Physiology and Endocrinology: Estrous synchronization and detection of estrus in cattle

M230 Efficacy of $PGF_{2\alpha}$ doses to induce luteolysis on day 5, 7, or 9 of estrus cycle in nonlactating Nellore cows. Marcos V. Biehl^{*1}, Alexandre V. Pires^{1,2}, Marcos V. C. Ferraz Junior², Jose R. S. Gonçalves³, Anibal B. Nascimento¹, Marcelo H. Santos², Vinicius N. Gouvea², Alexandre A. Miszura², Leandro H. Cruppe⁴, and Michael L. Day⁴, ¹University of São Paulo, Piracicaba, São Paulo, Brazil, ²University of São Paulo, Pirassununga, São Paulo, Brazil, ³Experimental Station Hildegard Georgina Von Pritzelwitz, Londrina, Paraná, Brazil, ⁴The Ohio State University, Columbus, OH.

The aim of this preliminary study was to evaluate the luteolytic competence of different PGF2a (PGF, Lutalyse) doses on d5, d7 and d9 of the estrous cycle. Nonlactating Nellore cows (n = 97) were synchronized with the 7-d estradiol benzoate + CIDR program. Cows received Estrotect patches at CIDR removal to determine estrus response. Presence of ovulatory follicle and its disappearance were confirmed 48 and 72 h after CIDR removal, respectively. Cows detected in estrus within 48 h and with confirmed ovulation 72 h after CIDR removal remained in the study (n = 68). Cows were assigned to treatments according to BW (407.9 ± 5.1) and BCS (3.01 ± 0.02) , scale 1 to 5). One of 4 PGF doses were administered either 5, 7 or 9 d after estrus and confirmed ovulation (a single 12.5, 25 or 50 mg dose or 2 25 mg doses 8 h apart of dinoprost trometamine), in a 3×4 factorial arrangement of treatments. Presence of a corpus luteum was determined by ovarian ultrasound scan and progesterone (P4) analyses (P4 \ge 1ng/mL) on either d 5, 7, or 9 (0h) of the estrous cycle. Blood samples were collected at 0, 24, 48 and 72 h after PGF administration to assess the incidence of luteal regression (defined as concentration of P4 < 1ng/mL at 72 h after PGF). Serum P4 concentrations were quantified using a chemiluminescent immunoassay. Data were analyzed using GLIMMIX procedure of SAS. Differences in proportion of cows that experienced luteal regression were not detected for day of cycle (P = 0.19); PGF dose (P = 0.13) or their interaction (P= 0.99). The incidence of luteal regression by day of cycle was 45.0%(9/20; 5 d), 66.6% (16/24; 7 d) and 70.8% (17/24; 9 d) and for PGF dose was 42.1% (8/19; 12.5 mg), 56.2% (9/16; 25 mg) 76.4% (13/17; 50 mg) and 75.0% (12/16; 25 + 25 mg given 8 h apart). Animal numbers used in the present study limited the capacity to determine significance of numerical differences that were observed. It is interesting that luteal regression was induced in less than 50% of cows on d 5 of the estrous cycle and in cows that received 12.5 mg of PGF. Additional research is necessary to determine the optimal dose of PGF and timing during the estrous cycle to ensure luteal regression in cyclic Nellore cows.

Key Words: corpus luteum, regression, Nellore cow

M231 Addition of gonadotropin-releasing-hormone treatment at the beginning and/or at the end of an estradiol-based protocol for timed artificial insemination in Nelore (*Bos indicus*) cows. Carla Cristian Campos*, Estevão Vieira de Rezende, Mayara Oliveira, Renata de Freitas Ferreira Mohallem, and Ricarda Maria dos Santos, *Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.*

