organization of the 5UTR and to identify PC promoter elements. The RPCI-42 Bovine Bacterial Artificial Chromosome (BAC) library was screened with oligonucleotide sequences corresponding to specific elements of the 5 UTR sequence of bovine PC and to a region of the coding sequence. Two BACs that hybridized to all probes were selected for further analysis. A partial restriction map of the BACs was made with oligonucleotides corresponding to the coding region and the 89 and 110 bp elements of bovine PC 5 UTR. The BAC fragments that hybridized to the oligonucleotide probes were isolated and sequenced. The sizes of the cloned genomic PC 5 UTR fragments were verified by PCR, using genomic DNA from four cows. Sequencing data confirms the existence of a 178 bp exon that contains the 68 and 110 bp sequence elements

of the 5 UTR for PC mRNA. The 178 bp exon appears to be the first transcribed exon in PC and the 68 and 110 bp 5 UTR sequences are most likely generated by alternative transcription start sites. Genomic sequence data also confirms that the 3 end of the 89 bp element is a discrete 41 bp exon. Regions within the genomic sequence adjacent to the 178 and 41 bp exons of the PC 5 UTR contain binding sites for TBP, Sp1, Ap1 and/or CEBP transcription factors. These data provide information about the arrangement of exons in the 5 UTR of PC and about putative promoter regions.

Key Words: Pyruvate Carboxylase Gene, Liver, Promoter

Physiology and Endocrinology: Reproductive Technologies and Management

W211   The effect of day six or day seven prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injections and using a disinfectant lubricant with Controlled Internal Drug Release (CIDR) inserts for estrus synchronization in dairy heifers.
W. M. Graves*[1], A. K. McLean[1], R. C. Smith[1], J. B. Rosenberg[2], and B. C. Beachnau[3], [1]*University of Georgia, Athens,* [2]*Fort Dodge Animal Health, IA,* [3]*Pfizer Animal Health, Portland, MI.*

Our objective was to compare d-6 versus d-7 prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injections and the effect of using a disinfectant lubricant with CIDR inserts (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY) placed intravaginally for 7 d. A total of 164 heifers at two locations received $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY, 5 mg; i.m.) on either d 6 or d 7 at the time CIDR inserts were removed. Two different types of lubricants, Safe Lube (H&W Products, Inc, Salem, OH) and Nolvalube (Fort Dodge Animal Health, Fort Dodge, IA) with Nolvasan disinfectant (0.1% chlorhexidine acetate) were used to measure effects on discharge at removal and conception. Insertion devices were dipped in Nolvasan solution (29.57mL per 3.785 L), excess fluid was shaken off, then dried with a paper towel prior to using Safe Lube for application. Insertion devices were just wiped and more Nolvalube added between heifers using the disinfectant lubricant. Data were analyzed by Chi-Square. Twenty-nine of 81 (35.80%) heifers treated with Safe Lube showed signs of vaginal discharge versus 18 of 83 (21.69%) treated with Nolvalube (P<.05). Sixty-six (81.48%) of those treated with Safe Lube were in estrus and 29 (43.94%) pregnant versus 76 (91.57%) in estrus and 40 (52.63%) pregnant for the Nolvalube heifers (NS, P=.11). Seventy-one of 82 (86.59%) heifers that were injected on d 6 were in estrus and 33 (46.48%) pregnant versus 71 of 82 (86.59%) injected on d 7 that were in estrus and 43 (47.89%) pregnant (NS). Day-6 heifers came into estrus in tighter synchrony. A total of four (5.63%) of the d-6 heifers were in estrus on d 8, 52 (73.24%) on d 9 and 15 (21.13%) on d 10. A total of four (5.63%) of the d-7 heifers were in heat on d 8, 45 (63.38%) on d 9, 18 (25.35%) on d 10 and four (5.63%) on d 11. Fewer discharges were seen using the disinfectant lubricant. The number of heifers in estrus and pregnant were similiar for the d-6 and d-7 $PGF_{2\alpha}$ injections.

Key Words: CIDR, $PGF_{2\alpha}$ Synchronization, Disinfectant Lubricant

W212   Development of a boar semen mobility assay.
A. A. Olivera, D. L. Fernandez*, and E. S. Fonda, *Department of Animal and Veterinary Sciences, California State Polytechnic University, Pomona.*

Eighteen ejaculates from six mature boars were used to develop a porcine sperm mobility assay that could be performed under field conditions. A modified densimeter was used to determine an index representing the changes in light absorbance after spermatozoa penetrated a resistance medium. The control values were subtracted from the experimental values to create the Net Mobility Index (NMI). Variables used in this experiment were incubation time (5, 10, 15 or 20 min), extender (Modena, X-Cell, MR-A or VSP), sperm concentration (1, 2, or 3 x $10^{(}8)$ cells/ml) and Accudenz Resistance Media concentration (3%, 6% or 9%). Inactivated sperm cells were used as controls. NMI values were lower (P<0.01) for inactivated sperm (3.16±0.22 vs 25.044 ± 0.39). NMI values increased (P<0.05) with incubation time (5 min, 13.78±0.39; 10 min, 23.806±0.37; 15 min, 29.00±0.38; 20 min, 32.31±0.37). However, sperm penetration tended to stabilize over time. NMI differed (P<0.05) among extenders (X-Cell, 31.42±0.37; Modena, 26.70±0.37; MR-A, 23.91±0.39; VSP, 17.53±0.39). Increasing sperm concentration

increased (P<0.05) NMI (1 x $10^{(}8)$, 19.20±0.33; 2 x $10^{(}8)$, 25.28±0.33; 3 x $10^{(}8)$, 30.19±0.33), however, neat ejaculate NMI (29.511±0.39) was similar to (P>0.05) NMI for 3x$10^{(}8)$ cells/ml. NMI differed (P<0.05) among the three concentrations of resistance media (3%, 28.71±0.33; 6%, 24.23±0.33; 9%, 21.73±0.33). We conclude that the changes in the light absorbance of the resistance media are an expression of the sperm mobility. Moreover, this technique has a potential application, under field conditions, to assess objectively sperm mobility and categorize males based on a Sperm Mobility Index.

Key Words: Sperm, Boar, Mobility

W213   In vitro production of Holstein embryos using Beltsville method sex-sorted sperm.
R. D. Wilson*[1], K. A. Weigel[1], P. M. Fricke[1], M. L. Leibfried-Rutledge[2], D. L. Matthews[1], J. J. Rutledge[1,2], and V. R. Schutzkus[1], [1]*University of Wisconsin Madison,* [2]*BOMED Inc, Madison, WI.*

Our objective was to explore the impact of sperm sorting on the efficiency of in vitro embryo production in Holstein cattle. Cull cows were used as donors, and ovaries were collected via colpotomy or at the time of slaughter. Oocytes were aspirated from the ovaries, and embryos were produced using sex-sorted semen from three Holstein sires. Embryos were transferred into recipient Holstein cows and heifers on the donors farm. Seven Wisconsin herds participated, and 365 embryos were produced from 104 donor cows. Only 272 were transferred, due to limited availability of recipients. On average, 3.5 ± 0.4 transferable embryos were produced per donor, including 1.4 ± 0.2 grade one embryos and 1.5 ± 0.2 grade two embryos. Individual farms averaged from 1.6 to 5.8 transferable embryos per donor. On average 43.7 ± 4 oocytes were collected per donor. The number of usable oocytes averaged 33.9 ± 3.4 and percent embryo cleavage (52.1 ± 1.9) were significant predicators of the number of blastocysts and number of transferable embryos. Preliminary pregnancy results show strong farm and sire effects. Overall conception rate was 36 percent for heifer recipients and 18 percent for milking cow recipients (P<0.05). To test the effect of sperm sorting on the percentage of embryos developing to blastocyst stage, oocytes were recovered from anonymous donors at a slaughterhouse and fertilized using non-sorted sperm or sex-sorted sperm from these sires. Oocytes (n=3312) fertilized using non-sorted sperm produced (P<0.05) more embryos developing to blastocysts than oocytes (n=1577) fertilized using sex-sorted sperm (20.1 ± 2.9 percent vs. 12.2 ± 2.3 percent, respectively). These results suggest that sexing sperm using the Beltsville method (fluorescence-activated cell sorting) method may have adverse effects on embryo development and conception rates in in vitro production systems.

