovine ovary.

Key Words: Cattle, Follicles, Superovulatory response

342 Follicular growth, corpus luteum lifespan, and free radical status in lactating Holstein cows exposed to heat stress. J. P. Trout*, L. R. McDowell, and P. J. Hansen, *University of Florida, Gainesville.*

Objectives were to determine if heat stress affected follicular growth, increased corpus luteum lifespan, and altered antioxidant status in lactating dairy cattle. Eleven multiparous lactating Holstein cows, 60 to 110 days in milk, were maintained in environmental chambers from days 11-21 of the estrous cycle. Cows were randomly assigned to one of two treatments: heat stress (HS, 40°C) or thermoneutral (TN, 20-24°C). Air temperature was modified on a daily basis (± 2°C) if target rectal temperatures (40.0-40.9°C) were not achieved. Rectal temperature at 4:00 PM was higher (P<.0001) for HS cows as compared to TN cows (40.3±.14°C vs. 39.0±.13°C) as was respiration rate (P<.0001; 97±4.7 breaths/min vs. 48±4.2 breaths/min). There were no differences (P>.05) in estrous cycle length (22.0±1.58 d vs. 24.8±1.45 d) or luteal lifespan (18.2±1.58 d vs. 20.7±1.44 d), although 5 of 6 TN cows had extended (≥23 d) estrous cycles vs. 1 of 5 HS cows. Cows were ultrasounded from days 11-21. HS cows had similar numbers of class 1 (3-5 mm), class 2 (6-9 mm), and class 3 (>9 mm) follicles per cow. There were also no treatment effects (P>.05) on size of the dominant follicle or second largest follicle. Treatment did not affect plasma vitamin E concentrations, but red blood cell (RBC) glutathione content was lower (P=.05) for HS cows (.58 ± .09 vs .86 ± .08 nmol/mg protein). Treatment did not affect concentrations of malondialdehyde in RBC or in a muscle biopsy collected at day 21. In conclusion, heat stress did not extend luteal lifespan or estrous cycle length of lactating Holstein cows. There was no evidence that HS increased generation of free radicals in lipid compartments, but the decrease in RBC glutathione may indicate that HS increases free-radical production in the cytosolic part of the cell. (Supported by USDA-CBAG Grant No. 95-34135-1860)

Key Words: Heat stress, Estrous cycle, Antioxidant

343 Comparison of insulin and pST on post-weaning follicular development in the primiparous sow. N. C. Whitley, A. B. Moore, and N. M. Cox, *Mississippi State University, Mississippi State.*

The objective was to compare effects of exogenous insulin and pST on follicular development after weaning. Crossbred primiparous sows nursing 8.8 ± 0.3 pigs an average of 21.3 ± 0.3 days received saline (1.5 cc i.m.; n=9), insulin (0.4 IU/kg BW s.q.; Eli Lilly Lente Iletin II; n=10) or pST (40 μg/kg BW i.m.; n=10) daily from days 1 to 5 after weaning (d 0). Sows were euthanized approximately 3 hours after the last injection and ovaries were collected. The diameter of each follicle ≥ 2 mm was measured, and fluid from the 20 largest follicles was collected for detection of IGF-I, estradiol and progesterone by radioimmunoassay. Total number of follicles was not affected by treatment, with 13.8, 14.5 and 12.6 follicles per ovary for saline, pST and insulin treatments, respectively (SEM=1.4). Average follicle diameter was increased by both pST (5.5 mm; P<0.01) and insulin (5.2 mm; P<0.09) when compared to saline treatment (4.6 mm; SEM=0.2). Both pST and insulin increased (P<0.0001) follicular fluid estradiol when compared to fluid from saline-treated sows. However, the increase was greater (P<0.004) for insulin (3-fold) when compared to pST (2-fold) treatment. Similarly, progesterone concentrations in follicular fluid were higher (P<0.002) for pST- and insulin-treated sows when compared to saline-treated sows (90.5, 140.7 and 52.3 ng/ml, respectively; SEM=15.5) with a greater (P<0.03) increase for insulin than for pST treatment. In contrast, follicular fluid IGF-I was increased (P<0.0001) in follicular fluid from pST-treated sows when compared to either insulin- or saline-treated animals. Average follicular fluid IGF-I concentrations were 117.7, 47.8 and 46.5 ng/ml for pST, insulin and saline treatments, respectively (SEM=3.5). In conclusion, both pST and insulin treatments positively influence follicular development, although perhaps through different mechanisms.

Key Words: Follicle, Primiparous sow, IGF-I

344 Insulin stimulates rapid 2-deoxyglucose transport in swine preovulatory ovarian follicles. H. Zhang, A. B. Moore, and N. M. Cox, *Mississippi State University, Mississippi State.*

Although granulosa cells are known to absorb glucose by facilitative diffusion carriers, less is known about regulation by insulin in cultured whole follicles. The study was designed to investigate the characteristics of preovulatory follicle glucose transport. Uptake of glucose analog, 2-deoxy-D-glucose (2-DG), was measured in follicles (4 mm diameter) from ovaries representing d 16-19 of the estrous cycle, which were considered non-atretic and potentially preovulatory. After preincubation for 60 min at 37°C in low glucose medium (Dulbecco's Modified Eagle), rates of ^{3}H 2-DG accumulation were measured as functions of incubation time (1, 5, 15 and 30 min in 10 mM 2-DG) and 2-DG concentration (10, 25 and 50 mM for 15 min). Accumulation of 2-DG (nmole/mg·min) was maximum at 1 min (P<0.05). Values at 5 min were lower and were similar to those at longer incubations. Uptake of 2-DG increased linearly from 10 to 50 mM in the whole follicle (r=0.98, P<0.001) as well as in the fluid compartment, while the accumulation of 2-DG in the cellular complex reached a plateau by 15 min with 25 mM 2-DG. When follicles were treated with 0, 50, 150 or 450 ng/mL of insulin in 10 mM 2-DG for 5, 15 and 45 min, rates of accumulation at 5 min were 85%, 55% and 71% higher in the treated follicles, respectively, and 32%, 43% and 52% higher in the treated follicles that were pre-exposed to insulin, respectively, relative to the controls (P<0.05). The highest accumulation occurred in the 5 min incubation except for control follicles (P<0.05). Our findings provide two insights. First, follicles rapidly accumulate 2-DG, apparently achieving equilibrium with incubation solutions by 5 min. Second, stimulatory effects of insulin on 2-DG uptake can be achieved in follicles without prior exposure to insulin.

Key Words: Insulin, 2-Deoxyglucose transport, Follicle

345 Influences of prepartum body condition score changes on reproduction in beef cows calving in moderate body condition. D. G. Morrison[1]*, J. C. Spitzer[2], and J. L. Perkins[3], *[1]Louisiana State University Agricultural Center, Rosepine, [2]Clemson University, Clemson, and [3]University of Arkansas, Fayetteville.*

Multiparous, spring-calving beef cows (n=250) were blocked by BW and body condition score (BCS; 1=emaciated to 9=obese) then allotted to receive either a high or low plane of nutrition from late summer to early winter during a 3 yr period. At the beginning of the last trimester of pregnancy, cows were grouped by BCS as follows: Group 1-BCS≤4, Group 2-BCS=5 or 6, Group 3-BCS≥7. Each group was managed so that each cow would calve at BCS=5. At the time of group assignment, mean BW and BCS differed (P<.01) among groups and were 480 kg and 3.6, 541 kg and 5.5, and 594 kg and 7.1 for Groups 1 to 3, respectively. Within 28d before calving, BW and BCS were similar among groups averaging 555 kg and 5.1. Prepartum BCS changes averaged 1.4, –.4, and –2.0 units for Groups 1 to 3, respectively (P<.01). Cows were managed as a single group after calving in each state. State effects were significant for both prepartum and postpartum BW and BCS changes. Significant state × BCS group interactions were noted before calving for BW, BCS, and changes in BW and BCS, but were caused by differences in magnitude among states. The percentage of cows with ovarian activity at the start of a subsequent breeding season was not affected (P>.20) by either state or BCS group and averaged 66%. Mean pregnancy rates at 20, 40, and 60d of a subsequent breeding season were: 55, 76, and 89% for Group 1; 51, 67, and 82% for Group 2; and 64, 79, and 89% for Group 3 (P>.34). Mean days to rebreeding were 89, 87, and 85 for Groups 1 to 3, respectively, (P=.70). Neither calf birth weight ($\bar{x}$=38.6 kg) nor adjusted weaning weight ($\bar{x}$=223.6 kg) were affected by prepartum BW and BCS changes. We conclude that reproductive performance of cows calving in moderate body condition is not influenced by large changes in body energy reserves during the last trimester of pregnancy.

Key Words: Beef cow, Body condition, Reproduction

346 Follicular growth and metabolic changes in beef heifers fed incremental amounts of polyunsaturated fat. R. L. Stanko[1,2*], P. Fajersson[1] L. A. Carver[3], and G. L. Williams[1], *[1]Texas A&M University Agricultural Research Station, Beeville, [2]Texas A&M University, Kingsville, and [3]Cargill MLPD, Elk River, MN.*

Increments in dietary fat consumption modify metabolic status and enhance follicular growth in cattle, independent of caloric intake or BW change. Objectives of this study were to determine the minimum level of dietary polyunsaturated (PU) fat needed to maximize follicular growth in sexually mature beef heifers. Forty Brahman × Hereford (F_1) heifers averaging (± SEM) 370 ± 15 kg were allocated randomly to 4 dietary groups (n = 10/group) in 2 replicates (REP 1, n=19; REP 2, n=21). Groups received complete mixed diets containing no added fat (Con), or 1.41 (Low), 2.87 (Med), or 4.21% (High) added fat for 61 d. All diets were formulated to result in .27 kg gain/day (0.3 Mcal NE_g; 9% total protein). Single blood samples were collected twice weekly to determine serum concentrations of insulin, somatotropin, and total cholesterol. Estrous cycles were synchronized with prostaglandin $F_{2\alpha}$ on d 20 (E1) and 50 (E2). Follicle populations were monitored with ultrasonography on d 1, 3, 5, 7, and 9 (d 0 = estrus) of each synchronized cycle, and the total number of large and medium-sized (≥ 5 mm) follicles (MLF) was recorded. Overall, the High fat group maximized follicular growth. However, a treatment × estrous cycle × replicate interaction ($P < .01$) was detected. In REP 1, High (3.6 ± .4) and Medium (3.3 ± .5) groups had greater (P<.05) numbers of MLF after E1 and E2 compared to Low (1.4 ± .2) and Con (1.4 ± .3) groups. However, during E1 of Rep 2, Con-fed heifers had a greater (P<.01) number (3.0 ± .4)of MLF than the other groups (1.4 ± .2, 1.8 ± .2, and 1.3 ± .2, respectively). This pattern was reversed after E2, and the High group exhibited a greater number (1.5 ± .2; $P < .03$) of MLF than Con (.8 ± .2). Neither ADG (.39 ± .2 kg/d) nor mean serum insulin (.71 ± .1 ng/mL) differed ($P>.1$) among dietary groups; however, mean serum somatotropin (64 ± 5.7 ng/mL) and total cholesterol (248 ± 14.2 mg/dL) were greater ($P<.05$) in High than in all other groups (31.3 ± 4.5 ng/mL and 205.3 ± 12 mg/dL, respectively). Dietary PU added at the highest level (4.21%) maximized ovarian follicular growth in this study.

