Physiology and Endocrinology: Regulation of Estrus

218   Use of digital infrared thermography to measure the skin temperature changes in estrus synchronized dairy cows. S. Talukder*[1], L. Ingenhoff[1], K. L. Kerrisk[1], S. C. Garcia[1], and P. Celi[1,2], [1]*The University of Sydney, Narellan, Australia,* [2]*The University of Melbourne, Parkville, Victoria, Australia.*

The primary aim of this study was to explore the potential use of digital infrared thermography for estrus detection in dairy cows. Twenty cows were synchronized using controlled internal drug release (CIDR, progesterone 1.9g) and prostaglandin $F_{2\alpha}$ (PGF$_{2\alpha}$, cloprostenol sodium 500μg). Vulva and muzzle skin temperatures were measured every 12 h from CIDR insertion to 32 h post PGF$_{2\alpha}$ injection and then every 4 h until ovulation occurred. Thermal images obtained with a FLIR T620 series infrared camera were analyzed using ThermaCAM Researcher Professional 2.9 software. All the images of vulva and muzzle were averaged for the following 5 periods: 1) 24 h before PG injection (B-PG), 2) 32 h after PG injection (A-PG), 3) 36 h after PG injection to 1st sign of estrus (PG-E), 4) time from estrus to ovulation (E-OV) 5) 4 h interval in which ovulation occurred (OV). The relationship of vulva and muzzle temperature at different periods was analyzed by linear mixed model using Genstat version 14. Temperature humidity index and time of the day were included as covariates. Vulva and muzzle temperature changed significantly ($P < 0.01$) between time periods. Muzzle skin temperature increased from A-PG to E-OV after which temperature decreased. A positive ($P < 0.01$, r = 0.74) relationship was noted between muzzle and vulva skin temperature. The mean time of onset of estrus and ovulation were 54.7 ± 14.4 and 87.3 ± 19.1 h respectively after PGF$_{2\alpha}$ injection. Ovulation occurred 30.7 ± 9.8 h after onset of estrus. Muzzle and vulva temperature also changed significantly ($P < 0.01$) as ovulation approached. The most distinct increase in vulva and muzzle temperature was observed 22 and 16 h respectively before ovulation. Therefore, digital infrared thermography could be a promising tool for estrus detection or prediction of ovulation in dairy cows. Further biometrical analyses are required to build prediction models and to test the accuracy with hormonal assay.

Key Words: infrared thermography, estrus, dairy cow

219   Presynchronizing PGF$_{2\alpha}$ and GnRH injections before a fixed-time artificial insemination CO-Synch + CIDR program. S. L. Hill*[1], S. L. Pulley[1], KC Olson[1], J. R. Jaeger[1], R. M. Breiner[1], V. R. G. Mercadante[2], G. C. Lamb[2], and J. S. Stevenson[1], [1]*Kansas State University,* [2]*University of Florida.*

We hypothesized that pregnancy outcomes may be improved by inducing luteal regression and ovulation before a timed AI (TAI) program in suckled beef cows. This hypothesis was tested by presynchronizing estrous cycles before initiating a TAI program with the objective to increase the proportion of cows starting the program in a high (≥ 1 ng/mL) progesterone status and increase pregnancy per TAI (P/TAI). Cows were assigned randomly to 2 treatments after stratification by breed, parity, and days postpartum. Cows at 4 locations (n = 803) were assigned to either: (1) control (100 μg GnRH i.m. and insertion of a progesterone-releasing intravaginal controlled internal drug release (CIDR) insert on d –10, 25 mg PGF$_{2\alpha}$ i.m. and CIDR insert removal on d –3, and 100 μg GnRH i.m. plus TAI on d 0); or (2) PrePGG (same as control with a pre-PGF$_{2\alpha}$ injection at d –20 and a pre-GnRH injection at d –17). Blood was collected on d –20, –17, –10, –3, and 0 for progesterone assays. Ovarian structures in cows at 1 location were scanned. Binomial data were analyzed by procedure GLIMMIX. The PrePGG cows were more likely ($P < 0.05$) than controls to have luteolysis after the initial PGF$_{2\alpha}$ injection (42.2 vs. 14.4%) and had reduced ($P < 0.05$) concentrations of serum progesterone (0.37 vs. 0.71 ng/mL) at the time of the presynchronizing GnRH injection. On d –10 and –3 more ($P < 0.05$) PrePGG cows had at least 1 CL than controls (63.9 vs. 41.9% and 62.7 vs. 45.3%, respectively) indicating that ovulation had occurred in response to pre-GnRH. The P/TAI at d 35 did not differ between PrePGG and control (44.4 vs. 44.0%, respectively). Final pregnancy outcome (82.6 vs. 82.6%) and pregnancy loss (5.4 vs. 3.6%) did not differ between PrePGG and control, respectively. Cows having BCS >5.0 at d –20 were more ($P < 0.01$) likely to become pregnant than thinner cows (49.8 vs. 38.8%). Cows that were ≥ 70 d postpartum also had a greater ($P < 0.01$) P/TAI than cows with fewer days postpartum at TAI (52.6 vs. 36.1%). In summary, luteal regression and ovulation were enhanced by presynchronization before the 7 d CO-Synch program; however, P/TAI was not increased.