Objective was to determine the effects of adding a gonadotropinreleasing-hormone (GnRH) treatment at the beginning and/or at the end of an estradiol-based protocol for timed artificial insemination (TAI) on pregnancy per AI (P/AI) in Nelore (*Bos indicus*) cows. The experiment was conducted in 2 beef cattle farms (A and B) located in a

central-western state in Brazil. The body condition score (BCS) evaluation and the ultrasound examination to determine ovarian status (follicle diameter <10 mm; $\geq 10 \text{ mm}$; or presence of corpus luteum - CL) were performed at the beginning of TAI protocol. Cows (n = 494) were randomly assigned to 4 groups: Control (n = 126), GnRH at day zero (D0) of the protocol (n = 123), GnRH at d 10 (D10) (n = 123), and GnRH in both times (D0+D10) (n = 122). The GnRH treatment consisted of one 50 µg i.m. injection of gonadorelin. All the cows were submitted to the following TAI protocol: Day 0 = insertion of intravaginal progesterone device previously used for 8 or 16 d and 2.0 mg of estradiol benzoate (EB) i.m.; Day 8 = progesterone device withdrawal, 750 IU i.m.injection of equine chorionic gonadotropin (eCG), 1.0 mg of estradiol cypionate (ECP) i.m. and 0.265 mg of sodium cloprostenol (PGF_{2a}) i.m.; Day 10 = TAI, performed by a single inseminator. Pregnancy was diagnosed by ultrasound 39 ± 10 d after TAI. Data were analyzed by GLIMMIX procedure of SAS. Overall P/AI was 42.7%. No effect of treatment was detected (P = 0.25) on P/AI, and it was 37.3% for Control, 45.5% to GnRH D0, 49.6% to GnRH D10 and 38.5% to GnRH D0+D10. An effect of farm was detected on P/AI, where Farm A had 47.3% and Farm B 38.2% (P = 0.02). Cows with follicles <10 mm had lower P/AI (16.4%; P < 0.01) than cows with follicles $\ge 10 \text{ mm}$ (46.2%) and the ones that had a CL (48.4%). The interactions between farm and treatments (P =0.77) or between ovarian status and treatments (P = 0.12) did not affect P/AI. In conclusion, gonadotropin-releasing-hormone treatment used at the beginning and/or at the end of an estradiol-based TAI protocol did not affect pregnancy per AI in Nelore (Bos indicus) cows.

Key Words: GnRH, Nellore, timed AI

M232 Using estrus-detection patches to optimally time artificial insemination improved pregnancy rates in suckled beef cows in a timed AI program. Scott L. Hill*¹, David M. Grieger¹, K. C. Olson¹, John R. Jaeger¹, Jason K. Ahola², Mariah C. Fischer², Teresa L. Steckler³, G. Allen Bridges⁴, Jamie A. Larson⁵, Carl R. Dahlen⁶, Sarah R. Underdahl⁶, George A. Perry⁷, William D. Whittier⁸, John F. Currin⁸, Jeffrey S. Stevenson¹, ¹Kansas State University, Manhattan, KS, ²Colorado State University, Fort Collins, CO, ³University of Illinois, Dixon Springs, IL, ⁴University of Minnesota, Grand Rapids, MN, ⁵Mississippi State University, Mississippi State, MS, ⁶North Dakota State University, Fargo, ND, ⁷South Dakota State University, Brookings, SD, ⁸Virginia Tech, Blacksburg, VA.

A multiple-location study examined pregnancy rates after delaying AI in suckled beef cows from 60 to 75 h when estrus had not been detected by 60 h after a 7-d CO-Synch + progesterone insert (CIDR) timed AI (TAI) program (d 0: progesterone insert [CIDR] concurrent with injection of GnRH; d 7: prostaglandin $F_{2\alpha}$ injection and removal of CIDR; and GnRH injection at TAI [60 or 75 h after CIDR removal]). A total of 1,519 suckled beef cows at 14 locations in 8 states (CO, IL, KS, MN, MS, ND, SD, and VA) were included. Before applying the TAI program, BCS were assessed. Estrus was defined to occur when an estrus-detection patch was > 50% colored (activated). Pregnancy was determined 35 d after AI via transrectal ultrasound. Cows in estrus 60 h (n = 689; 45.6%) after CIDR removal were inseminated and injected with GnRH (control). Remaining nonestrus cows were allocated to 3 treatments: (1) GnRH injection and AI at 60 h (Early-Early = EE; n = 281), (2) GnRH injection at 60 h and AI at 75 h (Early-Delayed = ED; n

(49.8%) were more (P < 0.05) likely to become pregnant than nonestrus herd mates when they were in the DD (71.6 vs. 39.1%), ED (70.2 vs. 46.0%), and EE (53.7 vs. 41.4%) treatments, respectively. Pregnancy rates also were greater (P < 0.05) in early-calving (>76 d postpartum) than later-calving (\leq 76 d postpartum) cows (57.5 vs. 51.4%) but were not affected by parity or BCS. Use of estrus-detection patches to delay AI in cows not in estrus by 60 h after CIDR removal optimized time of