Key Words: Sexed Semen, In Vitro Production, Dairy Cattle

W214   Induction of bilateral double ovulation to promote twinning in beef cattle.
M. Hogeg[1], A. Bor[2], Y. Lavon[1], M. Maman[1], S. Jacoby[2], and D. Wolfenson*[1], [1]*The Hebrew University, Rehovot, Israel,* [2]*Agricultural Research Organization, Bet Dagan, Israel.*

Beef cattle producers gain substantial economic benefits from cows that produce twins. Calf survival and birth weight are greater and incidences of abortion and dystocia are less in bilateral than unilateral twin pregnancies. A major objection to using gonadotropins to induce twins is the wide variation of ovarian response. The present study examined induction of bilateral-double or triple ovulation by stimulating follicle

growth with FSH, use of ultrasound-guided selective follicular aspiration to remove extra-large follicles, followed by supportive FSH to maintain co-dominance. In Exp. 1, Holstein cows were given short (three doses, n=6) or long (five doses, n=6) FSH treatment (50 mg Folltropin) every 12 h, from day (d) 3 of the cycle. On d 5, selective aspiration was performed, on d 6, $PGF_{2\alpha}$ was given, on d 7, two supportive 30 mg doses of FSH were given 12 h apart to maintain co-dominance, on d 8, GnRH was given and post-ovulation CL growth was determined. Short and long FSH treatments induced double or triple ovulation in 42% of the cows. In Exp. 2, a short FSH treatment was given only to cows (n=10) with 7 to 8 mm medium follicles on d 3, and supportive FSH (30 mg) was given on d 5 and 6. Two non-responding cows were excluded, and 6 out of 8 cows (75%) exhibited double or triple ovulation. Control cows (n=6) that did not receive FSH ovulated a single follicle, as expected. In Exp. 3, on Holstein heifers, 4 heifers with no supportive FSH doses ovulated a single follicle, and 50% of those that received 10 mg on d 5, 6 and 7 (n=5) or 30 mg on d 5 and 6 (n=5) exhibited double or triple ovulations. Overall for Exps 1-3: 3.0 medium and 1.8 large follicles were counted on d 4, 2.5 follicles were aspirated on d 5, and 70% of the cows exhibited bilateral ovulations. The results suggest that FSH combined with selective follicular aspiration can be used to induce bilateral twinning in beef cattle.

Key Words: Twins, Double Ovulation, Beef Cattle

W215   Effects of holding time prior to freezing on the motility, viability and membrane binding ability of ram sperm. P. H. Purdy*, *USDA-ARS-National Animal Germplasm Program, Fort Collins, CO.*

The United States sheep industry lacks infrastructure to effectively collect and store genetic resources in the national repository. Therefore, we investigated a methodology that could be used to ship diluted ram semen samples that were cooled and held at 5C for up to 48 hours prior to cryopreservation. Semen samples from 6 rams were collected and the concentration and motility were determined using spectrophotometry and computerized automated semen analysis (CASA), respectively. Samples were diluted to 400×10^6 cells per mL with a one-step Tris-egg yolk-glycerol media and cooled to $5°C$ over 2 hours using a styrofoam shipping box and commercial cold packs. The samples were maintained at $5°C$ in the shipping box, and aliquots were loaded into 0.5 mL French straws at 0, 24 or 48 hours after cooling, frozen in vapor 4.5 cm above liquid nitrogen for 12 to 13 minutes and plunged for storage. No differences between freeze times (0, 24, 48 h) were detected using ANOVA in post thaw motility (29, 31, 36%; P > 0.05), plasma membrane integrity (28, 35, 29%; P > 0.05) or live acrosomal integrity (99, 99, 99%; P > 0.05). Motility was assessed using CASA, and plasma membrane integrity and acrosomal integrity were simultaneously determined using the fluorescent stains propidium iodide and FITC-PNA, respectively, with flow cytometry. In addition, no differences were observed in the mean number of cells binding to a chicken oocyte membrane (461, 532, 319; P > 0.05) at time 0, 24 and 48 h, respectively. These results indicate that ram sperm may be held at 5C for up to 48 hours prior to freezing with no deleterious effects on post thaw motility, plasma membrane integrity and acrosomal integrity. In addition, the chicken oocyte membrane binding assay demonstrates a simple in vitro method to assess post-thaw ram sperm capacitation, acrosome reaction and binding ability. The combination of the shipping protocol and viability testing has the potential to ease the constraint on collecting and freezing ram semen.

Key Words: Ram Spermatozoa, Cryopreservation, Capacitation and Acrosome Reaction

W216   Effects of PGF presynchronization and CIDR on pregnancy rates in suckled beef cattle subjected to fixed-time insemination following estradiol and progesterone treatment to synchronize follicular growth, and PGF and estradiol cypionate treatment to synchronize ovulation. J. A. Small*[1], M. G. Colazo[2], J. P. Kastelic[3], and R. J. Mapletoft[2], [1]*Agriculture and Agri-Food Canada, Brandon, Manitoba,* [2]*WCVM-University of Saskatchewan, Saskatoon, Canada,* [3]*Agriculture and Agri-Food Canada, Lethbridge, Alberta.*

The objective was to determine effects of presynchronization and controlled-release progesterone on pregnancy rate in beef cattle subjected to a synchronization program based on estradiol, progesterone

(P4) and PGF treatment for AI without estrus detection (TAI). Cross-bred suckled beef cattle (n = 288) at 47 ± 14.8 d (mean ± standard deviation) post-partum were allocated to three treatment groups on the basis of parity (n=78, 66, 41, and 79 for Lactations 1, 2, 3 to 4 and 5 to 10, respectively), body weight (range 416 to 842 kg), and body condition score (range 3.5 to 6.0). All cattle were treated with 2.5 mg estradiol-17? and 100 mg progesterone (P4; both from Sigma Chemical Co, St. Louis, MO) im on Day 0 and 500 ug cloprostenol sodium (PGF; Estrumate, Schering-Plough, Pointe Claire, QC) and 0.5 mg estradiol cypionate (ECP; Professional Veterinary Laboratories, Winnipeg, MB) im on Day 7, with AI 56 h later (TAI Day 9.3). Experimental treatments were CIDR (1.9 g P4; Bioniche Animal Health, Belleville, ON) for 7 d on Day 0 (Groups 1 and 3), and PGF on Day -7 (Groups 2 and 3). All AI utilized frozen-thawed semen with proven fertility. From Days 11 to 54, the cattle were exposed to fertile bulls and frequently observed for estrus and breeding. Pregnancy was diagnosed by ultrasonography (Day 54) and transrectal palpation (Day 129). There was no interaction of parity and treatment (P>0.05). Pregnancy rate to TAI was lowest (P<0.05) for Group 2 (46.9, 31.2, and 45.3% for Groups 1, 2 and 3, respectively). Pregnancy rate to natural service was greatest (P<0.05) for Group 2 (46.9, 64.6 and 45.3%); most breedings occurred from 17 to 24 d after TAI (85.2, 59.1, and 72.4%; P>0.05). Cumulative pregnancy rates were similar (P>0.05) among groups (93.8, 95.8 and 90.6%). Pregnancy rate to TAI was significantly increased with a CIDR, but was not significantly affected by PGF presynchronization.

Key Words: Estrus Synchronization, Fixed-Time AI, Beef Cattle

W217   Effect of presynchronization with GnRH on fertility of holstein dairy heifers receiving synchronization of ovulation and timed artificial insemination (TAI). H. Rivera* and P. M. Fricke, *University of Wisconsin, Madison.*

To evaluate the effect of presynchronization with GnRH on the response of heifers receiving a hormonal protocol for synchronization of ovulation and TAI, Holstein dairy heifers (n=166) 14.9 ± 0.2 mo of age were randomly assigned to each of two treatments. Heifers (n=82) in the first treatment (GPG) received a hormonal protocol for synchronization of ovulation and TAI (100 μg GnRH, d 0; 25 mg $PGF_{2\alpha}$, d 6; 100 μg GnRH+TAI, d 8), whereas heifers (n=84) in the second treatment (GGPG) received the same treatment as GPG heifers but with the addition of 100 μg GnRH 7 d before the first GnRH injection of the protocol (d -7). Artificial insemination before scheduled TAI on d 8 was conducted for heifers in both treatments based on once daily assessment of removed tail chalk beginning on d 0. Although presynchronization increased (P<0.05) the proportion of heifers with $\geq$ 2 CL on d 0 (30% 25/84 vs. 5% 4/81), the proportion of heifers ovulating after the first GnRH injection on d 0 (39%, 31/80 vs. 40%, 32/80), the proportion of heifers undergoing luteal regression after $PGF_{2\alpha}$ on d 6 (89%, 58/65 vs. 94%, 64/68), the proportion of heifers ovulating after the second GnRH injection on d 8 (87%, 66/76 vs. 85%, 66/78), the proportion of heifers receiving AI before TAI (9.5%, 8/84 vs. 4.9%, 4/82), and conception rate 30 d after AI (51%, 43/84 vs. 45%, 37/82) did not differ between GGPG vs. GPG heifers, respectively. Serum progesterone (P4) concentration on d 0 was greater (P<0.05) for heifers with > 1 CL (3.9 ng/ml) compared to heifers with one CL (2.8 ng/ml); however, serum P4 did not differ at the $PGF_{2\alpha}$ injection on d 6 based on CL number (3.8 vs 3.6 ng/ml). In conclusion, presynchronization with GnRH 7 d before initiation of synchronization of ovulation using GnRH and $PGF_{2\alpha}$ failed to decrease the proportion of heifers expressing estrus during the protocol and did not improve synchronization response or fertility to the protocol. *Supported by Hatch project WIS04431 to PMF*

Key Words: Dairy Heifers, Synchronization of Ovulation, Timed Artificial Insemination

W218   Effect of termination of pregnancy on serum concentrations of pregnancy associated glycoproteins in beef cows. D. C. Busch*, J. A. Atkins, D. J. Schafer, J. F. Bader, D. J. Patterson, T. E. Parks, J. A. Green, and M. F. Smith, *University of Missouri, Animal Science Research Center, Columbia.*