Key Words: timed AI, presynchronization, luteolysis

220   Influence of estrus at fixed-time AI on accessory sperm numbers and embryonic development. E. L. Larimore*[1], S. G. Kruse[2], B. J. Funnell[2], S. L. Bird[1], O. L. Swanson[1], G. A. Bridges[2], and G. A. Perry[1], [1]*Department of Animal Science, South Dakota State University, Brookings,* [2]*North Central Research and Outreach Center, University of Minnesota, Grand Rapids.*

Increased accessory sperm numbers have been correlated with increased embryo quality, and estrous expression before timed-AI (TAI) resulted in increased pregnancy success. Thus, the objective of this experiment was to determine if estrous expression before TAI affected accessory sperm numbers and embryonic development. Beef heifers at UMN (Rep 1; n = 44, Rep 2; n = 44) and SDSU (Rep 3; n = 50) were developed in a dry-lot and fed approximately 125% NRC requirements from weaning to AI. Ovulation was synchronized using the 5 d CO-Synch + CIDR with TAI on d 0. Estrous expression was assessed twice daily with the aid of EstroTect patches. Immediately following TAI, half the heifers continued on the pre-insemination diet and the remaining heifers were restricted fed. On d 6 embryos were collected and evaluated to determine quality (IETS standards; 1 = excellent, 5 = degenerate) and stage (1 = unfertilized, 9 = expanded hatched blastocyst). Embryos were stained and evaluated to determine the number of dead blastomeres (propidium iodide), total blastomeres, and number of accessory sperm (Hoechst 33342). Data was analyzed using the Mixed procedures of SAS. There were no treatment by replication or treatment by estrus interactions for any data evaluated, thus all data were pooled. Estrous expression before TAI did not affect the percent of embryos recovered ($P = 0.21$; n = 61 and 26 for heifers that did and did not exhibit estrus, respectively), number of dead cells ($P = 0.86$), or total cells ($P = 0.13$). However, embryos from heifers that exhibited estrus had improved embryo quality ($P = 0.03$; 2.2 $\pm$ 0.1) and advanced embryo stage ($P < 0.01$; 4.4 $\pm$ 0.1) compared with heifers that did not exhibit estrus (2.8 $\pm$ 0.3 and 3.7 $\pm$ 0.2, respectively). Heifers that exhibited estrus also tended to have increased numbers of accessory sperm ($P = 0.06$; 23.2 $\pm$ 3.6) and percentage of cells alive ($P = 0.08$; 81.1 $\pm$ 2.4%) compared with heifers that did not exhibit estrus (12.1 $\pm$ 2.8 and 69.7 $\pm$ 6.4%). In summary, initiation of standing estrus before TAI resulted in improved embryo stage and quality and tended to improve accessory sperm numbers and percentage of live cells.