Key Words: Polyunsaturated fat, Follicular growth, Cattle

347 Evaluation of the effects of body condition on luteal activity and estrus in postpartum beef cows. M. L. Looper*, C. A. Lents, J. A. Vizcarra, and R. P. Wettemann, *Oklahoma Agricultural Experiment Station, Stillwater.*

Seventy-seven multiparous beef cows (Hereford and Angus × Hereford) calving in thin to moderate body condition score (BCS) were utilized to evaluate luteal activity. After parturition, blood samples were collected twice weekly to determine the occurrence of the first postpartum luteal activity (**LA**, progesterone ≥ .5 ng/mL). Cows were exposed to bulls and observed twice daily for behavioral estrus. Weight changes and BCS were determined at 2 wk intervals. LA was classified as normal if 4 consecutive samples had ≥ .5 ng/ml of progesterone or shortened if ≤ 3 consecutive samples had ≥ .5 ng/ml. Postpartum interval to first normal LA was shorter ($P < .0001$) for moderate condition (BCS ≥ 4.5) cows as compared with thin (BCS ≤ 4) cows (58.3 ± 3.2 vs 93.3 ± 5.1 d, respectively). Interval to first estrus was shorter ($P < .0005$) for moderate cows compared with thin cows (53.3 ± 3.7 vs 89.3 ± 5.6 d, respectively). First normal LA was preceded by estrus in 74% of the cows. BCS and postpartum weight change did not influence the incidence of estrus associated with first normal LA. At the first estrus, 72% of cows had normal LA, 16% had shortened LA, and 12% lacked LA. BCS and postpartum weight change did not influence the type of LA associated with the first estrus. Seventy-eight percent of cows had a transient increase in progesterone preceding the first normal LA. Only 72% of the first postpartum estruses were associated with normal luteal development.

Key Words: Postpartum Cows, Estrus, Luteal Activity

348 Effects of dietary fat and sire breed on weight gain and reproductive traits of beef heifers. M. A. Lammoglia[1*], R. B. Staigmiller[1], R. A. Bellows[1], E. E. Grings[1], J. W. Bergman[2], R. E. Short[1], M. D. MacNeil[1], S. E. Bellows[1], and B. L. Balsam[1], *[1]USDA-ARS, Miles City, MT and [2]Eastern Agricultural Research Center, Sidney, MT.*

Prepuberal F1 heifers [crossbred dams bred to Hereford (H), Limousin (L) or Piedmontese (P) sires; n = 246] were allotted to receive 1.9% (LF) or 4.4% (HF) dietary fat. Safflower seeds (37% oil; 80% linoleic acid) were the fat source. Rations were isocaloric and isonitrogenous and were fed from 250 d of age until puberty or breeding season started. Heifers gained .77 kg/day. Epididectomized bulls aided 2× daily estrous detection. Puberty defined: heifer showed estrus, had a CL, and serum progesterone (P4) concentration above 1 ng/mL between d 7–10 post estrus. Heifers were weighed every 28 d; a 60-heifer sample representing each breed type and diet were back fat measured using ultrasonography. Results:

	Hereford		Limousin		Piedmontese		Probability		
Variables:	LF	HF	LF	HF	LF	HF	Diet	Breed	Diet × Breed
Total gain (kg)	124	116	118	126	101	102	NS	.01	NS
Back fat thickness (mm)	2.9	3.8	2.7	3.1	2.1	2.6	.01	.01	NS
Progesterone (ng/mL)	2.9	3.4	3.5	3.7	3.1	3.8	.01	NS	NS
Puberty age(days)	383	382	400	412	382	356	NS	.01	.05
No. cycling heifers (%)	77	84	81	79	78	100	.06	NS	.06

In conclusion, 4.4% dietary fat increased back fat thickness, serum P4, and number of estrous cycling heifers before the breeding season. The HF diet increased number of estrous cycling F1 H and P heifers before the breeding season but not F1 L heifers. Puberty age was decreased in HF F1 P, but not F1 H or L heifers. Sire breed influenced total gain, back fat thickness and puberty age.

Key Words: Back fat thickness, Dietary fat, Puberty, Sire breed

349 Effect of induced hypothyroidism on ovarian response to superovulation of Brahman *(Bos indicus)* cows. A. Bernal*, G. V. Demoraes, T. A. Thrift, C. C. Willard, and R. D. Randel, *Texas A&M University Agricultural Research & Extension Center, Overton.*

Multiparous non-lactating Brahman cows (n=20) exhibiting normal estrous cycles were randomly assigned within BW and body condition score (BCS) to dietary treatments containing either 0 (C; n=10) or 4 mg/kg BW/d 6-n-propyl-2-thiouracil mixed with the concentrate (PTU; n=10). Water, minerals and Coastal bermudagrass hay were available free choice and each animal received 1.8 kg of a 5:1 corn:soybean meal concentrate daily. Dietary treatments began on d 10 of the estrous cycle. Ten d after the second standing estrus, the cows received daily i.m. injections of 25 IU of porcine FSH (Superov®) over a 3-d period. Estrus was induced by administration of $PGF_{2\alpha}$. Artificial insemination was performed at 12 and 24 h after observed estrus. Seven d after insemination, collection of embryos by non-surgical procedures was performed and the ovaries were removed via mid-flank laparotomy. Blood samples were collected on a weekly basis. Serum triiodothyronine and thyroxine (T4) concentrations were lower (P<.05) in PTU than in C by d 28 of treatment and remained low thereafter. Based on T4 concentrations on d 49, five cows were hypothyroid (H-PTU) and four were partially suppressed (P-PTU). Cows in the PTU group had greater (P<.1) average daily gain, greater (P<.05) paired ovarian weights, greater (P<.06) amount of luteal tissue, greater (P<.06) number of large follicles (≥ 4 mm), lower (P<.001) embryo recovery rate, lower (P<.1) percentage of fertilized ova or embryos and lower (P<.1) percentage of blastocysts collected than those in C. Further, H-PTU cows had a greater (P<.06) number of luteinized follicles and a greater (P<.05) number of corpora lutea than C. It was concluded that induced hypothyroidism improved weight gain and increased ovarian response to FSH in superovulated Brahman cows. Ovulation appeared to be impaired and enhanced ovarian response to FSH may have affected fertility in hypothyroid Brahman cows.

Key Words: Hypothyroidism, Superovulation, Cows

350 Effects of induced hypothyroidism on weight gains, lactation and reproductive performance of primiparous Brahman cows. T. A. Thrift, A. Bernal, and R. D. Randel*, *Texas A&M University, Overton.*

Eighteen primiparous spring calving Brahman cows (BW=425± 13.8kg, BCS=5.0 ± .2units) were utilized to study the effects of thyroid manipulation on weight gain, milk production, and reproduction. Nine cows served as controls. Nine cows were induced to become hypothyroid by daily ingestion of 4mg/kg BW of 6-n-propyl-2-thiouracil (PTU). Cows were stratified to treatment one day post-calving based on season of birth, BW, BCS, calf sex, and calf sire. The treatment period lasted for 84 d and was followed by a 56 d post-treatment period. Cow BW, BCS and calf weight were recorded biweekly. Milk production was estimated at 14, 28, 56, 84, 98, 112, and 140 d post calving. Weekly blood samples were obtained for analysis of triiodothyronine, thyroxine, and progesterone. Estrus was monitored twice daily with the aid of a fertile bull equipped with a chin ball marker. Hypothyroidism was effectively induced in all PTU cows during the treatment period. PTU cows gained more ($P<.05$) weight (54.6 ± 7.6kg) and tended ($P=.06$) to gain more body condition (.61 ± .17 units) than control cows (15.7 ± 7.6kg; .11 ± .17units) during the treatment period. Control calves gained at a faster rate (.85 ± .04kg/d; $P<.01$) than PTU calves (.7 ± .04kg/d) during the treatment period. Milk production was lower ($P<.05$) in PTU cows on d 56 and 84. During post-treatment all trends were reversed so that BW, BCS, calf weight, and milk production were similar between the two groups by d 140. Reproductive performance was not affected by induction of hypothyroidism. In conclusion, induction of hypothyroidism was successful in increasing cow weight and BCS gains and suppressing milk production during the treatment period but these changes were not successful in improving reproductive performance of primiparous Brahman cows.

Key Words: Thyroid, Reproduction, Cattle

351 Effects of induced hypothyroidism or hyperthyroidism on growth and reproductive performance of Brahman heifers. T. A. Thrift*, A. Bernal, and R. D. Randel, *Texas A&M University, Overton.*

Twenty-one prepubertal Brahman heifers (BW=302 ± 7.5kg, BCS=5.4 ± .2, age=498 ± 3.4d) were utilized to study the effects of thyroid function on growth and reproduction. Seven heifers were controls (C). Seven heifers were induced to become hypothyroid by ingestion of 4mg/kg BW of 6-n-propyl-2-thiouracil (PTU). Seven heifers were induced to become hyperthyroid by daily injections of triiodothyronine (T3, 1mg/d). Treatments were administered for 84 d followed by an 84 d post-treatment period. Biweekly blood samples were obtained via tail venipuncture for analysis of T3, thyroxine, and progesterone. BW, BCS, and rectal temperature (RT) were recorded weekly. Estrus was monitored twice daily with the aid of a fertile bull equipped with a chin ball marker. Hyperthyroidism and hypothyroidism were successfully induced in T3 and PTU treated heifers, respectively. During the treatment period PTU heifers gained the most BW and BCS (72.4 ± 5.4kg; .93 ± .15units), C heifers were intermediate (41.7 ± 5.4kg; .43 .15units) and T3 heifers the least (13.3 ± 5.4kg; –.36 ± .15units; $P<.05$). RT also decreased ($P<.05$) in PTU heifers (–1.94 ± .2°C) as compared to C (–1.2 ± .2 ° C) or T3 heifers (–.75 ± .2°C). No heifers exhibited estrus during the treatment period. During the post-treatment period, T3 heifers gained the most BW and BCS (93.9 ± 6.1kg; 1.14 ± .13units), C heifers were intermediate (67.0 ± 6.1kg; .86 ± .13units), and PTU heifers the least (22.2 ± 6.1kg; –.14 ± .13units; $P<.05$). The reversal in BW and BCS gains observed during the post-treatment period corresponded to periods of transient hypo- and hyperthyroidism in T3 and PTU heifers, respectively. Age and BW at puberty and pregnancy were similar between all treatment groups. T3 heifers were thinner (5.7 ± .2units; $P<.05$) at puberty and pregnancy than PTU heifers (6.6 ± .2 units). Induction of hypothyroidism resulted in significant increases in BW and BCS during the treatment period, but these increases were not sufficient to dramatically affect reproductive performance of Brahman heifers.

Key Words: Thyroid, Puberty, Cattle

352 Zeranol and growth hormone treatment differentially influenced mRNA levels of the obesity protein, leptin, and the GH receptor in growing wethers. S. R. Raymond[1]*, M. G. Thomas[1], J. A. Carroll[2], R. L. Matteri[2], and D. H. Keisler[1], *[1]University of Missouri-Columbia and [2]USDA-ARS, Columbia, MO.*

A leptin cDNA was recently cloned from adipose tissue of humans, rodents, and livestock. In rodents, percentage body fat is positively correlated to mRNA levels of leptin and negatively correlated to feed intake. Two compounds known to influence feed intake and fat content of a carcass in ruminants are zeranol (Z; an estrogenic growth promotant) and recombinant growth hormone (GH). The objective of these studies was to determine the effect of Z and GH on steady-state mRNA levels of leptin and the GH receptor (GHR) in adipose tissue of growing wethers. In two studies, 36 whiteface wethers weighing 45.8 ± 3 kg were assigned randomly to a control group (CT1; n = 8), a group implanted with 12 mg of zeranol (Z, n = 12; Ralgro®, Mallinckrodt Vet.), a second control group (CT2; n = 8) or a group injected with 120 mg of bovine GH (n = 8; Posilac®, Monsanto Co.). Two wk after treatment, subcutaneous fat was biopsied from CT1, CT2, Z and GH treated wethers. Weight gain was similar in Z and GH treated wethers relative to CT1 and CT2 treated wethers in both experiments [P = .7 and .9, (Z) 1.7 ± .6 = (CT1) 1.4 ± .3 and (GH) 1.7 ± .7 = (CT2) 1.7 ± .6 kg per 2 wk]. Treatment of wethers with Z resulted in a decrease in relative mRNA levels of leptin and GHR [(Z) .75 ± .1 < (CT1) 1.5 ± .2 and (Z)1.0 ± .2 < (CT2) 5.5 ± 1.4], whereas GH tended to increase ($P < .1$) the relative levels of these transcripts [(GH) 2.1 ± .6 > (CT1) 1.2 ± .2 and (GH) 6.9 ± 2.4 > (CT2) 2.9 ± .5] when compared to control treated wethers. These data provided evidence that compounds known to influence feed intake and lean growth in ruminants differentially influence responsiveness and signalling mechanisms of adipose tissue. Work supported in part by USDA Grant 96-35203-3209 and Postdoc Grant 95-37206-2119.