Pregnancy associated glycoproteins (PAGs) are produced by binucleate cells in the ruminant placenta and have been used to diagnose pregnancy in cattle from d 27 post insemination to term. Previous studies indicate that PAGs have a half-life of approximately 8 d following calving; however, we hypothesized that the half-life would be shorter during early

gestation. The objective was to determine the half-life of bovine PAGs following prostaglandin $F_{2\alpha}$ (PGF)-induced abortion on d 32 to 36 post insemination. Twenty-five crossbred cows were artificially inseminated and allotted to a control (n = 10) or PGF (n = 15) group by age, breed, and days post AI. Blood samples were collected, via venipuncture, from cows every other day from approximately d 20 (d 0 = estrus) to d 28 post AI, daily from d 29 to PGF injection, and every 8 h from PGF injection until serum concentrations of PAGs decreased below the pregnancy-detection threshold of the assay. The uteri of all cows were examined by transrectal ultrasonography at d 30 and at the time of each blood sample following PGF injection to determine the time the fetal heartbeat stopped and when the embryo was expelled from the uterus. Serum concentrations of PAGs were determined by ELISA and were first detected between d 24 and 28 post AI. Serum concentrations of PAGs increased similarly in the control and PGF groups from d 28 to the time of PGF injection. Following PGF treatment (0 h), serum concentrations of PAGs decreased ($P < 0.05$) by 32 h and remained low through 144 h. The half-life of PAGs (mean ± SD) following PGF treatment was 59.7 ± 36.4 h (range 32 to 180 h). Interval (mean ± SD) from PGF injection to loss of fetal heartbeat, loss of embryo, and estrus was 50.5 ± 9.3 h, 60.7 ± 10.1 h, and 97.1 ± 12.1 h, respectively. In summary, the half-life of PAGs (d 32 to 40) was determined to be 2.5 d and may provide an alternative method to transrectal ultrasonography for monitoring late embryonic/fetal mortality.

Key Words: Pregnancy, Prostaglandin, Beef Cattle

W219   Use of supplemental GnRH following timed-insemination on pregnancy rates in dairy cattle exposed to mild heat stress or cool season environments. T. Dickerson*, K. Graves, J. White, S. Bowers, A. Denson, S. Schmidt, and S. Willard, *Mississippi State University, Mississippi State.*

Reproductive efficiency in dairy cattle decreases when animals are exposed to heat-stress conditions. Studies were conducted in the summer and fall seasons of 2003 to evaluate the efficacy of supplemental GnRH injections post-breeding on pregnancy rates in lactating dairy cattle. Lactating dairy cows in Summer (n=44) and Fall (n=74) were synchronized using the Ovsynch protocol, bred (TAI), and assigned to one of three GnRH treatment groups: Control (CON; no GnRH post-TAI), GnRH on d 5 & 11 post-TAI (GnRH-5/11), or GnRH on d 5 post-TAI (GnRH-5). Blood samples were collected on specified days throughout each trial for evaluation of serum concentrations of progesterone (P4). Ultrasonography was performed on d 5, 11, and 17 post-TAI on a subset of cows (n=8/treatment) for both studies for determination of CL area and number. Ambient temperature and relative humidity were collected daily at 10 min intervals for calculation of temperature-humidity index (THI). Overall Summer THI (24-h) was 77 (mild heat stress). No differences (P>0.10) were observed for serum concentrations of P4, CL area, and CL number with respect to treatment. Pregnancy rates for CON, GnRH-5/11, and GnRH-5 were 7.0%, 20% and 29% respectively with no differences (P>0.10) relative to treatment. Retrospective analysis of P4, and removal of cows (n=11) failing to respond to Ovsynch, resulted in an adjusted pregnancy rate of 13%, 23% and 33 %, respectively. Fall THI (24-h) was 52 (no heat stress). On D14 post-TAI, GnRH-5/11 cows had higher (P<0.05) serum P4 than CON, but did not differ (P>0.10) from GnRH-5 cows. On D17 post-TAI, GnRH-5/11 cows had higher (P<0.05) CL numbers than either CON or GnRH-5 cows. CL area was greater (P<0.05) for GnRH-5/11 than CON cows, but did not differ (P>0.10) from GnRH-5 cows on D17 post-TAI. Pregnancy rates for CON, GnRH-5/11, and GnRH-5 were 33%, 19% and 33% respectively, with no treatment differences (P>0.10). In summary, supplemental GnRH influenced reproductive function (P4, CL number and area) in Fall cows (GnRH 5/11 group). However, overall pregnancy rates were not significantly improved by GnRH treatment in Summer or Fall.

Key Words: GnRH, Progesterone, Pregnancy

W220   Effect of prolonged in vivo incubation of sperm from high and low fertility bulls on pregnancy survival in lactating dairy cows. M. W. Macfarlane*, B. J. Macfarlane*, and J. R. Pursley, *Michigan State University, East Lansing.*

Fertility of lactating dairy cows is altered by timing of AI in relation to ovulation. However, it is not clear how bull fertility affects this relationship. This study was designed to determine the effect of prolonged in vivo incubation of sperm from high and low fertility bulls on pregnancy rates and pregnancy survival in lactating dairy cows. All cows (n = 907) received Ovsynch to precisely control ovulation. Cows were inseminated at either 8 h prior to (treated), or 16 h after (control), the final injection of GnRH of Ovsynch. Therefore, treated and control cows received AI approximately 36 and 12 h prior to ovulation, respectively. Based on ultrasound examinations, only cows with synchronized ovulations were used in the study. Cows were assigned to treatments based on parity and days in milk (DIM). Bulls (n=5) were selected based on estimated relative conception rate (ERCR: + 4 or -4) and were assigned equally to cows in each treatment by parity and DIM. Ultrasonography was used to diagnose pregnancy at 28 and 56 d after AI. Outcomes were pregnancy rate / AI (PR/AI; %) at, and pregnancy survival (%) between, 28 and 56 d post-AI. Semen (n = 5 straws per bull) was randomly chosen and evaluated post-thaw for numbers of live sperm and percent motility. There was an effect of bull on PR/AI at (P < 0.01), and pregnancy survival between (P < 0.01), 28 and 56 d post-AI. PR/AI (%) and pregnancy survival (%) was decreased in the bull with the lowest overall fertility in treated vs. controls (10 vs. 25 and 58 vs. 86, respectively), but was not affected in the bull with the highest overall fertility (45 vs. 44 and 91 vs 98, respectively). Numbers of live sperm per straw, percent motility, and total live motile sperm were different between bulls (P < 0.01). There were strong correlations between PR/AI and pregnancy survival (R = 0.68) and % motility and PR/AI (R=0.71). In summary, prolonged in vivo incubation of sperm prior to ovulation appears to have a negative impact in low but not high fertility bulls. It appears that pregnancy losses may be predetermined at the time of fertilization.

Key Words: Dairy, Fertility, Timing of AI

W221   Synchronization of beef heifers using CIDR with estradiol cypionate. D. M. Kniffen*[1], B. M. Neely[1], P. R. Tozer[2], and M. L. O'Connor[1], [1]*Penn State University, University Park,* [2]*Western Australian Department of Agriculture, Geraldton, WA.*

Improving the success rate of estrous synchronization programs may increase the use of artificial insemination (AI) in the beef industry. The time and effort required to detect estrus in beef females continues to be a roadblock to increased use of AI. Introduction of a intravaginal progesterone insert (CIDR) has provided an effective method of estrous synchronization. Improving the synchrony of estrus following CIDR removal without sacrificing fertility will further reduce the time commitment for an effective AI program. The objective of this study was to determine if synchrony of estrus following a CIDR protocol could be enhanced by administration of 2 mg estradiol cypionate (ECP) at CIDR insertion. Seventy Angus and Angus-based crossbred beef heifers of breeding age and weight received a CIDR device on d 0. On d 7, the CIDR was removed and PGF2α (25 mg) was administered. The treatment group (n=35) received ECP. Heifers were observed for estrus for 30 min at 2 h intervals for 72 h following CIDR removal. All heifers were inseminated by a professional technician soon after observed in estrus, generally within 12 h. The percentage of heifers observed in estrus within 72 h did not differ (P>0.10) between treatment (74%) and control (71%) groups. First service conception rates (49%) did not differ (P>0.10). However, the average interval to onset of estrus following CIDR removal differed (P<0.05; 45 h for treated vs 51 h for controls). ECP induced an earlier expression of estrus without affecting conception rate.