Key Words: embryo, estrus, accessory sperm

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221 Reducing handling in the 5-day timed AI program in dairy cows. J. S. Stevenson*, S. L. Pulley, and S. L. Hill, *Kansas State University, Manhattan.*

We hypothesized that 50 mg of prostaglandin $F_{2\alpha}$ (PG) on d 6 (not d 5 as previously tested) would induce luteolysis in a traditional 5-d Ovsynch-72 program (GnRH 5 d before [d 0] and 72 h after [d 8] 25-mg PG doses [d 5 and 6 after GnRH]; timed AI on d 8). Experiment 1 monitored luteal regression of original and GnRH-induced luteal tissue (CL) and blood progesterone (P4) after either of the 25-mg doses of PG (d 5 and 6; control; n = 31) or a single 50-mg dose of PG on d 6 (1 × 50 mg; n = 30). Estrous cycles were presynchronized (GnRH 7 d before 25 mg of PG) and 11 d later (62 to 71 d in milk) cows were enrolled in a 5-d Ovsynch-72 program and treatments were administered. Blood was sampled for P4 and luteal structures were measured by ultrasonography on d 0 (original CL) and d 5 through 9 to monitor new GnRH-induced CL. Data were analyzed as repeated measures using procedure MIXED. Control PG reduced ($P < 0.01$) luteal tissue area of original CL on d 6 and 7, but no difference was detected by d 9. In contrast, no differences were detected in luteal tissue area of the induced CL on d 5 through 9. Serum P4 on d 5 through 9 averaged: 5.6 vs. 6.0, 1.2 vs. 6.0 ($P < 0.01$), 0.29 vs. 0.65, 0.16 vs. 0.59, and 0.14 vs. 0.46 (±0.3 ng/mL), respectively, for control and 1 × 50 mg dose. Luteolysis occurred in all 31 controls, but luteolytic failure occurred in 2 of 30, 1 × 50 mg cows in which no CL were present on d 0 (1 or 3 new CL were present on d 5). Experiment 2 monitored luteolysis in non-pregnant repeat-service cows subsequently treated with the same 2 treatments as in Exp. 1. Serum P4 in 57 cows on d 5, 6, and 8 was: 5.4 vs. 6.6, 1.7 vs. 5.8 ($P < 0.01$), and 0.29 vs. 0.35 (±0.5 ng/mL), respectively, for control and 1 × 50 mg dose. Luteolysis occurred in 28/30 controls and in 26/26, 1 × 50 mg treated cows. Pregnancy outcome 32 d after AI for both experiments was 30/60 (50%) vs. 26/56 (46%) for control vs. 1 × 50 mg dose, respectively. We concluded that the single 50-mg dose was equivalent to the control based on actual luteal tissue regression, decreased P4, and pregnancy outcome.

Key Words: luteolysis, luteal, pregnancy

222 Effect of presynchronization using Ovsynch or a single GnRH injection 7 d before an Ovsynch56 protocol on fertility of lactating dairy cows at first service. P. D. Carvalho*, J. N. Guenther, M. J. Fuenzalida, M. C. Amundson, M. C. Wiltbank, and P. M. Fricke, *Department of Dairy Science, University of Wisconsin-Madison, Madison.*

Presynchronization strategies such as Presynch Ovsynch and Double Ovsynch increase fertility to timed artificial insemination (TAI); however, simpler presynchronization strategies could reduce costs and simplify reproductive management. Lactating Holstein cows (n = 400) were randomly assigned to one of 2 presynchronization treatments before beginning an Ovsynch56 protocol (GnRH (G1) at 70 ± 3 DIM; PGF 7 d later; GnRH 56 h after PGF; TAI 16 h later at 80 ± 3 DIM) for first TAI. Cows (n = 208) in the first treatment (Double Ovsynch; DO) were presynchronized using a modified Ovsynch protocol (GnRH at 53 ± 3 DIM, 7 d later PGF, 3 d later GnRH) ending 7 d before G1 of Ovsynch56. Cows (n = 192) in the second treatment (GGPG) were presynchronized using a single injection of GnRH 7 d before G1 of Ovsynch56 at 63 ± 3 DIM. Blood samples were collected at G1 and the PGF injection of the Ovsynch56 protocol to determine progesterone (P4) concentration and pregnancy diagnosis was performed by ultrasonography. Temperature humidity index (THI) was calculated based on data from a station located near the farm, and data were analyzed using PROC GLIMMIX of SAS. Overall, DO cows had greater ($P = 0.03$) pregnancies per AI (P/AI) compared with GGPG cows 32 d after TAI (50.2% vs. 40.8%). Treatment was not affected by parity (primiparous vs. multiparous), and pregnancy loss did not differ between treatments or parities. Interestingly, P/AI was similar for DO and GGPG cows during cool weather (THI < 72; 47.2% vs. 45.1%, respectively), whereas P/AI was greater ($P = 0.02$) for DO cows during heat stress (THI ≥ 72; 52.0% vs. 34.8%). Based on P4 at G1, more ($P < 0.001$) DO cows had P4 in a medium range (>0.5 to < 4 ng/mL) compared with GGPG cows (81.9% vs. 58.7%). In addition, more ($P < 0.001$) DO cows had high P4 (>4 ng/mL) at the PGF injection of Ovsynch56 compared with GGPG cows (69.0% vs. 36.8%). In conclusion, presynchronization with a modified Ovsynch protocol increased P/AI by increasing synchrony to the Ovsynch56 protocol particularly during heat stress compared with presynchronization with a single injection of GnRH.