Key Words: Leptin, Zeranol, Growth Hormone

353 Expression of interleukin-6 mRNA in peripheral tissues and hypothalamus following a lipopolysaccharide challenge in neonatal pigs. J. J. Klir* and R. L. Matteri, *Animal Physiology Unit, Agricultural Research Service, USDA, Columbia, Missouri.*

It is not known whether the proinflammatory cytokine interleukin-6 (IL-6) is fully induced in neonates exposed to bacterial infections. This study evaluated tissue-specific expression of IL-6 mRNA in spleen, liver, thymus, pituitary gland, and hypothalamus following a peripheral (ip) injection of lipopolysaccharide (LPS) from *E. coli* (150 μg/kg) in 1, 21, and 28 d old pigs. For each piglet injected with LPS there was one littermate of the same sex injected with saline as a control. At 3 h postinjection, animals were sacrificed, and tissue samples were collected. Slot blotting and scanning densitometry method was used to quantify IL-6 mRNA. All values were normalized to β-actin and expressed as relative units. There was a highly significant overall effect of LPS ($P = 0.0001$), tissue differences ($P = 0.0001$), and effect of age ($P = 0.0233$). Although LPS stimulated increased IL-6 mRNA expression in all tissues, the highest mRNA expression was detected in the pituitary gland. The most significant general age difference in mRNA induction in response to LPS in pigs was observed between 1 and 28 d of age ($P = 0.0064$). Levels of IL-6 mRNA expression in 1 vs. 28 d old LPS-injected pigs were 0.889±0.237 vs. 1.187±0.268 in the spleen, 2.915±0.921 vs. 3.569±1.312 in the liver, 3.989±1.226 vs. 6.677±2.217 in the thymus, 5.299±0.589 vs. 12.375±4.150 in the pituitary gland, and 3.663±0.514 vs. 11.762±5.118 relative units in the hypothalamus, respectively. These results indicate that, although neonatal pigs respond to bacterial LPS by increased expression of IL-6 mRNA as early as 1 d of age, the level of this expression is relatively low at this age. An undeveloped acute phase response could contribute to high mortality and morbidity due to bacterial infections occurring during the first few days after birth.

Key Words: Pig, Lipopolysaccharide, Interleukin-6

354 Steroids increase appetite, reduce hyperglycemia, but do not affect glucose kinetics during hypoglycemia in endotoxemic steers. C. D. McMahon, T. H. Elsasser[1], D. R. Gunter, L. G. Sanders, B. P. Steele, and J. L. Sartin*, *Auburn University, Auburn, AL and [1]U.S.D.A., Beltsville, MD.*

Endotoxin induces transient hyperglycemia, then chronic hypoglycemia and increased glucose tolerance. Rectal temperature, concentrations of cortisol and tumor necrosis factor$_\alpha$ (TNF$_\alpha$) are elevated. Furthermore, appetite is reduced resulting in tissue mobilization. Steroids enhance recovery from infection, but their mechanism of action is not known. Our objectives were to determine whether a steroid implant (20 mg estradiol and 200 mg progesterone, synovex-S, Syntex) given to endotoxemic steers would: (1) reduce hyperglycemia, block increased glucose tolerance, and block hypoglycemia, (2) reduce rectal temperatures, TNF$_\alpha$, and cortisol, (3) increase appetite, and reduce BUN, NEFA, and TAG. Holstein steers were assigned within a 2×2 factorial design as follows (n=5 per group): C/C, no steroid and vehicle; S/C, steroid and vehicle; C/E, no steroid and endotoxin (1 μg/kg i.v., *E. coli* 055:B5, Sigma); S/E, steroid and endotoxin. Steroid implants were given at 20 weeks (d 0) and serial blood samples (15 min) were collected on d 14 for 8 h, with endotoxin or vehicle injected after 2 h. Glucose (100 mg/kg) was injected i.v. at 6 and 24 h. Hyperglycemia was lower (P<0.05) in S/E (218 mmol·min·l^{-1}), than C/E (668 mmol·min·l^{-1}) between 30 and 150 min after endotoxin (SED=181). Steroids did not block hypoglycemia, or affect insulin concentrations and glucose removal rates, which were higher (P<0.05) in endotoxin treated steers at 6 h, and not different between groups at 24 h. Appetite was greater (P<0.05) in S/E (2.1 %BW), than C/E (1.1 %BW) (SED=0.3), and concentrations of NEFA, BUN, and TAG were lower (P<0.05) in S/E, than C/E 24 h after endotoxin. Steroids did not affect rectal temperature, or concentrations of TNF$_\alpha$ and cortisol. We conclude that gonadal steroids reduce initial hyperglycemia, increase appetite, and reduce tissue wasting, but do not affect TNF$_\alpha$, cortisol, or glucose kinetics during the hypoglycemic phase of endotoxemia.

Key Words: Endotoxin, Steroid, Glucose

355 Endocrine responses to weaning and post-weaning dietary changes in the neonatal pig. J. A. Carroll[1]*, T. L. Veum[2], and R. L. Matteri[1], *[1]Animal Physiology Research Unit, Agricultural Research Service, USDA and [2]University of Missouri, Columbia, MO.*

The objective of this study was to determine the effects of weaning and changing post-weaning diet composition on growth patterns and growth-related hormonal profiles in neonatal pigs. Forty-eight crossbred piglets were assigned to two groups(n=24/group) based on weaning at 2 or 3 wk of age. At the specified weaning time for each group, piglets were removed from the sow and placed on a commercial starter ration for the first 11 d post-weaning (Phase I diet). At d 12 post-weaning, the piglets were placed on a growing ration for the remainder of the study (Phase II diet). Body weights and blood samples were collected twice weekly from birth until 42 d of age. Serum hormone concentrations (GH, IGF-1, IGF-2, T3, T4 and cortisol) were determined by RIA. Serum concentrations of IGF-1, IGF-2, and ADG were reduced (P<.05) in both groups as a result of weaning, whereas serum concentrations of GH were elevated (P<.05) in both groups. Mild decreases in ADG occurred following the change from phase I to phase II diets (P<.05). Serum IGF-1 concentrations decreased following the phase I to II dietary change in the 2 wk weaning group (P<.05). The numerical increases in GH secretion following the change in diets were not significant in either group (P<.05). A developmental decline in serum T3 concentrations occurred from birth to d 18 in both experimental groups (P<.05). Serum T3 levels were not affected by weaning at either 2 or 3 wk of age, but did decline in both groups following the change in post-weaning diets (P<.05). Change in the patterns of T4 and cortisol secretion was not associated with weaning or the change in diets. These results demonstrate that growth-related endocrine function is affected by management practices associated with dietary changes in the neonatal pig.

Key Words: Porcine, IGFs, Weaning

356 Development of methods for measuring GRF and SRIF in hypophyseal portal blood of male red deer (Cervus elaphus). J. Webster*, T. Manley, S. Stuart, I. Corson, and J. Suttie, *AgResearch, Invermay Agricultural Centre, New Zealand.*

Growth in male red deer is seasonal, imposing limits on farmed venison production in New Zealand. Red deer grow rapidly in spring and slowly in winter despite ad-libitum high quality food. Previous studies showed that the GH pattern alters with growth state. To study the basis for the different GH patterns, we modified a procedure from sheep[1] to collect hypophyseal portal blood over 24 hours from undisturbed red deer. This is the first reported use of this method in red deer and problems encountered are described. Portal blood was collected from 6 deer into aprotinin (500 KIU/ml), centrifuged and plasma frozen. A GRF assay developed had a sensitivity of 2-4 pg/tube using tracer purified by HPLC. The SRIF assay had a similar sensitivity. Problems occurred with measurement of GRF and SRIF due to poor peptide recovery from portal plasma. Initially we used a MeOH/TFA extraction. A profile measured by this method had mean GRF and SRIF of 12.5 pg/ml and 47.5 pg/ml respectively and peptide recovery of 44-66%. GRF and SRIF patterns had no clear relationship with GH. Recovery of peptide added to jugular plasma during portal collection was poor and variable. A C18 Sep-Pak extraction had similar results. Acetone /HCl was tested and mean GRF recovery from jugular blood consistent at 50%. A profile measured with this method had mean GRF of 23.9 pg/ml and mean SRIF of 8.1 pg/ml. GRF and SRIF recoveries were poor at 28% and 12% respectively and GRF and SRIF patterns were not clearly related to GH. An affinity column method was also tested unsuccessfully. Further peptide extraction methods are being investigated. [1]Caraty et al., (1994) Methods in Neurosciences 20: 162-183.

Key Words: Red deer, Seasonal growth, Hypothalamic regulation

357 Porcine somatotropin (pST) elevates circulating gastrin concentrations in swine. F. C. Buonomo, D. L. Grohs, C. L. McLaughlin, and T. R. Hampton, *Monsanto Company, St. Louis, MO.*

A small number of hogs treated with pST develop esophagogastric ulcers. Since gastrin is a major physiological regulator of gastric acid secretion, and several lines of evidence indicate that the somatogenic effects of somatotropin on gastrointestinal growth and function may be mediated in part by gastrin, studies were initiated to investigate the relationship between pST and circulating gastrin concentrations in swine. Prior to initiation of these studies, a human gastrin radioimmunoassay was validated for the determination of serum porcine gastrin levels in a 24 h fasted swine model. In the first study, 6 cross-bred barrows (75 kg) received daily injections of either vehicle (sterile water for injection) or 6 mg of pST for a period of 10 days. On d 11, jugular blood samples were collected at 0, 30, 60, 90, 120 and 240 min post-injection. Circulating gastrin concentrations were significantly higher (~4.5-fold) in the pST-treated swine vs the control group at all time points evaluated. Thus, in a second study, blood samples were collected on an alternate day schedule for a period of 22 days from hogs treated in a similar manner starting at d 1 of injection. Hogs injected with 6 mg/d pST had higher (P<.05) serum gastrin levels by d 6 post-injection vs that of the control group. Serum gastrin levels continued to increase in the pST-treated hogs with the duration of pST administration (an additional 5 d). Moreover, gastrin concentrations continued to be elevated (P<.05) in the pST-treated hogs for 5 days following the cessation of injections and were numerically higher during the 11 day post-treatment period. These data suggest that the increased incidence of esophagogastric ulcer development associated with pST administration may be mediated via an elevation in circulating gastrin concentrations resulting in increased gastric acid secretion. This hypothesis is supported by the ability of concomitant oral administration of Omeprazole, a gastric acid secretion inhibitor, to attenuate pST-induced ulcer generation.

Key Words: Gastrin, pST, Swine

358 Benzimidazoles decrease esophagogastric ulceration in finishing hogs administered high doses of porcine somatotropin (pST). C. L. McLaughlin*, F. C. Buonomo, B. D. Vineyard, D. L. Hartzell, and C. A. Baile, *Monsanto Company, St. Louis, MO and University of Georgia, Athens.*

Although the benefits of pST use in improving growth performance and carcass composition are clear, esophagogastric ulceration as a consequence of pST treatment can also be increased under some circumstances. To test various anti-ulcer therapies, a pST-based model of ulceration was developed. Ten barrows per treatment group were administered 20 mg of pST plus the anti-ulcer therapy for six days and ulcers were scored using a 10-point system in which 0 = normal and 10 = total, severe ulcer. Recent evidence indicates that increased ulceration in pST-treated hogs may be due to increased circulating gastrin levels and subsequent increased acid secretion. Two benzimidazoles, which bind to parietal cell H^+/K^+- ATPase and inhibit acid secretion, decreased ulcer scores relative to pST alone. Ulcer scores in hogs receiving 40 mg of omeprazole (Prilosec®, Merck & Co., Inc.) by twice daily oral gavage plus 20 mg of pST/d were lower than those of hogs receiving 20 mg of pST/d alone (1.3 vs 7.3 score, SE=0.8, P<.05). In addition, ulcer scores of hogs injected twice daily with 40 mg/d timoprazole, an omeprazole analog, plus 20 mg of pST/day were lower than those of hogs receiving 20 mg of pST/d alone (.5 vs 8.0 score, SE=1.0, P<.05). On the other hand, other typical anti-ulcer therapies tested did not influence ulcer score when combined with pST treatment. Ulcer scores of hogs administered 20 mg of pST/d plus 125 or 250 mg of De-Nol twice daily, 400 micrograms of misoprostol/d by gavage, or 150 mg of ranidine IM three times/d did not differ from those of hogs receiving 20 mg of pST/d alone (average score of 7.9, SE=0.9). These results indicate that ulcers produced in hogs by high doses of pST can be prevented or mitigated by concomitant treatment with omeprazole or timoprazole, benzimidazoles that bind to the H^+/K^+- ATPase proton pump of the parietal cells, but not by other typical anti-ulcer therapies.