Key Words: Synchronization, Heifers, CIDR

W222   Synchronization of estrus in suckled beef cows using GnRH, prostaglandin $F_{2\alpha}$ (PG), and progesterone (CIDR): a multi location study. J. E. Larson*[1], G. C. Lamb[1], J. S. Stevenson[2], S. K. Johnson[2], M. L. Day[3], T. W. Geary[4], D. J. Kesler[5], J. M. DeJarnette[6], F. N. Schrick[7], and J. D. Arseneau[8], [1]*University of Minnesota, St. Paul,* [2]*Kansas State University, Manhattan,* [3]*The Ohio State University, Columbus,* [4]*USDA-ARS,* [5]*University of Illinois, Urbana,* [6]*Select Sires, Inc.,* [7]*University of Tennessee, Knoxville,* [8]*Purdue University, West Lafayette, IN.*

We determined whether a fixed-time AI (TAI) protocol could yield similar pregnancy rates to a protocol requiring detection of estrus and if adding a CIDR to GnRH + PG-based protocols would enhance fertility. Estrus in 2,630 suckled beef cows from 14 locations was synchronized and artificial insemination occurred after five treatments: 1) a CIDR for 7 d with 25 mg of PG at CIDR removal, followed by detection of estrus and AI for 84 h with any cow not detected in estrus by 84 h receiving

100 μg of GnRH and TAI at 84 h (Control; n = 511); 2) GnRH, followed in 7 d with PG, followed in 60 h by a second injection of GnRH and TAI (CO-Synch; n = 551); 3) CO-Synch plus a CIDR during the 7 d between the first injection of GnRH and PG (CO-Synch+CIDR; n = 547); 4) GnRH, followed in 7 d with PG, followed by detection of estrus and AI for 84 h, with any cow not detected in estrus by 84 h receiving GnRH and TAI at 84 h (Hybrid Synch; n = 513); and, 5) Hybrid Synch plus a CIDR during the 7 d between the first injection of GnRH and PG (Hybrid Synch+CIDR; n = 508). Pregnancy was diagnosed by transrectal ultrasonography and blood samples were collected (d -17 and -7, relative to PG) to determine cycling status. The percentage of cows cycling at the initiation of estrus synchronization was 67%. Pregnancy rates among locations ranged from 39% to 67%. Pregnancy rates were greatest for the Hybrid Synch+CIDR (57.9%) treatment, although not different from the CO-Synch+CIDR (53.6%) and Hybrid Synch (53.0%) treatments, but greater (P < 0.05) than the Control (52.3%) and CO-Synch (43.4%) treatments. Controls did not differ from CO-Synch+CIDR or Hybrid Synch. Overall, the Hybrid Synch+CIDR protocol consistently achieved the greatest pregnancy rates; however, CO-Synch+CIDR was a reliable TAI protocol that gives producers the option to eliminate detection of estrus when inseminating beef cows.

Key Words: Estrus Synchronization, Artificial Insemination, Beef Cows

W223   Synchronization of estrus in replacement beef heifers using GnRH, prostaglandin F$_{2\alpha}$ (PG), and progesterone (CIDR): a multi-location study. J. E. Larson*[1], G. C. Lamb[1], T. W. Geary[2], J. S. Stevenson[3], S. K. Johnson[3], M. L. Day[4], D. J. Kesler[5], J. M. DeJarnette[6], and D. G. Landblom[7], [1]*University of Minnesota, St. Paul,* [2]*USDA-ARS,* [3]*Kansas State University, Manhattan,* [4]*The Ohio State University, Columbus,* [5]*University of Illinois, Urbana,* [6]*Select Sires, Inc.,* [7]*North Dakota State University, Fargo.*

Our objectives were to determine whether a fixed-time insemination (TAI) protocol could yield similar pregnancy rates to a protocol requiring detection of estrus (EAI) and whether an injection of GnRH at CIDR insertion enhanced fertility. Estrus in 2,077 replacement beef heifers from 12 locations was synchronized and AI occurred after four treatments: 1) a CIDR for 7 d with 25 mg of PG on the day of CIDR removal, followed by detection of estrus and AI during 84 h. Heifers not detected in estrus by 84 h received 100 μg of GnRH and were inseminated (EAI; n = 517); 2) heifers were treated and inseminated as EAI heifers but also received GnRH at the time of CIDR insertion (GnRH+EAI; n = 504); 3) heifers received a CIDR for 7 d with PG on the day of CIDR removal, followed in 60 h by a second injection of GnRH and TAI (TAI; n = 531); and 4) heifers were treated and inseminated as TAI heifers but also received GnRH at CIDR insertion (GnRH+TAI; n = 525). Pregnancy was diagnosed by transrectal ultrasonography on d 30 to 35 and blood samples were collected (d 17 and 7, relative to PG) to determine cycling status. Percentage of heifers cycling at initiation of estrus synchronization was 91%, but the percentage of heifers cycling among locations ranged from 78 to 100%. Overall pregnancy rates among locations ranged from 38 to 74%. Pregnancy rates were 57.3, 54.5, 53.1, and 49.1% for GnRH+EAI, EAI, GnRH+TAI, and TAI, respectively. Although no differences in pregnancy rates among treatments were observed, the GnRH+EAI treatment most consistently achieved the greatest pregnancy rates. In addition, the GnRH+TAI protocol provides an alternative to estrous synchronize heifers with TAI as an option without detection of estrus.

Key Words: Estrus Synchronization, Artificial Insemination, Beef Heifers

W224   Ovulatory and reproductive characteristics of sows treated with an intravaginal GnRH agonist gel. K. Roski*[1], W. Flowers[1], G. B. Rampacek[2], D. L. Gregor[3], M. Swanson[3], and H. D. Hafs[3], [1]*North Carolina State University, Raleigh,* [2]*University of Georgia, Athens,* [3]*EIEICO, Inc, Radnor, PA.*

This study was designed to test the ovulatory and reproductive characteristics of sows treated with Ovugel (EIEICO, Radnor, PA), a gel containing a GnRH agonist administered intravaginally. The treatment groups received 100μg of GnRH agonist in varying viscosities of the gel, 0.6% (n=12), 0.9% (n=12), 1.2% (n=12), and 1.5% (n=12) respectively. A positive control group (n=11) recieved the 100μg of agonist in saline while the control sows received a vehicle of the 1.2% gel at 96h post weaning. Jugular cannulas were placed 48h before administration of the treatment. Blood samples were taken every 6h before treatment,

then every 2h for the first 12h after treatment, then every 6h for the next 18h. Estrus detection occurred every 6h through the entire study as well as real time ultrasonography every 4h to determine the time of ovulation. Control sows were bred based on the onset of estrus (n=9) and treatment sows were bred at +8 and +32h after the gel was administered. There were no differences in treatments in ovulation time (p=0.28), estrus length (p=0.60), farrowing rate (p=0.80), litter size (p=0.35), or LH surge (p=0.49). However, variation associated with ovulation time was significantly reduced (p=0.01) for saline, 1.2%, and 1.5% treatments compared with controls. The intravaginal absorption of GnRH in Ovugel or saline along with a timed insemination program is an effective method to reduce the interval over which ovulation occurs without overtly decreasing fertility.

Treatment	Saline + GnRH	Control	0.6% + GnRH	0.9% + GnRH	1.2% + GnRH	1.5% GnRH
Ovulation Time (h)	46.5 ±1.2	43.8 ±5.4	43.8 ±2.0	41.2 ±3.3	43.5 ±1.8	44.5 ±1.5
Estrus Length (h)	45.8 ±3.4	45.6 ±3.8	49.0 ±3.8	47 ±3.9	46.9 ±3.9	55.1 ±3.9
LH Surge (0-30h post treatment)	7/10	5/8	7/10	9/11	8/10	8/9
Farrowing Rate	8/8	9/9	11/12	10/12	11/12	10/11
Litter Size	12.9 ±0.7	9 ±1.2	11 ±1.3	11.6 ±1.0	11 ±0.5	10.6 ±1.4

Key Words: Ovulation, GnRH Agonist, Swine

W225   The effects of estradiol cypionate on expression of estrus in a follicular synchronization program. B. Dixon*, D. L. Ray, T. B. Hatler, and W. J. Silvia, *Department of Animal Science, University of Kentucky, Lexington.*

The objective of this experiment was to determine the effect of estradiol cypionate (ECP) on expression of estrus when used in conjunction with a presynchronization and estrus synchronization program. Ninety lactating Holstein (n=66) and Jersey (n=24) cows in varying stages of lactation were given two injections of PGF$_{2\alpha}$ fourteen days apart to presynchronize luteolysis. Twelve days following the last PGF$_{2\alpha}$ injection, a follicular synchronization program was initiated with an injection of GnRH (100μg, Factrel, Fort Dodge Animal Health), followed seven days later by PGF$_{2\alpha}$ (25mg, Lutalyse, Pfizer). Half of the cows were then assigned to receive an injection of ECP (1mg, Pfizer) 24 hours after the last PGF$_{2\alpha}$ injection. The other half received no ECP. Venous blood samples were collected three times per week for three weeks during the final phase of the synchronization for determination of progesterone levels. These samples were used to monitor luteolysis and formation of a new corpus luteum. Presynchronization failed to adequately synchronize the cycle of 31 out of 90 cows. ECP had a significant effect on expression of estrus among cows that were successfully presynchronized and ovulated. Of those cows that were properly synchronized for breeding, 100% (28/28) of cows treated with ECP expressed estrus, while only 81.8% (18/22) of untreated animals did so (P=.0318). For animals that fully regressed their corpus luteum and ovulated, expression of estrus was more tightly synchronized among cows receiving ECP (90.3% (28/31) expressing heat between 29 and 53 hours post ECP) than those that did not (53.9% (14/26)) (P=.0025). Of the 46 cows that appeared endocrinologically normal, expressed estrus and were bred, 15 conceived (32.6%). ECP had no effect on conception rate. The postovulatory rise in progesterone appears to be slower in nonpregnant animals than pregnant animals, with pregnant cows reaching progesterone levels 1.0 ng/mL higher 12 days after the final PGF$_{2\alpha}$ than animals that failed to conceive (P=.01). In conclusion, the addition of ECP to a synchronization program improves expression of estrus among cows that were successfully synchronized for breeding.