Key Words: synchronization, timed AI, dairy cow

223 Relationship of follicle size and concentrations of estradiol among cows that do and do not exhibit estrus during a fixed-time AI protocol. O. L. Swanson*[1], E. L. Larimore[1], B. L. Perry[1], G. D. Djira[2], R. A. Cushman[3], and G. A. Perry[1], *[1]Department of Animal Science, South Dakota State University, Brookings, [2]Department of Mathematics and Statistics, South Dakota State University, Brookings, [3]USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Cows that exhibited estrus around the time of fixed-time AI had greater pregnancy success compared with cows that did not. The objective of this study was to determine the relationship between follicle size and peak estradiol concentration between cows that did or did not exhibit estrus during a fixed-time AI protocol. Beef cows were synchronized with the CO-Synch protocol [GnRH (100 μg) on d −7, $PGF_{2\alpha}$ (25 mg) on d 0, and a second injection of GnRH 48 h after $PGF_{2\alpha}$ (d 2)] in 3 replicates (n = 80, 22 and 24). Follicle size (d 2) and ovulation (d 4) was determined by ultrasonography. Blood samples were collected every 3 (replicates 1 and 2) or 4 (replicate 3) h from $PGF_{2\alpha}$ injection to h 56. Estrus was detected by visual observation with the aid of estrus detection patches, and cows that ovulated were classified as exhibited estrus (n = 46) or did not exhibit estrus (n = 63). Data were analyzed using the GLM procedure in SAS with replicate as a co-variate. Among all cows ($P < 0.01$) and among cows that exhibited estrus ($P < 0.01$), there was a significant positive relationship between follicle size and peak estradiol concentration, but there was no relationship ($P = 0.60$) between follicle size and peak estradiol concentration among cows that did not exhibit estrus. Cows that exhibited estrus had a larger ($P = 0.02$) follicle (13.4±0.25 mm) and greater ($P < 0.01$) peak estradiol concentration (12.4 ± 0.54 pg/mL) compared to cows that did not exhibit estrus (12.6 ± 0.22 mm and 7.8 ± 0.46 pg/mL). There was no relationship between follicle size and estradiol concentration at the second GnRH injection among all cows ($P = 0.27$), cows that exhibited estrus ($P = 0.34$), and cows that did not exhibit estrus ($P = 0.34$), but cows that exhibited estrus (8.9 ± 0.56 pg/mL) had greater ($P < 0.01$) concentrations of estradiol compared to cows that did not exhibit estrus (5.8 ± 0.45 pg/mL). In summary, follicle size had a positive relationship with peak concentrations of estradiol, but only among cows that exhibited standing estrus.

Key Words: follicle size, estradiol, estrus

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224 Progesterone supplementation to dairy cows lacking a corpus luteum (CL) at the initiation of the Ovsynch protocol. R. S. Bisinotto*, N. Martinez, F. S. Lima, T. L. C. Pinto, R. S. Surjus, G. C. Gomes, E. S. Ribeiro, L. F. Greco, W. W. Thatcher, and J. E. P. Santos, *University of Florida, Gainesville.*