Key Words: pST, Benzimidazoles, Ulcers

359 Distribution of vitamin A and retinol-binding-protein (RBP) in various tissues of dogs and raccoon dogs. J. Raila[1]*, C. Eisenach[1], I. Buchholz[1], and F. J. Schweigert[2], *[1]Department of Physiology, Veterinary Faculty, University Leipzig, Germany and [2]Institute of Nutritional Sciences, University Potsdam, Germany.*

Carnivores are different to other species with regard to vitamin A(VA)-transport in blood, the distribution of VA in peripheral tissues and the excretion of VA with the urine. Raccoon dogs (*Nyctereutes procyonoides*)(n=11) and dogs (*Canis familiaris*) (n=6) of differ sex were investigated for their level of VA in plasma, urine and tissues.Organic extracts were subject to a rp-HPLC separation. Cytosolic preparations of tissues, plasma and urine were checked for RBP by Western blotting after separation on SDS-PAGE. The distribution of RBP in tissues was investigated with immunohistology using specific polyclonal antibodies raised against human RBP.We observed, that VA in the liver of raccoon dog was much lower (retinol: 5±4 μg/g; retinyl esters: 64±61 μg/g) compared to the kidney (retinol: 10±5 μg/g; retinyl esters: 281±150 μg/g). However, the concentration of VA in the liver (retinol: 26±21 μg/g; retinyl esters: 1532±700 μg/g) of the dog was higher than in kidney (retinol: 38±25 μg/g; retinyl esters: 66±16 μg/g). Retinyl esters in plasma and in tissues represented more than 50% and 90% of total vitamin A, respectively. RBP was demonstrated in plasma as well as in cytosol of liver and kidney. Additionally, we found, that in plasma and liver only one RBP-band with a molecular weight of 21 kDa was present, whereas in the kidney a second band of a lower molecular weight was present. RBP could be demonstrated immunohistologically in kidney, liver, lung and testis. In conclusion these results on vitamin A metabolism in canines raises several questions regarding generally accepted mechanisms involved in vitamin A homeostasis. Observation, that vitamin A is high in kidney, and the occurrence of a second RBP-band by Western blotting, might point to a central importance of the kidney in vitamin A metabolism of canines.

Key Words: Vitamin A, RBP, Carnivores

360 Effect of age on canine hepatic delta-6 and delta-5-desaturase activities. G. A. Reinhart[1], D. M. Vaughn[2], S. Lauten[2], S. F. Swaim[2], and M. G. Hayek[1], *[1]Research and Development, The Iams Company, Lewisburg, Ohio and [2]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, Alabama.*

Long chain polyunsaturated fatty acids play a key role in homeostasis of biological tissues and modulation of inflammatory reactions. The metabolic conversion of dietary 18-carbon fatty acids to the long chain 20- and 22-carbon polyunsaturated fatty acids has been shown in rodents and humans to be limited by fatty acid desaturation. The hepatic activity of delta-6-desaturase has also been reported to decline with age in rodents. The objective of this study was to determine if delta-6-desaturase and delta-5-desaturase activity varies in dogs with age. Nine purebred Pointers were used in the study. The dogs were selected to fit into one of three age groups: young (1–2 years), middle aged (4–5 years), and old (9–11 years). All dogs were normalized to a highly digestible, extruded diet (31% crude protein, 21% crude fat) for a 6 month adaptation period. At the conclusion of the adaptation period, liver biopsies were obtained for microsomal protein preparation. The microsomal protein was utilized in subsequent delta-5 and delta-6 desaturase enzyme kinetic studies using ^{14}C-linoleic acid as a substrate. Skin biopsies were also obtained for enzyme kinetic studies. The hepatic enzyme kinetic results demonstrated an age related decline in delta-6 and delta-5-desaturase K_m values. This was accompanied by an increase in delta-6 and delta-5 desaturase V_{max} values. As expected, there was no detectable delta-5 or delta-6-desaturase activity in the skin of dogs from any age group. This data suggests one possible mechanism by which the onset of chronic degenerative disease symptoms in dogs may be associated with lowered desaturase activities, and a possible benefit of directly providing polyunsaturated fatty acids that bypass these enzymes. Further understanding of the age related changes in canine lipid metabolism will enable more precise dietary fatty acid formulations for various lifestages of the dog.

Delta-6-Desaturase	Km	V_{max}	Delta-5-Desaturase	Km	V_{max}
Young	0.82	97.6	Young	2.87	56.6
Middle aged	0.62	96.9	Middle aged	1.05	39.3
Old	2.46	212.2	Old	4.76	107.9

Key Words: Canine, Lipid, Hepatic, Polyunsaturated, Fatty Acids, Enzyme

361 Influence of breed type on brown adipose tissue thermogenesis and iodothyronine 5'deiodinase activity in newborn calves. G. E. Carstens[1], E. H. McPhail[1], S.Kahl[2], S. B. Smith[1], and T. S. Rumsey[2], *[1]Texas A&M University, College Station and [2]USDA, Agricultural Research Service, Beltsville, MD.*

Conversion of thyroxine (**T_4**) to triiodothyronine (**T_3**) in brown adipose tissue (**BAT**) by 5′deiodinase (**5′D**) plays a critical role regulating BAT thermogenic capacity in newborn ruminants. Our objective was to determine the effects of breed type on BAT and liver 5′D activities, and plasma T_3 and T_4 concentrations in newborn calves. At 6 h of age, heat production was measured in Brahman (n = 7) and Angus (n = 10) calves prior to and following NE infusion (20 μg/kg/min; for 10 min) to assess BAT thermogenesis *in vivo*. Calves were killed at 12 h of age and perirenal BAT and liver samples collected to measure 5′D. Deiodinating activity was determined by quantifying the $^{125}I^-$ released from ^{125}I-labeled reverse-T_3 using assay conditions for type I (**5′D-I**; liver and BAT) and type II (**5′D-II**; BAT). Blood was collected at 6 h of age and analyzed for T_3 and T_4. Brahman calves had lower (P < .01) NE-induced thermogenic rates than Angus calves (52 vs 64 ± 3 cal/kg/min). However, Brahman calves had higher (P < .01) plasma T_3 (614 vs 377 ± 56 ng/dL) and higher (P < .05) T_4 (26.2 vs 16.2 ± 2 μg/dL) concentrations than Angus calves. Plasma T_4 levels were negatively correlated to NE-induced thermogenesis (r = –.41; P = .10), suggesting that elevated T_4 levels may play a suppressive role in regulating BAT. Compared to Angus calves, liver 5′D-I was lower (P < .05) in Brahman (20 vs 26 ± 1.8 nmol I/h/mg protein), whereas, BAT 5′D-I was higher (P < .05) in Brahman calves (.94 vs .60 ± .12 nmol I/h/mg protein). BAT 5′D-II was also higher (P < .01) in Brahman than Angus calves (4.8 vs 1.9 ± .51 pmol I/h/mg protein). To our knowledge, these data are the first to suggest that bovine BAT contains both types of 5′D. These data demonstrate that lower BAT thermogenesis rates in newborn Brahman calves is not due to a limit of T_3 generated by 5′deiodinase.

Key Words: Brown Fat, 5′Deiodinase, Thyroid Hormones

362 ***In vitro*** **liver synthesis and plasma levels of corticosteroid-binding globulin in the young pig.** J. Heo*, H. G. Kattesh, J.J.E. Doré, and J. D. Godkin, *The University of Tennessee, Knoxville.*

Plasma levels and the *in vitro* synthesis by the liver of corticosteroid-binding globulin (CBG) were compared in female pigs prior to weaning. Blood (4 ml) and two lobes of liver were collected from anesthetized pigs (4 pigs/age) at 3, 10, 20, 30 or 40 days following birth. Liver slices (200 mg) of uniform thickness (~0.5 mm) were placed in triplicate in dishes containing 5 ml of Williams medium E and incubated in a humidified 5% CO_2, 45% O_2, 50% N_2 atmosphere at 37°C for 12 hr. The concentration of CBG in the plasma (plCBG) and media (mCBG) from cultured liver slices was measured using an enzyme-linked immunosorbent assay (ELISA) for porcine CBG. Intra- and interassay coefficients of variation (n = 20) of CBG in a pooled plasma sample were 6.9% and 14%, respectively. The percentage of cytoplasmic lactate dehydrogenase activity leaked from liver slices into medium, used to monitor cell viability, ranged from 20 to 50%. Age-related changes in mean (±SE) values of CBG and TP were as follows:

Item/day	3	10	20	30	40
plCBG (ug/ml)	1.2 ± 0.2^{a}	1.4 ± 0.1^{a}	1.6 ± 0.1^{ab}	2.3 ± 0.3^{b}	1.6 ± 0.2^{ab}
mCBG (ng/mg)	2.9 ± 0.8^{ab}	4.4 ± 0.4^{b}	2.8 ± 0.2^{a}	2.3 ± 0.6^{a}	2.2 ± 0.5^{a}
TP (ug/mg)	11.0 ± 0.7	12.4 ± 0.2	10.6 ± 0.7	10.6 ± 0.5	12.0 ± 0.6

Plasma CBG levels increased ($P < 0.05$) 33% by day 30 relative to days 3 and 10. The concentration of CBG released from liver slices following 12 hr incubation was higher ($P < 0.01$) on day 10 compared to subsequent days measured. The amount of total protein (TP) released per unit weight of liver did not change with age. These results suggest that hepatic secretion of CBG may be regulated in a different manner from that of other proteins. Also, different patterns in CBG concentration in plasma versus *in vitro* synthesis by the liver may reflect an increase in CBG half-life in circulation and/or decrease in metabolic clearance with increasing age in the young pig.

Key Words: Pig, Corticosteroid-binding Globulin, Liver

363 **Circulating progesterone and intrafollicular steroid concentrations in dominant ovarian follicles following an injection of progesterone in cows.** B. R. Lindsey*, M. E. Wehrman, E. J. Melvin, J. A. Quintal, F. N. Kojima, E. L. Zanella, K. E. Fike, E.G.M. Bergfeld, and J. E. Kinder, *University of Nebraska, Lincoln.*

The objective was to determine effects of a single injection of progesterone (P4) at different times during the development of dominant ovarian follicles on circulating concentrations of P4 and on intrafollicular steroids of the dominant follicle. Estrus was synchronized to a common day in 36 multiparous cows assigned to 1 of 3 treatments or 1 of 3 corresponding control groups (n=6/group). Follicles ≥ 5 mm were ablated via transvaginal ultrasonography in all animals 7 days post-estrus (d0) to synchronize ovarian follicular growth. Treated and control animals received either 200 mg of P4 in 10 ml sesame oil or 10 ml sesame oil, respectively, as a single im injection on d2 (TRT1 vs CON1), d4 (TRT2 vs CON2), or d6 (TRT3 vs CON3) following follicle ablation. Blood plasma samples were obtained at 6 h intervals from d0 to d4, 6, or 8 for animals injected on d2, 4, or 6, respectively, for quantifying peripheral P4. Follicular fluid (FF) was aspirated *in vivo* from the largest 3 follicles of each animal via transvaginal ultrasonography 48 h following injections. Ratios of estradiol (E2):P4 were used to determine functional status of individual follicles [E2:P4>1 = estrogen active (EA); E2:P4<1 = estrogen inactive]. All treatment groups had greater mean circulating concentrations of P4 than their respective control groups during the 48 h following administration of treatments (TRT1, 8.1 vs CON1, 4.6 ng/ml, P<.0001; TRT2, 9.0 vs CON2, 4.6 ng/ml, P<.0055; TRT3, 7.8 vs CON3, 6.0 ng/ml, P<.0001). The number of EA dominant ovarian follicles within any treatment group was not different (P>.1) than their respective controls (TRT1, 5/6 vs CON1, 4/6; TRT2, 3/6 vs CON2, 4/6; TRT3, 3/6 vs CON3, 2/6). Administration of P4 either did not affect the number of EA dominant ovarian follicles, or treatment differences were not apparent within 48 h following treatment with P4.