Key Words: Synchronization, ECP, Estrus Expression

W226   Influence of milk production on conception following artificial insemination or embryo transfer in lactating Holstein cows. D. G. B. Demetrio*[1], J. L. M. Vasconcelos[1], C. A. Rodrigues[2], and J. R. Chiari[2], [1]FMVZ-UNESP, [2]Samvet Clinica e Embrioes, Brazil.

The aim of this trial was to evaluate conception following artificial insemination (AI) or embryo transfer (ET) in lactating Holstein cows. The trial was conducted at a dairy farm located in Descalvado, Sao Paulo, Brazil from October to December of 2003. Cycling cows (n=251) producing 33.5±7.2 kg milk/d with 174.4±116.2 DIM received PGF2α (Lutalyse[®], 25 mg, i.m.) and were assigned to two groups. Cows detected in estrus between 48 and 96h after PGF2α injection (n=106) were divided into: G1 (n=57); AI 12h after heat detection, or G2 (n=49); ET 6 to 8 d after detection of estrus. All cows from G2 received fresh embryos (grade 1 or 2) that were transferred by one trained technician. Milk production was measured daily, and the average of 7 d of production was used for anlaysis (starting on the day of the estrous detection). Pregnancy was detected 25 to 32 d after estrous detection by ultrasonography (Aloka SSD-500). Data were analyzed by a general linear models (GLM) procedure. Variables included in the model were milk production, previous number of AI, DIM, body temperature at d 7 and interactions. Conception was affected by treatment (P=0.07) and the adjusted results were 31.6±10.1% in G1 (IA) and 54.4±9.6% in G2 (TE). An interaction between milk production and treatment was detected (P<0.05). Cows with milk production under the average (33.5 kg milk/d) had similar conception rates: 49.1±14.1% (n=26) in G1 (IA) and 40.1±13.6% (n= 24) in G2 (TE). Cows over the average had different conception rates: 14.0±14.1% (n= 31) in G1 (IA) and 68.7±16.7% (n= 25) in G2 (TE). Variables included in the model: previous number of AI, DIM and body temperature at d 7 did not affect conception, but milk production had adverse influence on AI conception rate, but not on ET. This study suggests that embryo transfer can be used as a tool to increase conception rate in high milk production cows, rather than only on repeat breeder cows. This data also shows that milk production effects on conception could occur during the 7 d after ovulation.

Key Words: Embryo Transfer, Artificial Insemination, Milk Production

W227   Follicular diameter in postpartum Nellore cows treated with CRESTAR protocol. G. C. Perez*, R. M. Santos, J. L. M. Vasconcelos, E. T. N. Pereira, and D. B. Araujo, DPEA-FMVZ-UNESP, Botucatu, SP, Brazil.

The CRESTAR[®] protocol consists of a 9-d ear implant (3mg of norgestomet) combined with injection of estradiol valerate (5mg) and norgestomet (3mg) at the time of implant insertion. The objective of this study was to evaluate if body condition score (BCS), parity and cyclicity affects follicular diameter at the time of implant removal. Nellore cows (n = 415) averaging 119 ± 39.7 d postpartum, and with a BCS between 2.25 to 3.50 (1 to 5 scale), were maintained on pasture (Brachiaria decumbens) with mineral provided ad libitum. Ovarian structures were evaluated by transrectal ultrasonography using a B-mode scanner with a 7.5 MHz linear array transducer (Aloka SSD-500, Wallingford, CT). Cyclicity was determined before the beginning of the protocol by two ultrasound examinations 10 d apart. Cows showing luteal tissue at one of the exams were considered to be cycling. Follicular diameter and BCS were evaluated at the time of implant removal. Data were analyzed by a general linear models (GLM) procedure, and the variables included in the model were BCS, cyclicity, and parity. BCS influenced (P<0.05) follicular diameter at the time of the implant removal: 2.5 (8.9 ± 0.28mm; n = 78); 2.75 (9.5 ± 0.22mm; n = 137); 3.0 (9.5 ± 0.25mm; n = 109); 3.25 (10.2 ± 0.34mm; n = 55); 3.5 (9.7 ± 0.42mm; n = 36). Follicular diameter was not affected by parity [9.4 ± 0.27 mm in primiparous cows (n=87) vs 9.7 ± 0.14 mm in multiparous cows (n=328)] nor by cyclicity [9.7 ± 0.20mm in anestrous cows (n=201) vs 9.4 ± 0.20mm in cycling cows (n=214)]. Cows with lower BCS had smaller follicular diameter at the time of implant removal. Mechanisms controlling this phenomenon may be related to the injection of estradiol valerate which could delay the emergence of a new follicular wave by suppressing FSH or LH secretion and cause turnover of the dominant follicle in thin cows. Data suggest that cows with a low BCS may require a longer ear implant period and/or a smaller dose of estradiol valerate at the beginning of the CRESTAR[®] protocol.

Key Words: CRESTAR, Follicular Diameter, Nellore Cows

W228   Serum progesterone concentrations in Nellore x Angus heifers treated with 1.38-g or 1.9-g CIDR devices. R. M. Santos*[1], J. L. M. Vasconcelos[2], G. C. Perez[2], A. B. B. Maciel[2], and O. G. Sa Filho[2], [1]FCAV-UNESP, Jaboticabal, SP, Brazil, [2]FMVZ-UNESP, Botucatu, Brazil.

The aim of this study was to determine serum concentrations of progesterone (P4) during treatment with intravaginal devices containing either 1.38 g or 1.9 g of P4 for 25 d. Estrous cycles of Nellore x Angus heifers (n=26) were presynchronized using a modified Ovsynch protocol (GnRH - 50μg - 6d - PGF2α - 25mg - 48h GnRH - 50μg). Seven days after the second injection of GnRH, synchronized heifers (n=18) received PGF2α and 24 h later were assigned to either G1 (n=9; CIDR[®] 1.38 g) or G2 (n=9 CIDR[®] 1.9 g). Only heifers that had a regressed CL (determined by P4 concentrations on d 6, 7, 7.5 and 8) were used (5 heifers in G1 and 7 in G2). Serum P4 concentrations were quantified by RIA. Peak concentrations of P4 were evaluated at 0, 1, 4, 8 and 12 h after CIDR insertion, and every other day for 25 d. The decline in concentrations of P4 d was evaluated at 0, 1, 4 and 8 h after CIDR removal. Data were analyzed by a general linear models (GLM) procedure. Two cows from G2 lost the CIDR (at d 8 and 25, respectively) and were excluded from the anlaysis. Serum concentrations of P4 peaked within 1 h after device insertion, with peak concentrations higher (P<0.05) for G1 (10.2±1.21ng/mL) than for G2 (6.8±1.02ng/mL). Within 4 h concentrations of P4 were similar for G1 (6.3±0.64ng/mL) and for G2 (6.0±0.54ng/mL) and therafter through d 25. Concentrations of P4 ranged from 2-3 ng/mL between d 1 and 8 for G1 and until day 11 for G2, and then from 1-2 ng/mL until the day of insert removal. On d 25, mean concentrations were 1.02±0.12ng/mL and 1.12±0.12ng/mL for G1 and G2, respectively. The decrease in P4 concentrations after insert removal was similar between groups. One hour after inseert removal, P4 concentrations were 0.3±0.04ng/mL and 0.2±0.04ng/mL in G1 and G2, respectively. Although the 1.38-g CIDR produced a lower overall serum P4 concentration, the peak in P4 concentrations 1 h after CIDR insertion was higher than for the 1.9-G CIDR. This might be important in synchronization protocols, as rapid changes in circulating concentrations of P4 could affect LH pulsatility and turnover of the dominant follicle.

Key Words: CIDR, Progesterone, Beef Heifers

W229   Prolonged in vivo incubation of sperm affects fertility of lactating dairy cows and gender ratio of resulting offspring. M. W. Macfarlane*, B. J. Macfarlane*, and J. R. Pursley, Michigan State University, East Lansing.

The chance of a female calf being born is less than that of a male calf (female: male ratio of calves born is 46:54); and only 85% of female calves that are born successfully begin their first lactation. Therefore, increasing numbers of females available for herd replacement and marketing may increase sustainability and profitability of dairy enterprises. This study was designed to determine if prolonged in vivo incubation of sperm increases the percentage of female calves born. Lactating dairy cows (n=1606) from 3 farms received Ovsynch to precisely control ovulation. Cows were inseminated either in the uterine body or uterine horn ipsilateral to the ovary with the predicted pre-ovulatory follicle at either 8 h prior to (-8 group), or 16 h after (+16 group), the final injection of GnRH of Ovsynch (2 x 2 design). Therefore, cows in the -8 and +16 h groups received AI approximately 36 and 12 h prior to ovulation, respectively. Based on ultrasound examinations, only cows with synchronized ovulations were used in the study. Cows were assigned to treatments based on parity and days in milk (DIM). Ultrasonography was used to diagnose pregnancy at 28 and 56 d after AI. Outcomes were pregnancy rate/AI (PR/AI; %) at, and pregnancy survival (%) between, 28 and 56 d post-AI, and gender of resulting offspring. There was no effect of site of AI on PR/AI, so uterine body and uterine horn groups were combined within -8 and +16 groups. Overall, a greater % of female calves were born in the -8 h vs. +16 groups (55 vs. 45; P < 0.05). Farms 1 and 2, collectively, had greater (P < 0.05) % females born compared to farm 3 in both the -8 and +16 h groups (68 and 56 vs. 49 and 40, respectively). The +16 h group had a greater (P < 0.01) PR/AI (39 % vs. 29 %) than the -8 h group. Pregnancy survival (%) between 28 and 56 d post-AI was similar between 8 and + 16 (80 vs. 83, respectively) but was greater in cows receiving uterine body vs. uterine horn AI (85 vs. 79, respectively). In summary, prolonged in vivo incubation of sperm

prior to ovulation appears to improve female:male ratio of calves born, but reduces fertility.