= 270), or (3) GnRH injection and AI at 75 h (Delayed-Delayed = DD; n

= 279). Binomial data were analyzed using procedure GLIMMIX. More

cows (P < 0.05) that showed estrus by 60 h conceived to AI at 60 h than

those not showing estrus (65.9 vs. 44%). Further, more (P < 0.001) cows

showing estrus by 75 h conceived to AI (64.9 vs. 38.5%) than cows not

showing estrus. Control cows had a greater ($^{abc}P < 0.05$) pregnancy rates

 $(65.9\%^{a})$ than other treatments (ED = 54.6%^b, DD = 53.0%^b, and EE =

44.0%^c). Cows not in estrus by 60 h but with activated patches by 75 h

AI and improved pregnancy rates to TAI.

M233 Treatment of primiparous lactating dairy cows with GnRH before first insemination during summer heat stress.

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Objectives were to evaluate concentrations of progesterone (P4), ovulation incidence, fertility, and insemination pattern of primiparous dairy cows treated with GnRH before a presynchronization protocol during summer heat stress. At 60 ± 3 d postpartum, primiparous cows (n = 1,352) from 3 dairies were assigned randomly to 2 treatments (d 0): receiving GnRH (Gpresynch) or no GnRH (Control). Cows were presynchronized with 2 injections of prostaglandin F_{2a} (PGF) given 14 d apart beginning on d 7. Cows detected in estrus based on tail paint removal were inseminated. Cows not detected in estrus were enrolled in a Cosynch-72 protocol on d 35 (GnRH on d 35 and 45; PGF on d 42; timed AI on d 45). Incidence of uterine health disorders and mastitis recorded by farm personnel were collected. Cows were considered diseased if 1 disease event occurred before AI. Pregnancy diagnosis was conducted 36 ± 3 d after AI by transrectal ultrasonography. In a subgroup of cows, examinations of ovarian structures were performed on d -14, 0, and 7 (n = 161) and blood samples were collected on d 0 and 7 to determine concentration of P4 (n = 167). Logistic regression analysis was used for dichotomous outcomes and continuous variables were analyzed by ANOVA. The rate at which cows were inseminated was analyzed using the Cox's proportional hazard model. Ovulation risk to GnRH treatment was greater (P < 0.01) for Gpresynch than control cows. Percentage of cows that had a corpus luteum (CL) and concentration of P4 \geq 1 ng/mL on d 7 was (P < 0.01) greater for Gpresynch cows. Concentration of P4 on d 0 (P = 0.91) and d 7 (P = 0.31) did not differ between treatments. An interaction between treatment and disease tended (P = 0.08) to affect P4 on d7 because diseased cows treated with GnRH had greater P4 than diseased control cows. Controls tended (P =0.06) to have a faster insemination rate than Gpresynch cows [adjusted hazard ratio = 1.12 (95% CI = 1.00, 1.26)]. In addition, pregnancy per AI did not differ (P = 0.25) between treatments. Although GnRH treatment increased the percentage of cows with a CL, no difference in subsequent fertility was detected.

Key Words: dairy cow, gonadotropin-releasing hormone

M234 Treatment of multiparous lactating dairy cows with GnRH before first insemination during summer heat stress. Benjamin E. Voelz*, Lucas Rocha, Filippe Scortegagna, Jeffrey S. Stevenson, and Luís G. D. Mendonça, Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.