Key Words: Progesterone, Dominant Follicle, Intrafollicular Steroids

364 **Effect of GnRH, $PGF_{2\alpha}$, and norgestomet on follicular maturation and ovulation in suckled beef cows.** K. E. Thompson, D. M. Grieger, G. C. Lamb, and J. S. Stevenson*, *Kansas State University, Manhattan.*

Eighteen purebred cows were assigned randomly to three treatments to determine the effect of GnRH (G) and norgestomet (N) on follicular growth and ovulation. The treatments consisted of: 1) a 100-μg injection of G (d –7) plus a 6-mg ear implant of N, 25-mg injection of PG plus implant removal (d 0; GNPG); 2) an injection of G (d –7), an injection of PG (d 0; GPG); or 3) an injection of saline plus ear implant of N (d –7), an injection of PG and implant removal (d 0; SNPG). All cows in each treatment received a second injection of G 48 h after PG and were inseminated 16 h later. Blood samples were collected on d –18 and daily from d –7 to d +3 before intrarectal ultrasonography of ovarian follicles to determine cycling status and to monitor serum concentrations of progesterone (P4). On d –7 and d +2, blood was collected hourly for 6 h to monitor LH release after G. Pregnancy was determined 27 to 28 d after AI using ultrasonography. The number of cycling and noncycling cows ovulating (based on disappearance of dominant follicle and subsequent increase in serum P4) after the second injection of G were: GNPG (½ and 4/4); GPG (⅓ and ⅓); and SNPG (¾ and 2/2), respectively. Interactions of treatment × cycling status × day for concentrations of P4 were significant (P<.05). Treatment × cycling status means (ng/mL) for P4 for d –7 to d +3 in cycling and noncycling cows were: GNPG (1.51 and .58), GPG (1.33 and .38), and SNPG (2.40 and .26), respectively. Mean LH (ng/ml) for GNPG (9.6 ± 1.4) and SNPG (9.8 ± 1.4) differed (P<.05) from GPG (4.7 ± 1.4). The number of cycling and noncycling cows that became pregnant after timed AI were: GNPG (½ and ¾); GPG (⅓ and ⅓); and SNPG (2/4 and 0/2), respectively. We conclude that N increased the LH response to G, and G combined with N increased P4 in noncycling cows. Therefore, fertile ovulations were induced in cows after treatment with G and(or) N.

Key Words: GnRH, Norgestomet, Beef Cattle

365 **Effect of weaning weight on ovarian follicle populations in the cycling beef cow.** H. L. McLane*, N. R. Rohrbach, F. N. Schrick, and B. H. Erickson, *University of Tennessee, Knoxville.*

An experiment was conducted to identify possible associations between weaning weight (WWT) and ovarian follicle populations. Two and 3-yr old Polled Hereford cows (n=22; nonlactating and lactating) at random stages of their estrous cycle were treated with 25 mg of $PGF_{2\alpha}$ and two injections of a GnRH agonist to synchronize estrus (d=0). Ovaries were removed on d 8 of the cycle, measured, sectioned into four quarters and fixed in Bouin's solution. Eight 10μm thick sections, 100μm apart, were stained for light microscopic examination. Follicles were recorded as primary, growing or vesicular (normal or atretic, small, medium and large) for the whole ovary. Cows were divided into low, medium and high WWT groups based on their BW at 7-9 mo. Lactation status, age and cycle day interacted with WWT to affect the number of growing (P=.03), atretic vesicular (P=.03) and small atretic vesicular follicles (P=.02). Least square means of growing follicles for the medium WWT group (12,719±1550) differed from the low (3,792±1,976; P=.002) and high (7,685±1,809; P=.03). Numbers of total atretic vesicular follicles were not different for the WWT groups. Two-yr old cows had fewer small atretic follicles (18.9±4.0; P=.001) than 3-yr old cows (36.2±4.8; P=.001). When BW at ovariectomy was included with WWT in the statistical model, numbers of primary (P=.02), total atretic (P=.01), small (P=.02) and medium atretic (P=.02), and total vesicular follicles (P=.08) differed. These data imply that heifers of moderate WWT have a greater population of growing follicles at 2- and 3-yr of age, and support the use of WWT as a selection tool for reproductive potential.

Key Words: Follicle, Weaning weight, Beef cattle

366 Effect of hydroxycholesterol (OHC) analogues on progesterone (P4) production by bovine luteal cells. R. P. Del Vecchio and W. D. Sutherland*, *Agriculture & Agri-Food Canada, Brandon Research Centre, Brandon, Manitoba, Canada.*

Two experiments (EXP) were conducted to determine the effects of OHC analogues on bovine luteal cell P4 secretion. Corpora lutea were collected between d10-12 (EXP 1) or 16-18 (EXP 2) of the estrous cycle. The cells were dispersed and mixed large and small luteal cells were incubated in 1 ml of modified Ham's F-12. In EXP 1, cells were treated with 5μg of either cholesterol (C), 20α-OHC, 22(R)-OHC, 22(S)-OHC, 25-OHC and LH (0, 50ng). Medium was collected at 24, 48, 72, 96, 120 and 144h. Regardless of treatment, P4 production decreased (P<.05) over time. Addition of the OHC analogues increased (P<.0001) P4 production by 48h in all groups except C. By 96h, P4 production by all the OHC analogue treatments was greater (P<.05) than controls. The 22(R)-OHC treatment had the most, and C had the least significant effect. The addition of LH increased (P < .05) P4 beyond the respective OHC analogue treatment in all groups except 22(R)-OHC. In EXP 2, luteal cells were treated with 22(R)-OHC (0, .03, .06, .09, .12, .15, .3, .6, .9, 1.2, 1.5 μg) and LH (0, 50 ng) to determine a dose level for routine supplementation. A significant LH × 22(R)-OHC × d interaction showed that 22(R)-OHC increased (P<.0001) P4 production in a dose dependent manner. Addition of LH to luteal cells collected on d16 and 17 did not increase P4 production beyond the 22(R)-OHC only treatment. Unexpectedly, LH decreased P4 on d18 in the .90 μg (P<.07) and the 1.2, and 1.5 μg (P<.002) 22(R)-OHC treatment groups. These data show that medium supplemented with C or OHC analogues increases bovine luteal cell P4 output, with 22(R)-OHC being the most dramatic. The addition of LH failed to increase P4 production beyond the 22(R)-OHC only. The LH increased P4 in luteal cells collected on d10-12 but not 16-18 of the estrous cycle. Lastly, the interaction of LH plus higher doses of 22(R)-OHC seemed to inhibit P4 production by bovine luteal cells collected on d18.

Key Words: Corpora Lutea, Progesterone, Hydroxycholesterol

367 Immediate and delayed effects of heat stress on follicular development and function in lactating cows. Z. Roth, R. Meidan, A. Shaham-Albalancy, and D. Wolfenson*, *The Hebrew University of Jerusalem, Rehovot, Israel.*

Hyperthermia during the summer affects reproductive function. Small antral follicles take 40-50 days to develop into large dominant follicles; thus exposure to heat during the early stages of follicular development may later impair preovulatory follicle function. Two experiments were conducted to examine a possible delayed effect of heat stress on follicular development and steroidogenesis. In experiment 1, performed in the summer, lactating Holstein dairy cows were cooled (n=7; C) by sprinkling and ventilation, or heat-stressed (n=6; HS) by exposure to direct solar radiation during the entire estrous cycle. Following estrus, HS and C cows were cooled for 10 days. Follicular development was examined daily by ultrasonography. More medium-size (class 2, 6-9 mm) follicles were counted in the HS cows during the second (preovulatory) follicular wave of the first cycle than in C cows (P<0.05). This follicular wave emerged (in terms of number of class-2 follicles) one day earlier and declined 2 days later in HS cows relative to C cows (P<0.05). These findings indicate depression of dominance of the preovulatory follicle developed during a spontaneous estrous cycle under heat-stress conditions, confirming previous reports on dominance depression in a programmed estrous cycle. During the first wave of the subsequent cycle, less class-2 follicles developed but the rate of decline in their number in the HS group was slower (P<0.05) than in the C group, indicating a delayed effect of HS on follicular dynamics in the subsequent cycle. In experiment 2, performed in the winter, cows were heat-stressed in an environmental chamber (n=6) during days 2-6 of the cycle. On day 3 of the subsequent cycle, the ovaries were collected and medium-size follicles were classified as atretic or healthy: less healthy follicles were counted in HS (38%) than in C (56%) cows (NS). Dispersed granulosa and theca cells from healthy class-2 follicles were incubated for 6 h with testosterone (300 ng/ml) or forskolin (10 μM), respectively. Estradiol and androstenedione production were lower (P<0.05) in HS cows than in controls (0.3±0.15 vs. 0.96±0.25, and 0.22±0.04 vs. 0.89±0.19 ng/10^5 cells, respectively). Results suggest impairment of both follicular dynamics and steroidogenesis in cows previously subjected to heat stress; this delayed effect may be related to the lower fertility of dairy cows in the autumn.

Key Words: Dairy cows, Heat stress, Follicular development

368 The effect of plasma progesterone during the luteal phase on the characteristics of the subsequent cycle corpus luteum in dairy cows. A. Shaham-Albalancy[1], M. Rosenberg[2], Y. Folman[2], and D. Wolfenson[1]*, [1]*The Hebrew University and* [2]*Agricultural Research Organization, Rehovot, Israel.*

Conception in dairy cows is positively correlated with plasma progesterone (P_4) concentrations during the luteal phase preceding insemination. Plasma P_4 during the luteal phase affects dominant-follicle characteristics, which may alter the function of the corpus luteum (CL) developed following ovulation. The present study examined the effect of P_4 levels during the luteal phase on the characteristics of the subsequently formed CL. Lactating Holstein cows were allotted to four groups: (1) control, untreated cows (n=4); (2) high P_4 cows (n=5), in which two P_4-containing intravaginal devices (CIDRs) were inserted on day (d) 6, replaced on d 9 and 12, and removed on d 15 of the estrous cycle; (3) low P_4 cows (n=6), in which 3 $PGF_{2\alpha}$ injections given at 12 h intervals starting on d 3 created a low but progressively increasing plasma P_4 curve; in groups 1-3, $PGF_{2\alpha}$ was injected on d 14 and 15 to regress the CL; (4) constant low P_4 cows (n=5), in which low and steady P_4 curves were produced by injecting $PGF_{2\alpha}$ on d 6 and 7 and inserting 2 CIDRs on d 6, replacing them on d 9 and 12 and removing them on d 15. Mean±SE plasma P_4 concentrations in groups 1-4 during the treated cycle were: 3.2±0.3, 4.8±0.3, 2.3±0.2 and 2.0±0.2 ng/ml, respectively (p<0.05), and were similar among groups in the subsequent cycle. On d 13 of the subsequent cycle, the CLs were collected and 1-mm slices (2.3±0.1 mg tissue) were incubated for 16 h with medium only, LH (100 ng/ml), or LH+$PGF_{2\alpha}$ (100 ng/ml+1 μg/ml). Progesterone concentrations (μg/g tissue) in the incubation medium for high P_4, control, low P_4 and constant low P_4 groups, respectively, were for medium only: 19.7±2.1, 20.5±5.6, 17.7±4.0 and 19.4±4.1; for LH: 36.8 ± 8.0^a, 34.6 ± 12.9^a, 18.0 ± 4.9^b and 20.2 ± 5.0^b; and for LH+$PGF_{2\alpha}$: 47.9 ± 9.4^a, 19.5 ± 7.8^b, 17.2 ± 3.0^b and 20.4 ± 6.0^b, (p<0.05). Results indicate that CLs obtained from high P_4 and control groups secreted 87% more (p<0.05) P_4 in the presence of LH than the two low P_4 groups. The addition of $PGF_{2\alpha}$ did not reduce P_4 in the high P_4 group only. The reasons for these differences in CL function are unclear, as is their possible significance in the maintenance of the CL and fertility.