Key Words: Gender Ratio, Timing of AI, Pregnancy Rate

W230   Effect of eCG and/or calf removal after CRESTAR protocol in Nellore cows. G. C. Perez*, J. L. M. Vasconcelos, R. M. Santos, E. T. N. Pereira, and O. G. Sa Filho, *DPEA-FMVZ-UNESP, Botucatu, SP, Brazil*.

The CRESTAR® protocol consists of an ear implant (3mg norgestomet) for 9 days associated with an injection of estradiol valerate (5mg) and norgestomet (3mg) at implant insertion. The aim was to evaluate the effects of eCG injection at implant withdraw and/or calf removal (CR) between implant withdraw and TAI. Nellore cows (n=415) with 119±39.7 days postpartum (DPP), body condition score (BCS) between 2.25 to 3.5 (1-5) were assigned to 4 groups in 2x2 factorial (eCGxCR): G1(n=105) CRESTAR; G2(n=102) CRESTAR + CR (58h); G3(n=104) CRESTAR + eCG (Folligon®, 400UI) at implant removal and G4(n=104) CRESTAR + eCG at implant removal + CR (58h). TAI was performed in the 4 groups 50-58 h after implant removal. Ovarian structures and pregnancy were evaluated by ultrasound (Aloka SSD-500). Cyclicity was determined before the beginning of the protocol by 2 ultrasound examinations 10 days apart. Follicular development was determined by the difference between the follicle diameter on day of AI and on implant removal. Data were analyzed by general linear models, and parameters included in the model were DPP, CR, eCG, BCS, cyclicity, parity, calf gender and interactions. Cows that received eCG had better follicular development (1.8±0.2 vs. 1.2±0.1mm; P<0.01), higher estrus detection before TAI (42.1 vs. 26.4%; P<0.01), and higher synchronization rate (75.3 vs. 64.5%; P<0.05) than cows that did not received eCG. Calf removal did not affect these parameters. Conception at TAI was not affected by eCG (53.6% in treated cows vs. 50.3% in not treated) and by CR (51.2 with CR vs. 52.7% without). Independent of the treatment, BCS did not affect synchronization rate but influenced (P<0.05) conception at TAI (2.25 59.4%; 36.2%, 2.5 68.2%; 44.1%, 2.75 71.8%; 45.9%, 3.0 77.7; 58.8%; 3.25 74.1%; 57.2%, 3.5 68.1; 69.3%) respectively. The data suggest that eCG treatment improves synchronization rate by keeping the follicular development between implant removal and TAI, but not conception at TAI probably due to the negative effects of BCS in conception are higher than positive effects of eCG treatment.

Key Words: CRESTAR, Synchronization, Nellore

W231   Assessment of pregnancy in the mare using digital infrared thermography. S. Bowers*, S. Gandy, B. Anderson, P. Ryan, and S. Willard, *Mississippi State University, Mississippi State*.

Digital infrared thermal imaging (DITI) is a non-invasive diagnostic technique that is used to detect symmetry and asymmetry in surface temperature gradients. The objective of this study was to investigate the use of DITI to determine whether temperature differences exist between pregnant and nonpregnant mares. On the day measurements were obtained, each pregnant mare (n=10; beginning at 292.4 ± 1.4 d of gestation) was paired with a nonpregnant mare (n=17). Mares were normalized to environmental conditions prior to imaging. Ambient (AMB) temperature, DITI measurements (left and right flank and wither temperatures and background temperature), and rectal temperatures (RT) were obtained every 7 d for 5 weeks before parturition. Measurements were obtained beginning in March and continued through May with AMB temperatures ranging from 4.2 °C to 28.9 °C. There were no differences (P>0.10) in temperature of the left and right side within group (pregnant vs. nonpregnant) therefore data were pooled within group for analysis. Background temperature was positively correlated with flank and wither temperature (R=0.48, R=0.58, respectively; P<0.01) and was positively correlated with AMB temperature (R=0.92; P<0.01). RT tended to be positively correlated with AMB temperatures (R=0.32; P<0.10) but were not correlated to flank or wither temperatures. Pregnant mares had a higher (P<0.01) flank temperature than the nonpregnant mares (36.0 ± 0.2°C vs. 34.2 ± 0.2°C, respectively). Moreover, the difference (2.4°C) in flank temperatures between the pregnant and nonpregnant mares was greater when the AMB temperature dropped below 19°C. Flank temperature and wither temperature were positively correlated with one another (R=0.72; P<0.01) and were positively correlated with AMB temperature (R=0.48, R=0.64, respectively; P<0.01). However, wither temperatures did not differ (P>0.10) between the pregnant

(33.9 ± 0.6°C) and nonpregnant mares (33.8 ± 0.3°C). In conclusion, pregnant mares had higher flank temperatures than nonpregnant mares regardless of environmental conditions. This suggests that DITI measurements may have value in detecting or confirming pregnancy (late gestation) in some species.

Key Words: Thermography, Equine, Pregnancy

W232   Optimizing sperm concentration to maximize monospermy and minimize polyspermy with bovine *in vitro* fertilization. M. Barcelo-Fimbres* and G. E. Seidel, Jr., *Colorado State University, Ft Collins*.

We evaluated sperm concentrations and times of fertilization for bovine IVF. Slaughterhouse oocytes were matured in a chemically defined medium (CDM) similar to SOF (J. Anim. Sci. 78:152) plus 0.5% fatty acid-free BSA and hormones (M-CDM) for 23 h, at 38.5C in 5% CO_2 in air. Then oocytes and frozen-thawed sperm, centrifuged through a Percoll gradient, were co-cultured for 4 or 18h in F-CDM (CDM + heparin). Presumptive zygotes were cultured in CDM-1 (CDM + nonessential amino acids (NEAA), 0.5 mM glucose and 10 μM EDTA) at 38.5°C in 5%CO_2 /5%O_2 /90%N_2 . After 5 h, half of the presumptive zygotes were fixed and stained with aceto-orcein to evaluate fertilization (FR) and polyspermy (PS). After 48h the rest were cultured 120h in CDM-2 (CDM + NEAA and essential amino acids and 2 mM glucose). Oocytes (N=960) were randomly allocated to a 2 x 3 x 4 factorial design: time of fertilization (4 and 18 h), sperm dose (1, 0.33, and 0.11 x 10^6 sperm/mL), and sperm source (4 bulls). Percentages were arcsin transformed and analyzed by ANOVA. Respective main effect means for 1, 0.33, and 0.11 x 10^6 sperm/mL were: FR (72%, 62% vs. 42%); cleavage (CR) (89%, 81% vs. 58%); PS (24%, vs. 2% and 0%), and blastocysts/oocyte (BR) (17%, 21% vs. 9%) (all P<0.01). There was a difference (P<0.01) between 18 and 4 h in FR (69% vs. 48%), PS (13% vs. 4%), and CR (62% vs. 54%), respectively. The bull effect was not significant (P >0.05) for FR (53%, 57%, 62%, 62%), or PS (8%, 9%, 8%, 10%), but CR were higher (P <0.01) for 3 of the bulls (83%, 80%, 76% vs. 58%), and the BR was higher (P<0.05) for two bulls (20%, 20%, vs. 10%, 10%). FR and CR were correlated, r^2 =0.62. Parthenogenetic, control cleavage (4.1%) stopped at the 8-cell stage. The optimal sperm concentration was 0.33 x 10^6 /mL. The higher dose caused considerable PS; although the lower concentration decreased PS, it also decreases FR. The longer fertilization time resulted in a higher FR, but more PS.

Key Words: Bovine, IVF, Polyspermy

W233   Fixed-time insemination utilizing an Eazi-Breed™ CIDR® in combination with gonadotropin-releasing hormone or estradiol cypionate. K. J. Stutts*[1], D. W. Forrest[1], and C. R. Looney[2], [1]*Texas A&M University, College Station*, [2]*OvaGenix, Navasota, TX*.