Objectives of the experiment were to evaluate concentrations of progesterone (P4), ovulation incidence, fertility, and insemination pattern of multiparous dairy cows treated with GnRH before a presynchronization protocol during summer heat stress. At 49 ± 3 d postpartum, multiparous cows (n = 1,941) from 3 dairies were assigned randomly to 2 treatments (d 0): receiving GnRH (n = 965; Gpresynch) or no GnRH (n = 976; Control). Cows were presynchronized with 2 injections of prostaglandin $F_{2\alpha}$ (PGF) given 14 d apart beginning on d 7. Cows detected in estrus based on tail paint removal were inseminated. Cows not detected in estrus were enrolled in the Cosynch-72 protocol on d 35 (GnRH on d 35 and 45; PGF on d 42; timed AI on d 45). Incidence of uterine health disorders and mastitis recorded by farm personnel were collected. Cows were considered diseased if one disease event occurred before AI. Pregnancy diagnosis was conducted 36 ± 3 d after AI by transrectal ultrasonography. In a subgroup of cows, ultrasonography examinations of ovarian structures were performed on d-14, 0, and 7 (n = 351), and blood samples were collected on d 0 and 7 to determine P4 concentrations (n = 361). Logistic regression analysis was used for dichotomous outcomes and continuous variables were analyzed by ANOVA. The rate at which cows were inseminated or became pregnant was analyzed using the Cox's proportional hazard model. Ovulation risk to GnRH treatment was (P < 0.01) greater for Gpresynch than control cows. Percentage of cows that had a corpus luteum (P = 0.01) and P4 concentration ≥ 1 ng/mL at first PGF (P = 0.03) was greater for Gpresynch than control cows. Concentrations of P4 on d 0 (P = 0.30) and d 7 (P = 0.54) did not differ between treatments. Furthermore, insemination rate did not (P =(0.52) differ between treatments. In contrast, control cows tended (P =0.07) to become pregnant at a slower rate than Gpresynch cows [AHR = 0.81 (95% CI = 0.64, 1.02)]. Treatment with GnRH before a presynchronization protocol using PGF injections increased the percentage of cows bearing a CL at first PGF, but did not improve pregnancy per AI.

Key Words: dairy cow, gonadotropin-releasing hormone

M235 Presynchronization strategy using prostaglandin F_{2a} and GnRH to improve fertility in a resynchronization program based on detection of estrus. Lucas S. Rocha, Jeffrey S. Stevenson, and Luís G. D. Mendonça*, Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.

Objectives were to evaluate pregnancy per AI (P/AI) and pattern of insemination of 2 resynchronization protocols to maximize insemination rate based on estrus detection in lactating dairy cows. Holstein cows (n = 1,929) from 3 herds were assigned randomly to 2 presynchronization (Presynch) protocols based on their ear tag number. At non-pregnancy diagnosis (day of enrollment [d 0]), odd-numbered cows (P7GPG; n = 967) received a Presynch treatment of $PGF_{2\alpha}$ (PGF) and were enrolled in a resynchronization protocol on d 7 (GnRH on d 7 and 17; PGF on d 14; timed AI [TAI] on d 17). Even-numbered cows (P7GGPG; n = 962) received a Presynch treatment of PGF on d 0 and GnRH on d 7, and were enrolled in a resynchronization protocol on d 14 (GnRH on d 14 and 24; PGF on d 21; TAI on d 24). Detected estrus based on tail paint removal was conducted once daily during the study period and cows detected in estrus were inseminated within 1 h of detected estrus. Cows not detected in estrus by d7 (P7GPG) or d14 (P7GGPG) received their assigned TAI treatment. Cows were examined by transrectal ultrasonography to determine pregnancy status 36 d after AI. Binary outcomes were analyzed by logistic regression. The rate at which cows were inseminated was analyzed using the Cox's proportional hazard model. No treatment difference was detected for the percentage of cows inseminated based on detected estrus (72.2%). Presynch treatment (P < 0.01), TAI (P < 0.01), and the interaction between TAI and Presynch treatment (P = 0.02) affected P/AI. Cows inseminated in estrus were more likely to become pregnant than TAI cows (30.5 vs. 23.1%). No difference was detected in P/AI for cows inseminated at estrus (30.5%). In contrast, for cows receiving a TAI, P/AI was (P < 0.01) greater for P7GGPG than P7GPG cows (28.9 vs. 17.6%). Hazard of insemination was affected (P < 0.01) by treatment because P7GPG cows were inseminated sooner than P7GGPG cows (AHR = 1.70 [95% CI = 1.53 to 1.89]). Presynchronizing cows with GnRH delays AI but improves P/AI of cows submitted to a TAI protocol.