Key Words: Dairy cows, Progesterone, Corpus luteum

369 Endocrine profiles for bovine somatotropin (bST) and insulin-like growth factor I (IGF-I) in cows treated with incremental doses of sustained-release recombinant bST (rbST) for reproductive studies. C. R. Bilby* and M. C. Lucy, *University of Missouri, Columbia.*

Dairy cows treated with low doses of rbST have improved postpartum reproductive performance. The objectives were to evaluate incremental doses of rbST (Posilac®; Protiva, St. Louis, MO) for increasing serum bST and IGF-I and to perform a preliminary study of conception rate in cows treated with a low dose of rbST at insemination. Study 1: Pregnant lactating Holstein cows (n=18) were randomly assigned to one of four treatments: Control (n=5; saline); 100 mg rbST (n=5; 0.2 dose); 200 mg rbST (n=5; 0.4 dose); or 500 mg rbST (n=3; 1 dose). Serum and milk were collected on alternate days from 8 d prior to injection to 14 d after injection. Serum concentrations (ng/ml) of bST and IGF-I were measured by radioimmunoassay. There was a treatment-by-day interaction for serum bST (P<0.001) and IGF-I (P<0.001). Serum bST and IGF-I were 1.75±0.31 and 77.71±7.69, respectively for control cows. Greatest serum bST and IGF-I were 3.95±0.53 on d 6 and 92.60±5.71 on d 6 for 100 mg, 3.87±0.59 on d 6 and 119.45±5.71 on d 8 for 200 mg and 7.75±0.68 on d 8 and 134.34±7.37 on d 8 for 500 mg, respectively (day 0=injection). On d 14, serum bST and IGF-I were 2.99 ±0.59 and 84.08±5.71 for 100 mg, 1.72±0.53 and 90.24±5.71 for 200 mg and 3.57±0.68 and 101.02±7.37 for 500 mg, respectively. Increased serum IGF-I was associated with increased milk IGF-I. Study 2: Cows were injected with either 1/3 dose rbST (167 mg) or saline at insemination. Conception rate for rbST and control was 48.6% and 42.5% for herd 1 (n=75 beef cows), 40.0% and 25.9% for herd 2 (n=57 beef cows), 16.7% and 21.1% for herd 3 (n=37 beef cows) and 65.0% and 57.9% for herd 4 (n=39 dairy cows). Herd affected conception rate (P<0.002), but conception rates for rbST (43.7%; n=103) and control (37.1%; n=105) did not achieve statistical significance. In summary, low doses of rbST caused increases in serum bST and IGF-I that may be physiologically relevant in reproducing cattle.

Key Words: Somatotropin, IGF-I, Reproduction

370 Suckling reinitiated lactation in beef cows after an early postpartum hiatus of milking or suckling. G. C. Lamb[1], B. L. Miller[1], J. M. Lynch[1], D. M. Grieger[1]*, J. S. Stevenson[1], and M. C. Lucy[2], *[1]Kansas State University, Manhattan and [2]University of Missouri, Columbia.*

We determined whether lactation could be reinitiated after renewed suckling of cows that were neither milked nor suckled for 4 wk. Fifty-three Angus × Hereford cow-calf pairs were suckled ad libitum for 13 to 18 d postpartum and then assigned to six treatments for 4 wk: 1) calf was weaned (calf weaned; CW; n = 9); 2) calf was present continuously with dam but contact with the udder was prohibited (calf restricted; CR; n = 9); 3) calf was present continuously with dam (calf present; CP; n = 9); 4) same as CR but dam was suckled twice daily by her calf (CR+2×S; n = 8); 5) same as CW but dam was milked twice daily (CW+2×M; n = 9); 6) same as CR but dam was milked twice daily (CR+2×M; n = 9). On the last day of treatment, calves in CR, CP, CR+2×S, and CR+2×M were separated from their dams for 8 h. Upon reunion of cow and calf (0 min), blood was collected from –60 to +60 min to assess hormonal changes. Thereafter, calves suckled ad libitum their own dam, except when milked twice in 24 h after 40 I.U. of oxytocin at reinitiation of suckling (0 wk), 1, and 5 wk. Prolactin and cortisol were increased (P<.05) in CP, CR+2×S, and CR+2×M cows during 20 min after milking and reunion with their calves. Concentrations of IGF-I did not differ among treatments; however, cows that were neither milked nor suckled during treatment had more (P<.01) IGF-I than lactating cows (71.8 ± 4.6 ng/ml vs 56.0 ± 3.5 ng/ml). At 0 wk, energy corrected milk was greatest in CP (7.1 ± 1.0 kg/d) cows, followed by CR+2×M (5.8 ± .9 kg/d), CR+2×S (5.2 ± .7 kg/d), and CW+2×M (3.2 ± 1.0 kg/d) cows; no milk was harvested from CW and CR cows. After 1 wk of renewed suckling, milk synthesis in CW (2.4 ± .8 kg/d) and CR (1.6 ± .8 kg/d) cows was reinitiated, and by 5 wk, nearly normal composition and yield of milk were harvested in CW (3.7 ± .8 kg/d) and CR (3.0 ± 1.0 kg/d) cows. We concluded that renewed suckling in early postpartum cows that are neither milked nor suckled during a 4-wk hiatus reinitiated lactation.

Key Words: Milking, Suckling, Lactation

371 A Possible Indicator of Endometritis in Dairy Cattle in Honduras. R. C. Seals[1]*, I. Matamoros[2], and G. S. Lewis[1], *[1]Virginia Polytechnic Institute and State University, Blacksburg and [2]Escuela Agricola Panamericana, Zamorano, Honduras.*

The objective of this study was to determine whether temporal relationships between 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) and progesterone (P_4) can be used to improve the prediction and early diagnosis of uterine infections (endometritis), which often reduce reproductive performance. Beginning after calving and continuing on a 3 ×/wk schedule, jugular blood was collected from dairy cows, their reproductive organs were examined per rectum, and uterine swabs were taken. As cows became available, they were assigned to one of three groups. 1) Control cows (CON; n=10) had no signs of uterine infection, 2) treated (TRT; n=11) cows developed spontaneous endometritis and were treated after diagnosis, and 3) untreated cows (UT; n=10) developed spontaneous endometritis and were not treated. Cows in the TRT and UT groups were diagnosed with endometritis on d 18.4 ± 2.1 (mean ± SEM) postpartum. Mean concentrations of PGFM were less ($P < .05$; SEM = 350 pg/mL) from d 1 to 7 postpartum in cows that developed endometritis (TRT, 1,340 and UT, 1,550 pg/mL) than in CON cows (2,160 pg/mL). Also, mean concentrations of PGFM were less ($P < .05$; SEM = 83 pg/mL) from d 22 to 35 postpartum in cows that developed endometritis (TRT, 280 and UT, 270 pg/mL) than in CON cows (440 pg/mL). No differences were detected in serum P_4, days to first estrus, or days to first AI. In conclusion, reduced concentrations of PGFM during certain postpartum periods may be a useful tool for the prediction and early diagnosis of endometritis.

Key Words: Cattle, Endometritis, Indicator

372 Effect of $PGF_{2\alpha}$, estradiol-17β or $PGF_{2\alpha}$ + indomethacin, estradiol-17β or tamoxifen on progesterone, pregnancy specific protein B (PSPB) and pregnancy status in 90 day pregnant ewes. P. J. Bridges[1], Y. S. Weems[1], B. R. LeaMaster[1], R. G. Sasser[2], D. L. Vincent[1], and C. W. Weems[1], *[1]University of Hawaii, Honolulu and [2]University of Idaho, Moscow.*

A model for regulation of ovine placental secretion of progesterone has been proposed (Weems et al., Prostaglandins 43:203, 1992; 46: 277, 1993 and 48:377, 1994) whereby estradiol-17β regulates placental secretion of PSPB, PSPB regulates placental secretion of PGE and PGE regulates placental secretion of progesterone in 90 day pregnant sheep. The objective was to determine the effects of $PGF_{2\alpha}$ or estradiol-17β or $PGF_{2\alpha}$ + estradiol-17β, indomethacin or tamoxifen on secretion of progesterone, PSPB or pregnancy at 90 days in sheep. Ewes were fitted with jugular venous and vena cava catheters and received vehicle, $PGF_{2\alpha}$ (8 mg/58 kg/BW) i.m. at 0 hr, estradiol-17β (500 μg) i.m. every 6 hr, or $PGF_{2\alpha}$ at 0 hr + estradiol-17β, indomethacin (100 mg) or tamoxifen (40 mg) i.m. every 6 hr. There were 5 ewes in each group. Jugular venous and inferior vena cava blood were collected every 6 hr from 0-60 hr, every 20 min from 62-66 hr and at 72 hr for analysis for progesterone or PSPB by RIA. Profiles of progesterone in jugular venous or PSPB in vena cava plasma were analyzed by a split-plot ANOVA for repeated measures. Profiles of progesterone in $PGF_{2\alpha}$ + indomethacin-treated ewes were lower ($P \leq 0.06$) than in control ewes and did not differ between the other 5 treatment groups. There was a treatment × time effect ($P \leq 0.05$) for PSPB in vena cava plasma. PSPB increased ($P \leq 0.05$) at 6 hr and declined with time in the control, $PGF_{2\alpha}$ and $PGF_{2\alpha}$ + estradiol-17β groups. PSPB increased ($P \leq 0.05$) at 6 hr in the $PGF_{2\alpha}$ + indomethacin, estradiol-17β and $PGF_{2\alpha}$ + tamoxifen groups and remained elevated for the remainder of the 72 hr sampling period. Two of 5 ewes in the $PGF_{2\alpha}$ + estradiol-17β group aborted. These data support the model that estradiol-17β may regulate placental PSPB secretion and that PGE may regulate placental secretion of progesterone.

Key Words: Ewe, Progesterone, Placenta

373 Plasma progesterone response following ACTH administration in the pregnant Brahman heifer. S. T. Willard[1]*, D. C. Lay, Jr.[2], T. H. Friend[3], D. A. Neuendorff[1], and R. D. Randel[1], *[1]Texas A&M University, Overton and [3]College Station, and [2]Iowa State University, Ames.*

Previous reports of adrenal progesterone (P_4) contributions during late gestation in cattle, and ACTH-induced P_4 responses in the non-pregnant heifer, prompted an investigation to evaluate the plasma P_4 response and the ratio of plasma cortisol (CT):P_4 following an ACTH challenge in mid-gestation pregnant Brahman heifers. Twenty-three pregnant heifers (139 ± 5 d of gestation) received one of the following treatments: 0 (saline; n=5), 0.125 (n=4), 0.25 (n=5), 0.5 (n=4) and 1.0 (n=5) IU of ACTH per kg BW. Blood samples were collected at –15, 0, 15, 30, 45, 60, 75, 105, 135, 165, 195, 255 and 315 min post-ACTH challenge. Plasma P_4 was quantified by RIA. Pre-ACTH P_4 did not differ ($P > .10$) among ACTH treatment groups (pooled: 12.1 ± 0.6 ng/ml). Among peak P_4 values at 15 min post-ACTH infusion, control P_4 (9.6 ± 1.2 ng/ml) tended to be lower ($P < .07$) than 0.5 IU ACTH-treated heifers (13.3 ± 1.1 ng/ml), and were lower ($P < .02$) than 0.25 and 1.0 IU ACTH-treated heifers (14.7 ± 1.1 and 22.2 ± 3.7 ng/ml, respectively). During the primary P_4 response period (0-75 min post-ACTH), the area under the curve (AUC) was greater ($P < .05$) for 1.0 IU ACTH-treated heifers than all other groups. The CT:P_4 ratios were lower (Time × Treatment, $P < .01$) for control heifers than all ACTH-treated heifers. Among ACTH-treated heifers, the CT:P_4 ratio response and CT:P_4 ratio AUC were similar ($P > .10$) following ACTH challenge. These data suggest acute increases in ACTH elevate plasma P_4, most likely of adrenal origin, in mid-gestation pregnant heifers, while the CT:P_4 ratio remains relatively constant irrespective of ACTH dose (0.125 – 1.0 IU).