The objective of this study was to evaluate the efficacy of four estrus synchronization protocols utilizing an intravaginal progesterone-releasing device (CIDR) in combination with estradiol cypionate (ECP) or gonadotropin-releasing hormone (GnRH) on conception rate to a fixed-time insemination in beef heifers. Angus-cross heifers (n=1906) that averaged 13 mo of age and 320 kg BW were randomly assigned to one of four treatments. Treatment (TRT) 1 heifers received a CIDR insert and ECP (1 mg i.m.) on d 0. $PGF_{2\alpha}$ (25 mg) was injected (i.m.) and CIDRs were removed on d 9. A second injection of ECP (0.5 mg) was administered on d 10 and heifers were fixed-time inseminated on d 11. TRT 2 heifers received the same hormonal regimen as TRT 1 except that CIDRs were removed and $PGF_{2\alpha}$ was injected on d 7 followed by the second injection of ECP on d 8 and insemination on d 9. TRT 3 heifers received a CIDR insert and GnRH (50 μg i.m.) on d 0. $PGF_{2\alpha}$ (25 mg) was injected and CIDRs were removed on d 7. A second injection of GnRH (50 μg) was administered and the heifers were inseminated on d 9. TRT 4 heifers received the same hormonal regimen as TRT 3 except that $PGF_{2\alpha}$ was injected on d 6. Beginning 14 d after insemination, heifers were exposed to fertile bulls for 60 d. Conception to the timed insemination was determined by transrectal ultrasonography 30 d after bull removal. Conception rates to fixed-time insemination were different (P<.02) across all treatments. Conception rates to fixed-time insemination for TRT 1, 2, 3, and 4 were 32.4%, 19.6%, 43.2%, and 50.4%, respectively. Overall pregnancy rates for TRT 1 (84.6%), 3 (89.0%), and 4 (87.7%) were higher (P<.0001) than TRT 2 (69.8%). These data indicate that conception rates to timed AI were higher for the CIDR

protocols with GnRH than with ECP, and that injecting $PGF_{2\alpha}$ on d 6 rather than d 7 when using the GnRH/CIDR protocol resulted in a higher conception rate to fixed-time insemination. *Supported by Pfizer Animal Health.*

Key Words: Estrus, Synchronization, Heifers

W234   Deep uterine horn AI improves fertility of lactating dairy cows. J. R. Pursley*, *Michigan State University, East Lansing.*

Currently, fertility levels of lactating cows are approximately 50 % of heifers. This study was designed to determine if fertility of lactating dairy cows could be increased if semen was deposited in the uterine horns compared to the uterine body. Lactating Holstein cows (n=833) from 3 farms were randomly assigned to receive AI in either the uterine body or in the uterine horn by splitting the straw between both horns and depositing the semen near the greater curvature of each horn. Cows were assigned to the two treatments by DIM and parity. Cows received AI following a detected estrus or following Ovsynch (GnRH, 7 d PGF, 48 h GnRH, 16 h AI). Technicians from each farm received training in uterine horn AI prior to the start of the trial. Pregnancy was determined by the farm veterinarian between 35 and 65 d post-AI. Pregnancy rate/AI was greater (P < 0.01) in the uterine horn group compared to uterine body (50 vs. 35 %). There was no effect (P > 0.05) of parity or DIM on pregnancy rate/AI. Pregnancy rate/AI was similar (P > 0.05), and there was no effect (P > 0.05) of treatment, between AI following Ovsynch vs. AI following detected estrus. There was an effect (P < 0.05) of farm on pregnancy rates/AI, but there was no treatment by farm interaction (P = 0.89). All farms had increases in pregnancy rates/AI in cows inseminated in the uterine horns and ranged from 26 to 43 %. In summary, uterine horn AI appears to increase pregnancy rates/AI in lactating dairy cows.

Key Words: Uterine Horn AI, Fertility, Dairy Cows

W235   Addition of ECP or calf removal to a modified MGA + CO-Synch protocol for synchronization of ovulation in beef cows. S. K. Johnson, K. R. Harmoney, and J. S. Stevenson*, *Kansas State University, Manhattan.*

A study was conducted in 735 suckled beef cows to determine if synchronization of ovulation could be improved with estradiol cypionate (ECP) or 48 h calf removal in a modified MGA + CO-Synch protocol. All cows were fed MGA (0.5 mg·hd^{-1}) daily for 14 d (d -32 to -19) and received GnRH (100 μg) on d -7, PGF (25 mg) on d 0, and one fixed time AI at 72 h after PGF. Treatments were applied in a 2 x 2 factorial arrangement of four treatments. Calves either remained with cows (**S**) or were removed for a 48 h period (**CR**), beginning 24 h after PGF until after the fixed time AI. Cows either received ECP (**E**; 1 mg) at 24 h after PGF or GnRH (**G**; 100 μg) concurrent with fixed time AI. Concentrations of progesterone (P4) were determined in serum samples collected on d -17, -7, 0, and 3. If P4 was $\geq$ 1 ng·ml^{-1} on d -17 and/or d -7, cows were classified as cycling. Three locations were studied in year 1 and 2 locations were repeated in year 2. Pregnancy rate to AI (PR) was determined by transrectal ultrasonography on d 33 to 35. The interaction of location nested within year by treatment was significant. The combination of E and S was the least variable of the 4 treatments, with PR ranging from 40 to 58% whereas the other treatments ranged from 29 to 66% from lowest to highest response per location/year. Pregnancy rate was similar for E and G-treated cows (48% and 45%, respectively). Cycling status influenced the response to calf removal. Noncycling CR-treated cows had greater (P<0.05) PR rate than S cows (37% vs. 27%, respectively). Noncycling E-treated cows had greater (P<0.05) PR for CR than S. Cycling E-treated cows had greater (P<0.05) PR for S than CR. Within cycling classification, G-treated cows had a similar response to CR or S. Use of CR in synchronization protocols is most appropriate in herds that anticipate a higher proportion of noncycling cows. Use of ECP to synchronize ovulation did not improve overall PR and required an additional animal handling.

Key Words: Fixed Time AI, Estrogen, Calf Removal

W236   Effect of an addition of a CIDR insert to the Heatsynch protocol on ovulation rate, pregnancy rate and pregnancy loss in lactating dairy cows. K.N. Gavao*, S.O. Juchem, R.L.A. Cerri, A.C. Coscioni, M. Villasenor, and J.E.P. Santos, *University of California Davis, Tulare.*

Objectives were to determine the effects of incorporating a CIDR insert in a pre-synchronized (27 and 41 days in milk (DIM), PGF_{2a} Heatsynch protocol (55 DIM, GnRH; 62 DIM, PGF_{2a}; 63 DIM, estradiol cypionate; 65 DIM, timed AI) on ovulation rate (OR), display of estrus, pregnancy rate (PR), and pregnancy loss (PL) in lactating cows. Holstein cows, 675, from two herds were randomly assigned to treatments. Blood was sampled at 41 and 55 DIM for analysis of progesterone to determine cyclicity. The CIDR was inserted at the time of injection of GnRH, and removed at the the time of injection of PGF_{2a} of Heatsynch. Ovaries were scanned at the time of PGF_{2a} injection and 2 and 7 d later. Pregnancy was diagnosed at 27 and 41 d after AI. Data were analyzed by the LOGISTIC and GLM procedures of SAS (2001). Addition of a CIDR to the Heatsynch protocol eliminated cows displaying estrus prior to the last PGF_{2a} injection (0 vs 2.01%) and decreased proportion of cows bearing a CL at the last PGF_{2a} injection (86.3 vs 93.9%; P<0.001). CIDR did not affect the proportion of cows displaying estrus after the last PGF_{2a} injection (77.2 vs 73.8%; P=0.11) or OR (85.6 vs 86.6%; P=0.30). PR were similar for CIDR and controls on d 27 (35.8 vs 38.8%; P=0.68) and 41 (29.3 vs 32.3%; P=0.33) regardless of cycling status prior to Heatsynch (P>0.15). Pregnancy loss was similar for CIDR and controls (18.3 vs 16.8%; P=0.77). Cyclic cows had higher PR than anovulatory cows at 41 d after AI (33.8 vs 20.4%; P = 0.03) because of lower PL (16.0 vs 30.3; P=0.06). When cows were stratified based on estrus prior to timed AI, at timed AI or no estrus, PR on d 27 (36.9 vs 45.4 vs 17.0%; P<0.001) and 41 (34.2 vs 37.2 vs 12.0%; P<0.001) differed, which might be related to the higher OR (90.0 vs 94.3 vs 63.1%; P<0.001) in cows displaying estrus. PL tended to be lower for cows displaying estrus either prior to or at timed AI than those not in estrus (7.3 vs 18.0 vs 29.6%; P=0.10). Incorporation of a CIDR insert into the Presynch/Heatsynch protocol did not improve PR in lactating dairy cows. Improvements in PR in the Heatsynch protocol are expected when more cows display estrus, thereby increasing synchronization and OR.