Key Words: estrus, presynchronization, resynchronization

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M236 Progesterone concentration at each treatment during an Ovsynch protocol affects fertility to timed AI in lactating Holstein cows. P. D. Carvalho*, A. H. Souza, M. C. Wiltbank, and P. M. Fricke, *Department of Dairy Science, University of Wisconsin*-

Our objective was to assess the association between progesterone (P4) concentration at each treatment of an Ovsynch protocol and pregnancies per AI (P/AI) to timed AI (TAI) in lactating Holstein cows. Data from 7,792 cows from 14 experiments in which P4 was measured during an Ovsynch protocol [GnRH (G1); 7 d PGF_{2α} (PGF); 56 h, GnRH (G2); 16 h, TAI] were analyzed. Cows receiving exogenous P4 or that received 2 injections of PGF or an increased dose of PGF were excluded from the analysis. The association between P4 during the Ovsynch protocol and P/AI to TAI was analyzed independently because P4 was not measured for all cows at every time point in all experiments. Data were analyzed by logistic regression using the GLIMMIX procedure of SAS. At G1, cows (n = 6,144) were stratified into 9 P4 categories from 0 to \geq 7 ng/mL using 0.5 ng/mL increments. Overall, P/AI differed (P < 0.01) among P4 categories at G1 with fewer P/AI for cows with P4 < 0.5 ng/mL (30%; 375/1,259) or P4 > 7.0 ng/mL (30%; 115/381) compared with cows with intermediate P4 (41%; 1,839/4,504; calculated relative decrease in P/AI of 27%; 41-30 = 11/41 = 27%). At PGF, cows (n = 3,383) were stratified into 9 P4 categories from 0 to \geq 8 ng/mL using 1.0 ng/mL increments. Overall, P/AI differed (P < 0.01) among P4 categories at PGF with a 51% relative decrease in P/AI for cows with P4 < 1.0 ng/mL (21%; 140/681) compared with cows with P4 > 1.0 ng/mL (43%; 1,151/2,702). At G2, cows (n = 3,148) were stratified into 8 P4 categories from 0 to ≥ 0.7 ng/ mL using 0.1 ng/mL increments. Overall, P/AI differed (P < 0.01) among P4 categories at G2 with a 66% relative decrease in P/AI for cows with P4 > 0.4 ng/mL (14%; 61/435) compared with cows with P4 < 0.4 ng/mL (41%; 1,125/2,713). Based on this large data set, suboptimal P4 concentrations could be identified at G1 in 26% of cows (26% lower P/AI), at PGF in 21% of cows (51% lower P/AI), and at G2 in 14% of cows (66% lower P/AI). We conclude that achieving optimal P4 during an Ovsynch protocol may allow for a dramatic increase in fertility in lactating dairy cows. Supported by USDA NIFA Hatch project 23144.

Key Words: progesterone, fertility, Ovsynch

M237 Timed AI improves reproductive performance and reduces cost per pregnancy in dairy heifers. Thiago V. Silva¹, Fabio S. Lima², William W. Thatcher¹, and Jose E. P. Santos^{*1}, ¹University of Florida, Gainesville, FL, ²University of Illinois, Urbana, IL.

Objectives were to evaluate the effects of implementing timed AI for first insemination on reproduction and cost per pregnancy in dairy heifers.

Non-pregnant Holstein heifers (n = 611) at 400 d of age from 3 farms were allocated randomly to AI after detected estrus (Control, n = 306) or to timed AI for first AI (TAI, n = 305) followed by detection of estrus. Heifers in TAI were enrolled in the 5-d timed AI protocol on study d -6 [d -6, GnRH and an intravaginal progesterone insert; d -1, PGF_{2a} and insert removal; d 0, PGF_{2a}; d 2, GnRH + AI). Starting on study d 0, heifers had tailheads painted daily with chalk for detection of estrus. Control heifers received PGF_{2a} if not inseminated within 7 d of moving to the breeding pen. The study lasted 84 d to allow 4 21-d estrous cycle periods of breeding. A herd budget was created to determine cost per pregnancy. Data were analyzed by ANOVA, logistic regression, and the Cox's proportional hazard model using the MIXED, GLIMMIX, and PHREG procedures of SAS and models included the effects of treatment, farm, and treatment by farm interaction. The interval to first AI was 8 d shorter (P < 0.01; 10 vs. 2 d) and the 21-d cycle insemination rate was greater (P < 0.01) for TAI than Control heifers (82.4 vs. 91.4%). Pregnancy at first AI (PAI; Control = 58.3% vs. TAI = 62.8%) and all AI (Control = 56.0 vs. TAI = 57.4%) did not differ (P > 0.15) between treatments. Implementing timed AI increased (P < 0.01) the 21-d cycle pregnancy rate from 47.9% in Controls to 57.2% in TAI heifers and reduced (P < 0.01) median d to pregnancy (Control = 14.0 vs. TAI = 2.0). In fact, the hazard of pregnancy improved (P < 0.01) 60% for TAI than Control heifers (adjusted HR = 1.60; 95% CI = 1.35-1.89), resulting in increased (P < 0.01) proportion of pregnant heifers by 84 d of breeding (Control = 85.2 vs. TAI = 91.5%). The cost per pregnancy for was \$17.16 less expensive (P < 0.01) for TAI than Control heifers. Implementing timed AI for first service followed by detection of estrus improved reproductive performance and reduced the cost per pregnancy when compared with heifers managed under detected estrus only.