Objectives were to evaluate the effects of supplemental progesterone (P4) on fertility responses of Holstein cows lacking a CL at the initiation of the Ovsynch-56 program (d-10 GnRH, d-3 PGF$_{2\alpha}$, h-16 GnRH, d0 AI). Cows had their ovaries evaluated by ultrasonography on d-10 and those without CL were assigned randomly to receive 0 (Control; n = 270) or 2 controlled internal drug-release (CIDR) inserts containing P4 from d-10 to d-3 (2CIDR; n = 261). Cows with CL on d-10 were used as positive controls (Diestrus; n = 756). Cows had their ovaries scanned on d-3 to detect newly formed CL. Blood was sampled on d-10, −9, −7, −5, −3, and 0 and P4 concentrations determined by RIA. Estrus was detected based on removal of tail chalk. Pregnancy was evaluated 32 and 60 d after AI. The LOGISTIC and GLIMMIX procedures of SAS were used to analyze binomial and continuous responses. The use of 2 CIDR inserts increased ($P < 0.001$) P4 concentrations between d-9 and −3 compared with Control (2.57 vs. 0.75 ng/mL), but concentrations were less than those of Diestrus (4.45 ng/mL). Ovulation to the first GnRH was greater ($P < 0.001$) for Control and 2CIDR compared with Diestrus (61.9, 57.1, and 35.9%, respectively), which resulted in a greater ($P < 0.001$) proportion of cows bearing a new CL on d-3 (71.5, 65.9, and 39.0%, respectively). Nonetheless, a greater proportion of Diestrus cows had CL on d-3 compared with Control and 2CIDR (89.6, 71.5, and 65.9%, respectively). Pregnancy per AI was less for Control than for Diestrus and intermediate for 2CIDR cows (Table 1). However, this trend was observed only in cows that were not in estrus at AI.

Table 1. Fertility responses (%; no.) to treatment

	Treatment			
	Control	2CIDR	Diestrus	*P*-value
Estrus at AI	59.3 (270)	66.7 (261)	55.8 (756)	0.70
Pregnant d 32				
Overall	26.7 (270)[b,B]	31.8 (261)[A]	33.6 (756)[a]	0.002
Estrus at AI	35.0 (160)	36.2 (174)	35.8 (422)	0.93
Not in estrus at AI	14.6 (110)[b]	23.0 (87)[a]	30.8 (334)[a]	0.001
Pregnant d 60				
Overall	23.0 (270)[b]	27.7 (260)[b]	31.5 (752)[a]	0.002
Estrus at AI	29.4 (160)	32.2 (174)	33.4 (419)	0.62
Not in estrus at AI	13.6 (110)[b]	18.6 (86)[B]	29.1 (333)[a,A]	0.002
Pregnancy loss	13.9 (72)[a]	12.2 (82)[a]	5.2 (250)[b]	0.03

Key Words: anestrus, progesterone, dairy cow

225 Resumption of postpartum ovarian cyclicity in dairy cows and its relationship with acute phase proteins, uterine health and lipolysis during the transition period. C. C. Brauner*[1], A. R. T. Krause[1], M. E. Lima[1], E. G. Xavier[2], A. Schneider[1], E. Schmitt[3], E. Schwegler[1], M. M. Weschenfelder[1], P. Montagner[1], F. A. B. Del Pino[1], M. N. Corrêa[1], and L. F. M. Pfeifer[3], *[1]Universidade Federal de Pelotas, NUPEEC, Pelotas, RS, Brazil, [2]Granjas 4 Irmãos S/A, Rio Grande, RS, Brazil, [3]Empresa Brasileira de Pesquisa Agropecuária EMBRAPA, Porto Velho, RO, Brazil.*

The aim of this study was to evaluate the resumption of postpartum cyclicity and its relationship with acute phase proteins, uterine health and lipolysis during the transition period of dairy cows. Twenty multiparous Holstein cows were enrolled in this study from a commercial dairy farm