Key Words: Progesterone, ACTH, Adrenal

374 Comparison of endocrine and metabolite profiles among Angus, Brahman, and Senepol cows. P. Alvarez[1]*, T. D. Hamilton[1], R. E. Stewart[1], C. C. Chase, Jr.[2], A. C. Hammond[2], T. A. Olson[3], E. J. Bowers[2], and L. J. Spicer[1], *[1]Oklahoma State Univ., Stillwater, [2]USDA, ARS, Brooksville, FL, and [3]Univ. of Florida, Gainesville.*

Concentrations of FSH, insulin, estradiol (E_2), glucose and plasma urea nitrogen (PUN) were determined in multiparous lactating Angus (**A** temperate Bos taurus n = 11; BW = 407 ± 9 kg), Brahman (**B** tropical Bos indicus n = 9; BW = 490 ± 10 kg) and Senepol (**S** tropical Bos taurus n = 11; BW = 478 ± 8 kg) cows during July and August in central Florida. Body condition score (5.0 ± .2) and age (6.5 ± .4 yr) did not differ among breeds. Length of the estrous cycle was greater ($P < .05$) in **S** (21.8 ± .6 d) than **A** (18.6 ± .6 d) or **B** (19.5 ± .6 d) cows. Blood plasma samples were collected daily during an estrous cycle and analyzed for FSH, insulin, E_2, glucose, and PUN on d 0 to 20. Plasma concentrations of insulin were greater ($P < .001$) in **B** (.59 ± .04 ng/mL) than in **A** (.44 ± .04 ng/mL) and **S** (.49 ± .03 ng/mL) cows, but did not differ between **A** and **S** cows. Plasma concentrations of E_2 did not differ ($P > .10$) among genotypes but both day and breed × day affected ($P < .05$) E_2 ; peak E_2 levels were reached later in the estrous cycle of **S** than **A** or **B** cows. Plasma concentrations of FSH were greater ($P < .05$) in **A** (.63 ± .05 ng/mL) than in **B** (.46 ± .06 ng/mL) and **S** (.44 ± .05 ng/mL) cows, and both day and breed × day affected ($P < .05$) FSH. The secondary surge of FSH seemed more distinct in **A** cows than in **B** or **S** cows. Plasma glucose concentrations were greater ($P < .05$) in **S** (76.7 ± 1.84 mg/dL) than in **A** (69.0 ± 1.94 mg/dL) cows, but did not differ ($P > .05$) between **B** (74.3 ± 2.06 mg/dL) and **A** or **S** cows. Concentrations of PUN were greater ($P < .001$) in **B** (15.4 ± .52 mg/dL) and **S** (15.4 ± .46 mg/dL) cows than in **A** (11.9 ± .49 mg/dL) cows. In conclusion, plasma concentrations of hormones and metabolites differ among Brahman (Bos indicus), Senepol (tropical Bos taurus) and Angus (temperate Bos taurus) cows maintained in a subtropical environment.

Key Words: Beef Breeds, FSH, Insulin

375 Effect of dopamine antagonist on serum GH, IGF-I, and prolactin concentrations in lactating Holstein cows. A. Ahmadzadeh*, M. A. Barnes, R. M. Akers, F. C. Gwazdauskas, and M. L. McGilliard, *Virginia Polytechnic Institute and State University, Blacksburg.*

Fluphenazine (FLU), a dopamine receptor antagonist, was used to test the effect of endogenous dopamine on serum GH, IGF-I, and prolactin concentrations. Eighteen lactating cyclic Holstein cows were randomly assigned to receive (i.v.) one of three treatments: saline (C; n=6), .1mg/kg BW FLU (LD; n=6), or .5 mg/kg BW FLU (HD; n=6). On d 10 or 11 post-estrus (d 0=estrus), blood samples were collected at 15 min intervals for 4 h before and 4 h after FLU or saline. Mean serum progesterone in all cows was 3.1±.3 ng/ml. Mean serum GH and prolactin concentrations did not differ among the groups prior to treatments ($P > .1$). Neither dose of FLU affected mean serum GH concentration (LD: 4.6±.4 and HD: 5.4±.4 ng/ml) compared to the saline-treated cows (C: 4.8±.4 ng/ml). However, both doses of FLU increased ($P < .01$) serum prolactin concentration (LD: 29.1±2.5 and HD: 26.9±2.5 ng/ml) compared to saline-treated cows (C: 5.0±2.5 ng/ml). Mean serum IGF-I concentration was not altered by FLU (from 49.6±4.9 before FLU to 50.9±5.1 ng/ml after FLU in HD group and from 74.8±4.9 before FLU to 67.1±4.9 ng/ml after FLU in LD group). Serum IGF-I concentration remained unchanged in saline-treated cows (from 78.2±4.9 before saline to 81.9±4.9 ng/ml after saline). These data suggest that a dopamine mediated mechanism for modulation of GH secretion is absent in lactating cyclic Holstein cows. However, endogenous dopamine may play an inhibitory role in prolactin secretion in lactating dairy cows.

Key Words: Dopamine antagonist, GH, Prolactin

376 The relationship of porcine fecal progesterone concentrations to porcine serum progesterone concentrations. T. A. Armstrong*, V. S. Hedgpeth, J. H. Britt, and W. L. Flowers, *North Carolina State University, Raleigh.*

The objective was to determine the temporal relationship between serum and fecal progesterone (P4) concentrations in the pig. In the first experiment, P4 levels in serum and feces were quantified for sows at various stages of lactation following hCG injections. Extraction of P4 from fecal samples was done by the addition of 10 mL of 0.01 M PBS gel to 1.0 g of fecal material and agitation at room temperature for 18 h. The liquid and solid fractions were separated via centrifugation, and the supernatant was analyzed for P4 via an RIA. In early and mid-lactation, the serum P4 following hCG increased ($P < 0.01$) to peak levels of 12.4 ± 2.0 ng/mL at d 14 and 7.6 ± 1.2 ng/mL at d 18, respectively. In contrast, fecal P4 did not show an increase ($P < 0.01$) over time. When hCG was administered in late lactation, both serum and fecal P4 increased ($P < 0.01$) to peak levels at d 21, 13.4 ± 1.3 ng/mL and 24.4 ± 3.0 ng/g, respectively. In a second experiment, 28.26 mg of P4 was injected into an intact (INT) and ovariectomized (OVX) gilt. No differences ($P < 0.9$) over time in P4 were observed for the INT gilt. In contrast, the OVX gilt had an increase ($P < 0.01$) in serum P4 8 h post-injection. However, in fecal samples no differences ($P < 1.0$) were observed over time. These data demonstrate an inconsistent temporal relationship between P4 in serum and feces. In situations where P4 decreased, fecal concentrations were not a reliable estimator of serum values. In situations where serum P4 increased above 10 ng/mL, fecal and serum values exhibit a positive relationship.

Key Words: Progesterone, Feces, Serum

377 The equine endometrial cycle: a morphometrial, ultrastructural, enzyme- and immuno-histological investigation. G. Raila[1]*, H.-A. Schoon[1], D. Schoon[1], S. Özgen[1], H. Aupperle[1], E. Klug[2], and O. Strankmeyer[2], *[1]University Leipzig and [2]Tierärztliche Hochschule Hanover, Germany.*

Aim of this study is the detailed characterization of the mare's endometrial cycle using light and electron microscopy, immuno- and enzymehistochemistry and morphometry. The study includes endometrial biopsies from 14 mares (actively cycling mares n=13; ovariectomized mare n=1) collected throughout one cycle every 3 days.The proliferation phase is characterized by an increase in the epithelial height (28,3 ± 3,2 μm), the alkaline phosphatase activity and numerous mitoses. An intensive nuclear expression of Ki-67 antigen (1,8 ± 0,2 IRS [immune reactive score]), estrogen receptors (3,9 ± 1,6 IRS) and progesterone receptors (4,7 ± 2,8 IRS) in epithelial cells with a maximum on the 5th day post ovulationem is detectable. Ultrastructurally, nonciliated epithelial cells with longish-oval nuclei, rich in heterochromatin and with filiform mitochondria dominate. In the secretory phase, a continuous decrease in epithelial height (20,0 ± 4,3 μm), less mitoses and an increase in the activity of the acidic phosphatase and the non-specific esterases are observed. The expression of Ki-67 antigen (0, 58 ± 0,13 IRS), ER (0,78 ± 0,40 IRS) and PR (1,1 ± 0,6 IRS) in the epithelia decline. Elektronmicroscopically, the glandular epithelia show distinct signs of secretion: numerous secretory vacuoles, edematous round mitochondria and dilated dictyosomes. Additionally ciliated cells without any secretory signs appear on the 9th day. By means of morphometry, the day of the cycle can be determined in biopsies with a probability of 81%. The following parameters are most suitable: height and width of the epithelial cells, areal percentage of the glands in a defined area, density and diameter of the glands. The discriminant analysis shows an increase in the accuracy up to 97% if the serum estradiol and progesterone values are also taken into account.

Key Words: Equine endometrial cycle, Ultrastructure, Immunohistology

378 Onset of estrus and luteal function in peripubertal heifers given an intravaginal progesterone releasing insert with or without a subsequent injection of estradiol benzoate. S. K. Johnson[1]*, M. L. Day[2], J. M. Lynch[3], J. E. Kinder[3], R. Rasby[3], R. E. Short[4], R. P. Wettemann[5], and H. D. Hafs[6], *[1]Fort Hays State University, Hays, KS, [2]Ohio, [3]Heartland Cattle Co, NE, [4]USDA, Montana, [5]Oklahoma, and [6]New Jersey.*

Peripubertal heifers (n=317) of European breeding at six locations were used to determine the effects of a 7-day progesterone (P4) treatment alone or in combination with an injection of estradiol benzoate (EB) on behavioral estrus and luteal function. Each replicate was targeted to begin when approximately 40% of the heifers had reached puberty. Within replicate, heifers were assigned by age, weight and genotype to receive one of the following treatments on day 0; 1) an intravaginal insert containing P4 for 7 d(P; n=103), 2) an intravaginal insert containing P4 for 7 d plus an injection of 1 mg EB im at 24 to 30 h after insert removal (PE; n=105) or 3) a sham insert for 7 d (C; n=109). Serum concentrations of P4 were determined on d -7, 0, 8, 15 and 22. Heifers with concentrations of P4 > 1 ng/ml on d -7 and d 0 were excluded from the study. Average weight and age (±SD) of heifers at the start of the trial were 292 ±45 kg and 365 ±38 d, respectively. Treatment with P4 increased (P+PE vs C; P<.01) the proportion of heifers that were in estrus (59% vs 21%) and formed functional CL (55% vs 12%) after insert removal. A greater (P<.01) proportion of heifers in the PE than P groups were in estrus (81% vs 37%) and formed functional CL (66% vs 44%) after insert removal. Of the heifers exhibiting estrous activity, a greater proportion (P<.05) of heifers in the PE (94%) than P (80%) groups were active between d 9 - 11. There was no difference among treatments in the proportion of heifers in standing estrus that formed CL. Neither age, weight, nor body condition score interacted with treatment. Short-term treatment with P4 alone or in combination with EB increased the proportion of heifers in estrus and forming functional CL. Synchrony of estrus was greater in heifers that received EB as well as P4. (Supported by InterAg, Hamilton, NZ)

Key Words: Heifer, Progesterone, Puberty

379 The effect of male stimuli on the time and duration of estrus and luteinizing hormone (LH) in hair sheep ewes. R. W. Godfrey*, J. R. Collins, and E. L. Hensley, *Agricultural Experiment Station, University of the Virgin Islands, St. Croix.*

Hair sheep ewes were used to evaluate the influence of the ram on the duration and timing of estrus and LH levels around estrus. Ewes were given two i.m. injections of prostaglandin F2a (PGF; 15 mg) 10 d apart. On the d of the second PGF (0 h) ewes were placed in groups with one of three types of teaser animal equipped with marking harness. One group of ewes (n = 16) was exposed to an epididymectomized ram (RAM), one group of ewes (n = 16) was exposed to an epididymectomized ram wearing an apron to prevent intromission (APRON) and the third group of ewes (n = 17) was exposed to an androgenized ovariectomized ewe (T-EWE). Jugular blood samples were collected from ewes at 6-h intervals for 96 h. Plasma was harvested and LH concentration was determined by RIA. Estrus detection was conducted at 6-h intervals. A ewe was considered to be out of estrus when she no longer stood to be mounted by the teaser animal. There was no difference (P > .10) in the proportion of ewes expressing estrus (79.6%) or having an LH surge (85.7%) among the treatments. Neither the time to estrus nor the duration of estrus were different (P > .10) among APRON, RAM or T-EWE groups (40.9 ± 3.1 vs 43.5 ± 3.3 vs 42.3 ± 3.5 h, respectively, and 26.5 ± 2.0 vs 24.1 ± 2.2 vs 29.1 ± 2.3 h, respectively). The time to the LH surge was similar (P > .10) among APRON, RAM and T-EWE groups (49.9 ± 4.0 vs 47.7 ± 4.1 vs 51.3 ± 4.0 h, respectively). The magnitude of the LH surge was greater (P < .01) in T-EWE than in APRON or RAM ewes (105.3 ± 4.7 vs 87.1 ± 4.7 vs 82.2 ± 4.8 ng/ml, respectively). The time from estrus to the LH surge was not different (P > .10) among APRON, RAM or T-EWE ewes (9.7 ± 2.2 vs 9.3 ± 2.3 vs 11.1 ± 2.5 h, respectively). These results show that the expression and the duration of estrus are not influenced by the ram in hair sheep. In contrast, the LH surge appears to be enhanced in ewes exposed to an androgenized ewe.