Key Words: dairy heifer, timed AI, reproduction

M238 Addition of a second dose of prostaglandin F_{2a} to a fixed-time AI protocol improves fertility of anestrous dairy cows. Francisco R. Lopes Jr.*, Marcos H. C. Pereira, Anderson K. Munhoz, and José L. M. Vasconcelos, *Sao Paulo State University, Botucatu, SP, Brazil.*

This study was conducted to evaluate if addition of a second prostaglandin (PG) F_{2a} dose improves fertility of anestrous lactating Holstein cows submitted to an estradiol/progesterone (P4) based timed AI (TAI) protocol. Ovaries were scanned by ultrasound to determine if a corpus luteum (CL) was present at the protocol initiation (d-11) and on the day of PGF_{2a} (d -4). Cows without CL on d -11 and d -4 were classified as anestrous (n = 436). Anestrous cows were submitted to the TAI protocol: d-11: 2 intravaginal P4 devices (1.9g P4, CIDR, Zoetis) and 2.0 mg of estradiol benzoate (EB) i.m. (Estrogin, Farmavet); d-4, 25mg of PGF2a i.m. (Lutalyse, Zoetis) and withdrawal of one CIDR; d-2 withdrawal of the second CIDR and 1.0 mg i.m. of estradiol cypionate (ECP, Zoetis); on d 0 TAI. On d-4, cows were randomly assigned to 1 of 2 treatments: one dose of PGF_{2a} on d -4 (1PGF) or 2 doses of PGF_{2a} (2PGF), the first on d-4 and the second on d-2. Rectal temperature was measured on the day of TAI and 7 d later. The average rectal temperature was calculated and the cows were divided into 2 groups: without hyperthermia (<39.1°C) or with hyperthermia (≥39.1°C). Pregnancy was diagnosed 60 d after AI. Binomial variables were analyzed using the GLIMMIX and continuous using the MIXED procedures of SAS. The 2PGF treatment tended to improve the synchronization rate (80.2% [n = 218] vs. 72.4%)[n = 218] P = 0.07). An interaction (P = 0.05) between treatment and hyperthermia was observed for pregnancy per AI (P/AI) on d 60. For hyperthermic cows, P/AI were 9.1% (n = 123) and 9.4% for 1PGF and 2PGF, respectively. For cows without hyperthermia, P/AI were 16.3%

(n = 95) and 30% (n = 88) in 1PGF and 2PGF, respectively. When only ovulated cows were included in the analysis, then an interaction (P = 0.05) existed between treatment and hyperthermia on P/AI at 60 d. For cows with hyperthermia, P/AI were 13.8% (n = 80) and 12.1% (n = 100) in 1PGF and 2PGF, respectively. For cows not suffering of hyperthermia, P/AI were 19.4% (n = 78) and 34.8% (n = 75) in 1PGF and 2PGF, respectively. The addition of a second PGF dose during the TAI protocol increased fertility in anestrous dairy cows without hyperthermia on the first 7 d after AI.

Key Words: anestrous, $PGF_{2\alpha}$, TAI

M239 Effect of a second treatment with prostaglandin $F_{2\alpha}$ during the Ovsynch protocol on pregnancy to the timed AI.