with annual rolling herd average of 7,891 ± 1.18 kg of milk. To assess the cyclicity resumption, blood samples were collected weekly from 16 to 44 d during the postpartum period to evaluate the concentration of progesterone. Cows were classified as either ovulatory (OC group), consisting of cows that ovulated up to 44 ± 2 d (n = 12) or anovulatory (AC group), those cows that did not ovulate in the same period (n = 8). Blood samples were collected weekly from day −21 relative to calving to 30 d postpartum aiming to evaluate the concentration of acute phase proteins (haptoglobin, paraoxonase and albumin), as well as, glucose and NEFA. Endometrial cytology was performed at 37 ± 2 postpartum days, to assess the uterus health considering the polymorphonuclear (PMN) cells count, using uterine low volume flushing. Data were analyzed by MIXED PROCEDURES of SAS. The OC had lower ($P = 0.05$) PMN percentage in endometrial cytology than AC with 26.3% vs. 53.4% PMN cells in the uterine flushing, respectively. The AC had lower ($P = 0.03$) concentrations of albumin during the prepartum (2.42 ± 0.07 vs. 2.64 ± 0.05 g/dL) and postpartum period (2.22 ± 0.09 vs. 2.52 ± 0.07 g/dL $P = 0.01$), and higher ($P = 0.04$) concentrations of haptoglobin during the prepartun (0.69 ± 0.16 vs. 0.23 ± 0.13 g/L) and tended ($P = 0.09$) to have lower activity of paraoxonase during the postpartum period (87.81 ± 9.11 U/L vs. 108.41 ± 7.45 U/L) than the OC group. No differences ($P > 0.05$) were observed of NEFA and glucose concentrations during the transition period. In conclusion, cows that return earlier to estrous cyclicity have reduced concentrations of haptoglobin and proportion of PMN in endometrium, and increased concentrations of albumin, but no differences in blood NEFA were observed between OC and AC.

Key Words: inflammation, ovulation, NEFA

226 A missense mutation in growth differentiation factor-9 (GDF9) increases ovulation rate in sheep. M. P. Mullen* and J. P. Hanrahan, *Animal and Grassland Research and Innovation Centre, Teagasc, Athenry, Co. Galway, Ireland.*

The Finnish Landrace is a well-known high prolificacy sheep breed and has been used in many countries to increase fecundity of local breeds. All of the evidence adduced to date suggests that mutations with a large effect on ovulation rate are not responsible for the exceptional prolificacy of Finnsheep. The objectives of this study were 1) to ascertain if any of the 10 established mutations with large effects on ovulation rate in sheep or 2) if any other DNA sequence variants within the candidate genes GDF9 and BMP15, are implicated in the high prolificacy of the Finnish Landrace breed using material from lines developed by divergent selection on ovulation rate. Genotyping results showed that none of 10 known mutations, FecX^G, FecX^B, FecG^H, FecB^B, FecX^I, FecX^H, FecX^L, FecT^T, FecG^E or FecX^R, were present in the set of 108 Finnsheep tested and thus do not contribute to the exceptional prolificacy of Finnsheep. However, DNA sequence analysis of GDF9 identified a previously known mutation, V371M, segregating at significantly different frequencies between high and low ovulation rate lines. Subsequently analysis of Belclare sheep revealed a significant association V371M and ovulation rate ($P < 0.001$). Heterozygous carriers of V371M, a missense mutation in GDF9, exhibited increased ovulation rate (0.24 s.e. 0.084) relative to the wild type. This finding brings to 11 the number of mutations that exhibit large effects on ovulation rate in sheep and to 3, including FecB^B and FecG^E, the number of known mutations within the TGFβ superfamily with a positive effect on prolificacy in the homozygous state without any associated sterile phenotype. These results further highlight the central role of members of the TGFβ superfamily in the control of fertility in mammals.

Key Words: fecundity, growth differentiation factor-9 (GDF9), control of fertility

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227   Effect of exogenous FSH on endogenous FSH secretion and testicular development in prepubertal bulls. B. R. Harstine*[1], L. H. Cruppe[1], F. M. Abreu[1], M. D. Utt[1,3], R. S. Cipriano[1], C. Premanandan[2], J. M. DeJarnette[3], and M. L. Day[1], [1]*Department of Animal Sciences, The Ohio State University, Columbus,* [2]*Department of Veterinary Biosciences, The Ohio State University, Columbus,* [3]*Select Sires Inc., Plain City, OH.*