Key Words: CIDR Insert, Synchronization, Dairy Cows

W237   Use of milk progesterone following artificial insemination in a resynchronization program for early rebreeding in dairy cows. A. Denson*, T. Dickerson, S. Bowers, S. Schmidt, K. Graves, K. Moulton, and S. Willard, *Mississippi State University, Mississippi State.*

Studies have revealed that the implementation of OvSynch protocols improves synchronization of ovulation and reduces days to first service and days open in dairy cows. Resynchronization of non-pregnant animals in a shortened time-frame post-AI may be beneficial in decreasing days open. The objective of this study was to use milk progesterone (P4) at d 21 following OvSynch and timed-AI to identify pregnant cows and resynchronize non-pregnant cows to accelerate rebreeding. Holstein (n=30) and Jersey (n=10) cows were synchronized using OvSynch and bred AI 16 h post-GnRH. All cows were administered GnRH at d 14 post-AI for resynchronization as part of a subsequent OvSynch protocol. Milk samples were collected on d 21 post-AI to diagnose cows as pregnant or non-pregnant based on qualitative high or low milk P4 (Accufirm). Cows having low P4 were administered $PGF_{2\alpha}$ followed 48 h later with a second administration of GnRH and AI 16 h later. Cows with high P4 were presumed pregnant and not administered $PGF_{2\alpha}$ or GnRH. Blood samples were obtained from all cows on d -9, -2, 0 (AI), 7, 14, 21 through 25 and 28. Blood serum was collected for P4 analysis by RIA. Pregnancy status of cows was confirmed at d 42 post-AI by ultrasonography (US). According to the milk P4 results, 47.5 % of cows were not pregnant to OvSynch/AI based on low milk P4 levels. Serum P4 on d 21 post-AI was low (<1 ng/ ml) in 68.4 % of these presumably non-pregnant cows by RIA. Of the 52.5 % of cows with high milk P4 on d 21 post-AI, 85.7 % of the cows exhibited high (>1 ng/ml) serum P4 by RIA. Serum (RIA) and milk P4 on d 21 did not differ (McNemar's test, P>0.10) in pregnant vs. nonpregnant determinations. US at d 42 following the first insemination revealed an overall pregnancy rate of 22.5 %, which represented a 42.9 % pregnancy rate for cows previously identified as pregnant by milk P4 (i.e., US vs. milk P4 disagreed in accuracy of pregnancy rate; McNemar's test, P<0.01). In summary, use

of milk P4 at d 21 post-AI as a selection criteria to identify pregnant cows as part of resynchronization programs may not be accurate enough to justify its use.

Key Words: OvSynch, Milk Progesterone, Synchronization

W238   Use of CIDR with a timed insemination protocol in lactating dairy cows during summer in Mexico. F. Moreira*, R. Flores, and J. Boucher, *Pfizer Animal Health,*.

The objective was to evaluate whether addition of EAZI-BREED CIDR Cattle Inserts (CIDR) to the Ovsynch program increases first service pregnancy rate (PR) in lactating dairy cows. The study was conducted in 5 dairies in Central Mexico. Cows were inseminated from June through September, 2001. Within trial site and within parity, cows were assigned to either an Ovsynch (n=255) or an Ovsynch-CIDR group (n=255) for their first service. Ovsynch was initiated at 50 ± 3 d postpartum with an injection of 100 μg of GnRH (2 mL, i.m.; CYS-TORELIN), an injection of 25 mg of $PGF_{2\alpha}$ (5 mL, i.m.; LUTALYSE Sterile Solution) 7 d later, a second 100 μg injection of GnRH 48 h later, and timed insemination 8 to 20 hours after the second GnRH injection. Ovsynch-CIDR cows received the same treatments but also received a CIDR Insert (1.38 g of progesterone) which was administered with the first GnRH injection and removed 7 d later at the $PGF_{2\alpha}$ injection. Cows were palpated for pregnancy 40 to 45 d after timed insemination. An interaction between treatments and parity was detected (P=0.023). The interaction indicated that PR was increased for Ovsynch-CIDR compared to Ovsynch in primiparous cows (38.2% and 20%, respectively; P=0.024) but no differences were detected in multiparous cows (22.3% and 27.5%, respectively; P=0.375). A subset of cows (n=466) was classified as anestrus or cyclic based on progesterone concentrations of two blood samples collected 7 d prior to and at the first GnRH injection. For anestrous cows, PR was 18.8% for Ovsynch (6/32) and 18.4% for Ovsynch-CIDR (7/38). For cyclic cows, PR was 23.5% for Ovsynch (47/200) and 29.1% for Ovsynch-CIDR (57/196). Such effects were further subdivided according to parity but none were significant. Addition of CIDR increased first service pregnancy rates of primiparous but not multiparous lactating dairy cows submitted to an Ovsynch program. Because cows were inseminated during summer, heat stress might have affected embryonic survival but the study was not designed to specifically address such an effect.

Key Words: CIDR, Ovsynch

W239   Efficacy of an injection of Dinoprost Tromethamine when given subcutaneously in two different sites on luteal regression in lactating Holstein cows. R. C. Chebel*[1], J. P. Reynolds[1], R. L. A. Cerri[1], J. Versteeg[2], H. M. Rutgliano[1], and J. E. P. Santos[1], [1]*University of California Davis, Tulare*, [2]*Pfizer Animal Health*.

The objective of this study was to compare the efficacy of administration of 25 mg of dinoprost tromethamine sterile solution (Lutalyse, Pfizer Animal Health) through different routes and in different sites on luteal regression. Lactating Holstein cows, 109, were synchronized with one injection of PGF2a 14 d prior to enrollment (study d 0). On study d 0, cows were examined by ultrasonography to determine presence of a CL in one of the ovaries. Cows with CL were blocked by parity and d in milk and, within each block, randomly assigned to receive PGF2a as an i.m injection in the semimenbranous/semitendinous muscle (CON); subcutaneous injection in the cervical area (SC); and subcutaneous injection in the ischiorectal fossa (SCI). Blood was sampled at 0, 12, 24, and 48 h after treatments for measurement of plasma progesterone concentrations using a validated EIA. Luteal regression was considered when progesterone decreased to less than 1.0 ng/ml. Relative changes in progesterone (0h=100%) were evaluated after PGF2a injection. Continuous and binomial data were analyzed by the MIXED AND LOGISTIC procedures of SAS (2001), respectively. CL regression for CON, SC, and SCI at 24 (65.7 vs 64.1 vs 68.6%; P=0.92) and 48 h (94.3 vs 86.8 vs 88.6; P=0.55) after treatment did not differ. Relative changes in progesterone (0 = 100%) at 12, 24, and 48h after treatment were 50.4, 34.5, and 27.7% for CON, 53.0, 33.1, and 28.4% for SC, and 52.4, 32.5, and 30.6% for SCI, and no effects of treatment (P=0.98) or treatment by time interaction (P=0.87) were observed. Injection of 25 mg of dinoprost tromethamine subcutaneously in the cervical area or ischiorectal fossa resulted similar changes in progesterone and luteal regression when compared with an i.m. injection in the semimenbranous/semitendinous muscle.

Key Words: Prostaglandin, Luteolysis, Dairy Cows

Production, Management and the Environment: Reproduction and Health Management

W240   A herd health management program resulted in decreased days open in first lactating cows in northern Thailand. K. Kreausukon*[1], V. Punyapornwitthaya[1], P. Kattapan[2], and W. Suriyasathaporn[3], [1]*Clinic of Ruminant, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai Province, Thailand*, [2]*LumPhoon Office of Department of Livestock Development, Thailand*.

The herd health management program (HH) is used to improve production performance in dairy farms, especially in Europe and North America. In northern Thailand, the HH program was introduced a few years ago by the faculty of Veterinary Medicine, Chiang Mai University. Therefore, the goal of this study was to evaluate the HH program on reproductive performance in the primiparous crosbred holstein cows. Data from 27 farms in Lamphun province during 1999 to 2003 were used to compare reproductive efficiencies before and after participating in the HH program that started in October 2001. Data on calving date, insemination date, and pregnancy check date were recorded. Calving and breeding season were divided into 3 seasons based on Thai Meteorological Department data, summer (mid Feb.–mid May), rainy (mid May-mid Oct.), and winter (mid Oct.–mid Feb.). The HH program factor was divided into two groups: before and after participating in the program. Factors associated with pregnant cows within 120 days postpartum were evaluated by Cox model. Results from 177 cows showed that median of days open of cows before and after the program were 155 and 129 days, respectively. Results from the Cox's model showed that conception rate after participating in the HH program was increased significantly (HR = 1.96). In addition, conception rate of cows inseminated durung the rainy season was lower than in summer (P = 0.07, HR = 1.82) and winter (P < 0.05, HR = 2.28). In conclusion, the HH management program improved reproductive performance of primiparous cows in northern Thailand.

Key Words: First Lactating Cows, Days Open, Cox Model

W241   Pregnancy rates and serum cortisol concentrations of relocated recipient cows in an embryo transfer program. J. L. Lopez*, E. Gonzalez, and D. L. Fernandez, *California State Polytechnic University, Pomona*.

Embryo transfer pregnancy rates are lower than pregnancy rates reported for AI or natural service, resulting in a significant economic loss to producers. Recipient cows in embryo transfer programs are routinely relocated. Relocation includes transportation, social regrouping of the animals, climate changes and dietary changes, among others, the stresses of which constitute relocation stress. Because stress affects reproduction, we hypothesized that relocation stress may play an integral role in the success rates of embryo transfer pregnancies. Recipient cows from Montana and Oregon (n=17) were relocated to California State Polytechnic University, Pomona (CPP) and cows from CPP (n=57) were used as non-relocated controls. All cows were treated identically in regards to diet, management and climate after arriving at CPP. Cows were synchronized for estrus and were implanted with Day 7 embryos 7 d after behavioral estrus. Blood samples were collected, centrifuged and the serum stored at -20°C until assayed for cortisol by RIA. The cows were palpated *per rectum* for evidence of pregnancy 60 days after implantation (Day 67) and another blood sample was collected. There was no difference (χ^2= 2.42, p > 0.10) in pregnancy rates between the control (61%) and the relocated (41%) cattle. Cortisol levels were not different (1.66 ± 0.40 vs. 1.67 ± 0.20 ngml[-1], respectively, p > 0.10) between