Key Words: Sheep, Estrus, Behavior

380 Progesterone (P4) and luteinizing hormone (LH) levels in hair sheep after estrous synchronization with either progesterone or prostaglandin F2α (PGF). R. W. Godfrey[1], E. L Hensley[1]*, J. R. Collins[1], and J. E. Wheaton[2], *[1]Agricultural Experiment Station, University of the Virgin Islands, St. Croix and [2]Department of Animal Science, University of Minnesota, St. Paul.*

Hair sheep ewes (St. Croix White and Barbados Blackbelly) were used to evaluate P4 and LH around the time of synchronized estrus. Three estrous synchronization protocols were used: 1) two 15 mg injections (i.m.) of PGF 10 d apart (n = 18), 2) controlled internal drug release (CIDR) devices containing 300 mg progesterone for 12 d (n = 18) or 3) intravaginal sponges containing 500 mg P4 for 12 d (n = 18). On the day of the second PGF injection or CIDR or sponge removal sterile rams were placed with the ewes and jugular blood samples were collected from ewes at 6-h intervals until the time of ovulation, which was determined by laparoscopy. Blood samples were collected daily for 16 d after estrus (d 0). Plasma was harvested and stored at –20°C until LH and P4 concentrations were determined by RIA. The decline in P4 after the second PGF injection or CIDR or sponge removal was similar (P > .10) among the treatment groups. The time to the preovulatory LH surge was similar (P > .10) among CIDR, PGF and sponge treated ewes (40.6 ± 3.0 vs 46.9 ± 3.4 vs 36.2 ± 3.1 h, respectively). The magnitude of the LH surge was greatest (P < .04) in CIDR treated compared to PGF and sponge treated ewes (94.7 ± 10.9 vs 58.7 ± 10.9 vs 47.0 ± 10.2 ng/ml, respectively). The time from estrus to the LH surge and the time from the LH surge to ovulation were not different (P > .10) among CIDR, PGF or Sponge treated ewes (14.2 ± 2.1 and 25.9 ± 2.7 h vs 13.9 ± 2.4 and 22.9 ± 3.1 h vs 12.3 ± 2.2 and 22.4 ± 2.6 h, respectively). Progesterone levels through d 16 after the synchronized estrus were not different (P > .10) among CIDR, PGF and sponge treated ewes. None of the estrous synchronization methods used had a detrimental effect on either LH or P4 levels before or after estrus, indicating that they would be acceptable for use in hair sheep under tropical conditions.

Key Words: Sheep, Progesterone, Luteinizing Hormone

381 Fertility of suckled beef cows after timed breeding following treatment with GnRH, $PGF_{2\alpha}$, and norgestomet. K. E. Thompson*, J. S. Stevenson, D. M. Grieger, G. C. Lamb, T. J. Marple, L. R. Corah, D. A. Nichols, and R. M. McKee, *Kansas State University, Manhattan.*

Purebred Angus, Hereford, and Simmental cows were used to test a new estrus-synchronization program consisting of GnRH, $PGF_{2\alpha}$ (PG), and norgestomet. Cows were inseminated after detected estrus, or in the absence of detected estrus, inseminations were made at one fixed time after a second injection of GnRH. The treatment consisted of a 100-μg injection of GnRH and a 6-mg ear implant of norgestomet on day –7. The ear implant was removed and a 25-mg injection of PG was administered on d 0. In the absence of detected estrus, the time-bred group received a second injection of GnRH 48 h after PG and was inseminated 16 h later. Blood samples were collected 11 d before the onset of treatment, before the first GnRH injection, and before the PG injection. Based on serum concentrations of progesterone, the first two blood samples were used to determine cycling activity of the cows and the third blood sample was used to determine whether the treatment induced an ovulation after the first GnRH injection. Pregnancy was diagnosed between days 33 and 43 after insemination using transrectal ultrasonography. The treatment induced luteal function in 10 of 36 anestrous cows. Proportions of cows detected in estrus and resulting conception and pregnancy rates are illustrated below. We conclude the use of GnRH, PG, and norgestomet in a timed breeding program can eliminate the necessity of detected estrus. In addition, the treatment induced estrus in 28% of the noncycling cows.

		Heat detection + time bred		
	Estrus bred	Estrus bred	Time bred	Total
% In Heat	79.5 (78)[c]	100 (16)	14.3 (70)	30.2 (86)
% Conception[a]	67.7 (62)	68.8 (16)	57.1 (70)	59.3[x] (86)
% Pregnant[b]	53.8 (78)	68.8 (16)	57.1 (70)	59.3 (86)

[a]No. of pregnant cows/no. of cows inseminated during 144 hours after PG.
[b]No. of pregnant cows/no. of cows synchronized.
[c]No. of cows.
[x]Different (P=.06) from estrus-bred cows.

Key Words: GnRH, Norgestomet, Beef cattle

382 Synchronization of first wave follicles and timing of ovulation in beef cattle following melengestrol acetate (MGA) and two injections of prostaglandin $F_{2\alpha}$ (PGF). F. N. Kojima*, W. A. Ricke, J. E. Williams, D. J. Patterson, and M. C. Lucy, *University of Missouri, Columbia.*

To facilitate timed AI programs in beef cattle, synchronization of estrus and timing of ovulation was evaluated after short-term treatment with MGA combined with two injections of PGF. The specific objective was to synchronize timing of ovulation of first wave follicles after the second injection of PGF. Beef heifers (n=12) and cows (n=6), at random stages of the estrous cycle, were fed MGA (0.5 mg/hd/d) for 7 d and injected with 25 mg of PGF on the last day of MGA. A second injection of PGF was given 11 d after cessation of MGA (7-11 program). Estrous activity was observed every 6 h for 6 d after the last day of MGA and 5 d after the second injection of PGF. Development of the first wave follicles and CL were monitored by real-time ultrasonography every other day from the last day of MGA to the second injection of PGF and every 12 h thereafter for 5 d to determine the timing of ovulation. Synchronized estrus within 7 d after MGA treatment and the interval to the onset of estrus did not differ ($P > .10$) between heifers (10/12 and 96 h) and cows (4/6 and 84 h). After the second injection of PGF, 6/12 heifers and 3/6 cows were synchronized. The interval to the onset of estrus was not different ($P > .10$) between heifers (54 h) and cows (64 h). All animals exhibiting estrus after the second injection of PGF ovulated first wave follicles and timing of ovulation did not differ ($P > .10$) between heifers (80 h) and cows (96 h). Animals that failed to respond to the second injection of PGF had early developing CL that were not responsive to PGF. In summary, the 7-11 program in the present study did not produce a satisfactory synchronization rate after the second injection of PGF. However, short-term feeding of MGA combined with two injections of PGF might be useful and have potential for estrous synchronization in beef cattle when the adequate interval from MGA to the second injection of PGF is determined.

Key Words: Beef cattle, MGA, Estrous synchronization

383 Estrous synchronization in beef cattle with melengestrol acetate (MGA) and an injection of progesterone (P4). K. E. Fike*, M. E. Wehrman, B. R. Lindsey, E. G. Bergfeld, E. J. Melvin, J. A. Quintal, E. L. Zanella, and J. E. Kinder, *University of Nebraska, Lincoln.*

The hypothesis was that estrous synchronization of beef cattle using a combination of MGA and an injection of P4 and estradiol (E2) to regress persistent ovarian follicles would improve pregnancy rate (number conceived/number in group) to AI as compared to only feeding MGA or $PGF_{2\alpha}$ 10 d apart. During two years (1995–96), Angus-cross heifers (n = 52) and cows (n = 39) or composite cows (n = 288) were assigned to receive either 1) MGA for 17 d (d 0 = 1st d of MGA) plus an injection of 200 mg P4 and 1 mg E2 in sesame oil (vehicle) on d 11 to regress persistent ovarian follicles (MGA + P4), 2) MGA for 17 d plus vehicle on d 11 (MGA), or 3) two injections of $PGF_{2\alpha}$ 10 d apart (d 7 and 17; PG). P4 concentration was assessed from blood samples obtained on d 0, 7, and 17 to indicate estrual status (anestrus or estrual) before estrous synchrony. Estrous detection occurred every 6 h for 7 d following end of treatment. Females showing estrus were inseminated 6 to 12 h after detection. Conception to AI was determined by ultrasonography 35 to 40 d later. Treatment and estrual status interacted ($P < .05$) to affect estrous synchrony and pregnancy rates. Treatment and year interacted ($P < .10$) among estrual females to affect pregnancy rate. Estrous synchronization using MGA + P4 and E2 differentially improves estrous synchronization and pregnancy rates among anestrous and estrual beef cattle while maintaining typical conception rates.

Item	MGA	MGP + P4	PG
Synchrony rate, %			
Anestrus	66.1[a]	81.4[b]	28.0[c]
Estrual	76.6[a]	89.9[b]	92.3[b]
Conception rate, %	50.0[a]	62.7[ab]	67.4[b]
Pregnancy rate, %			
Anestrus	33.9[a]	45.8[a]	16.0[b]
Estrual*	37.5	60.9	64.1

[a,b,c]Differ within rows: $P \leq .10$.
*Treatment × year: $P < .10$.

Key Words: Estrous Synchronization, Melengestrol Acetate, Beef Cattle

565 Spermatozoal sex ratio in Holstein ejaculates. J. E. Chandler*, R. W. Adkinson, and H. C. Steinholt, *LSU Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge.*

The polymerase chain reaction and image analysis were used to examine percent of Y-chromosome bearing sperm within two ejaculates from each of ten sires. The Y-chromosome specific primers, BRY1a and BRY1b, were used to amplify a 194 bp DNA segment from frozen semen. The contents of five straws (200 million sperm) from each ejaculate were pooled and the DNA was isolated for PCR. After PCR, samples of amplified DNA were electrophoresed on horizontal gel units. Five lanes on each gel contained replicate samples of amplified DNA from each ejaculate. There was also one lane each for the molecular weight marker, positive control (pooled spermatozoal DNA), and cow leucocyte DNA (negative control). Ethidium bromide in the agarose gel stained the DNA and fluoresced under UV light. The gels were photographed with a silicon intensified video camera. The gel images were contrast enhanced by using an image analysis program. The image analysis program was used to measure the average fluorescent intensity of the 194 bp corresponding area of each lane. Images were corrected for background and compared to a 50% Y chromosome standard constructed from a pool of semen from all bulls. The relative amount of Y chromosomal DNA in the sperm samples was found by comparing sample DNA to the 194 bp band of the positive control. There were differences in intensities of amplified DNA between bulls and between ejaculates within bulls. The percent Y chromosome bearing sperm per individual ejaculate ranged from 24 ± 9.8% to 84 ± 9.8% with overall mean and standard error of 50.0 ± 4.6%. One-fifth of the ejaculates differed ($P < .05$) from the overall mean. Thus, selection and use of ejaculates with naturally skewed sex ratio, as screened by PCR, could enhance calf production with the desired sex ratio.

Key Words: Sperm, Sex ratio, PCR