In prepubertal bulls, FSH facilitates testis maturation and a transient proliferation of Sertoli cells. Two experiments (Exp) examined the effects of exogenous FSH on hormone secretion and testis development of Angus-cross bulls. Exogenous FSH treatment consisted of IM injection of 30 mg NIH-FSH-P1 (Folltropin-V) in a 2% hyaluronan solution (pFSH). In Exp 1, bulls (50 ± 6.5 d age; d 0) received either pFSH (FSH, n = 5) or saline (control, n = 5) on d 0 and 3.5. Blood samples to assess FSH and testosterone (T) concentrations (CONC) were obtained every 6 h for 24 h after pFSH and every 12 h thereafter. Peripheral FSH CONC measured using a bovine FSH RIA were greater ($P < 0.05$) in the FSH than control treatment 6 h after pFSH and tended to be greater ($P \leq 0.08$) 12 h after pFSH. FSH CONC from 18 to 84 h after pFSH and T CONC throughout did not differ between treatments. In Exp 2, bulls were treated with pFSH (FSH, n = 11) or saline (control, n = 11) every 3.5 d from 35 ± 2 to 91 ± 2 d age. Blood samples were collected before each treatment to quantify T and FSH CONC. Body weight (BW) and scrotal circumference (SC) were measured weekly. Bulls were castrated at 93 ± 2 d age. Seminiferous tubule diameter, testis composition, and number of Sertoli cells per tubule cross section (GATA-4 positive staining) were determined from fixed and stained histological sections. FSH CONC did not differ between treatments from 35 to 67.5 d age, but increased ($P < 0.05$) in the FSH treatment at 70 d age and remained elevated and greater than control bulls through 91 d age. CONC of T, BW, SC, testis wt and volume, percent of parenchyma comprised of tubules, and tubule diameter did not differ between treatments. However, number of Sertoli cells per round tubule cross section was greater ($P < 0.05$) in the FSH than control (33.35 ± 0.9 vs. 28.27 ± 0.9 cells) treatment. In summary, exogenous pFSH treatment from 35 to 91 d of age increased endogenous FSH secretion and the number of Sertoli cells at 93 d of age. It is suggested that exogenous FSH altered endocrine mechanisms regulating endogenous FSH secretion and augmented Sertoli cell proliferation in young bulls.

Key Words: FSH, Sertoli cell, testes

228   Effect of estradiol benzoate on estrus intensity, estrus response and fertility in CIDR-treated crossbred heifers. M. U. Mehmood*[1], A. Y. Qamar[1], N. Ahmad[1], A. Sattar[1], and M. Abdullah[2], [1]*Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan,* [2]*Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.*

Crossbred dairy animals are capable of sustaining higher levels of production than native buffalo, Sahiwal, Cholistani and Desi breeds, but also more environmentally adaptable to the harsh climates of Pakistan when compared with exotics (Friesian or Jersey). Unfortunately as replacement heifers are not lactating, they are often ignored by the owners in terms of feeding and evaluating the onset of puberty. Therefore, the objectives of the present study were to determine the effect of estradiol benzoate (EB) on estrus response, intensity and fertility in CIDR (Controlled Internal Drug Releasing Device; 1.38g Progesterone; Pfizer, USA) treated crossbred heifers. Crossbred (Sahiwal × Friesian) heifers with average body condition score of 2.5 ± 0.5 (1–5), were placed at 4 different farms near Lahore, Pakistan. All the heifers were treated with CIDR on d 0 and were injected $PGF_{2\alpha}$ (Dalmazine, FATRO, Italy; 0.075 mg/mL, im), on d 6 followed by removal of CIDR on d 7. Estradiol benzoate (100 μg/mL, im Sigma Aldrich Germany) was administered 24 h after the CIDR removal (n = 50) treated with EB (group-I) while the other group (n = 50) was without EB treatment (group-II). Estrus detection was carried out by visual observation; twice daily for at least 30 min. All heifers were artificially inseminated 48 and 60 h after CIDR removal. Pregnancy status was determined by ultrasonography (HS 1500V; Honda; Japan, with 7.5 MHz probe) between d 30 and 40 post-AI. Estrus response and pregnancy rate were analyzed using Chi-squared, while estrus intensity scored by Mann-Whitney test. Estrus response was 100% in both groups. Estrus intensity was scored at the time of AI as 1 = Low, 2 = Medium, 3 = High based upon the heat signs. Estrus intensity was highly significant ($P = 0.01$) in group I (2.9 ± 0.1) compared with group II (2.0 ± 0.7). Pregnancy rate was 54% (27/50) in group I and 36% (18/50) in group II heifers ($P = 0.07$). It is concluded that estradiol benzoate in CIDR protocol intensified signs of estrus and pregnancy rate. It is implied that CIDR could be a good tool to enhance fertile estrus.

Key Words: crossbred heifers, CIDR, estradiol benzoate