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Lack of complete regression of the corpus luteum (CL) after prostaglandin $F_{2\alpha}$ (PGF) treatment can reduce fertility during timed AI (TAI) protocols. The main objective of this study was to evaluate the effect of a second treatment with PGF during the Ovsynch protocol on fertility to the timed AI (TAI). A total of 2,148 lactating dairy cows were used on 11 dairy farms in 4 different regions of the USA, 3 in WI, 3 in CA, 2 in NY, one in PA, one in NM, and one in TX. Cows were synchronized with Ovsynch (GnRH [Gonadorelin acetate; 100 µg/ mL; GONAbreed, Parnell]-7d-PGF[Cloprostenol sodium; 250 µg/mL; estroPLAN; Parnell]-56h-GnRH-16h-TAI) for TAI at 50 ± 3 DIM. The cows were randomized to receive: (1) No additional treatments with PGF = Ovsynch, or (2) A second PGF treatment at 24 h after the first PGF treatment = PROCEPT. Statistical analyses were performed using PROC Logistic in SAS, with treatment and parity as factors. In the overall logistic regression model there was no significant effect of Farm (P = 0.712); although, treatment effects on individual farms ranged from positive (P = 0.01) to a tendency for a negative effect (P = 0.12). There was also no interaction of parity and treatment in the overall model (P = 0.741). Overall, there was a tendency (P = 0.068) for an effect of treatment on P/AI with an increase from 33.3% in Ovsynch compared with 36.1% in PROCEPT (2.8% absolute increase; 8.5% relative increase [2.8/33.3]). There was no effect of treatment in primiparous cows (37.1% [99/267] vs. 38.2% [99/259]; Ovsynch vs. PROCEPT; P = 0.393). However, there was a tendency for an effect of treatment in multiparous cows (32.0% [259/809] vs. 35.4% [288/813]; 10.7% relative increase; P = 0.073). When multiparous cows were evaluated by parity, it was found that there was an effect of treatment in cows of second and third lactation (P = 0.047) but no effect of treatment in older cows (P = 0.517). Thus, treatment with a second PGF during the Ovsynch protocol tends to increase pregnancies per TAI producing about 10% more pregnancies per TAI in multiparous cows, with a significant increase in second and third lactation cows.

Key Words: Ovsynch, prostaglandin $F_{2\alpha}$, fertility

M240 Efficacy of different fixed-time AI protocols using GnRH, estradiol, and progesterone in lactating dairy cows.

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This study compared the reproductive efficiency of dairy cows submitted to fixed-time AI (FTAI) protocols using GnRH and/or different esters of estradiol. A total of 1,029 lactating Holstein cows (358 primiparous and 671 multiparous; BCS 2.99 ± 0.01 , 159.8 ± 3.4 DIM; 36.4 ± 0.29 kg/d of milk; LS means ± SEM) were housed in freestall barns and randomly assigned to 1 of 4 treatment groups using a completely randomized design with a 2×2 factorial arrangements of treatments. Cows were assigned to 1 of 2 treatments at the beginning of the FTAI protocol (GnRH [G] or estradiol benzoate (EB]) and 1 of 2 treatments at the end of the protocol (EB or estradiol cypionate [ECP]), resulting in: G-EB (n = 263), G-ECP (n = 277), EB-EB (n = 259) and EB-ECP (n = 230). All cows received an intravaginal progesterone (P4) implant at the start of the protocol (d 0) and it was removed on d 8. All cows also received PGF_{2 α} (500 µg) on d 7 and d 8. At the end of the protocol, treatment with ECP was on d 8 or treatment with EB was on d 9 with FTAI on d 10. Statistical analyses were performed using Proc GLIM-MIX of SAS 9.3. There were no interactions between treatments on pregnancies per AI (P/AI) at 30 and 60 d pregnancy diagnoses (P >0.05). At 30 d, there was no effect of EB or GnRH at start of protocol (33.9 vs. 37.0%; P = 0.30) and no effect of treatment at end of protocol (33.1 vs. 37.9%; EB vs. ECP; P = 0.11). At 60 d, there were no treatment effects at start (29.8 vs. 32.2%; EB vs. GnRH; P = 0.43) or end (30.3 vs. 31.7%; EB vs. ECP; P = 0.63) of the protocol. For pregnancy loss (d 30 to 60 of pregnancy), there were no interactions (P > 0.05) and no effect of treatments at start (P = 0.72), however, there was an effect of treatment at end (8.6 vs. 15.7%; EB vs. ECP; P = 0.04) of the protocol. Independent of treatments, DIM tended to affect P/AI at 30 d (P = 0.08) and at 60 d (34.8 vs. 27.4%, low vs. high DIM; P = 0.01), but did not affect pregnancy loss (P = 0.05). We concluded that in lactating Holstein cows, the use of EB or GnRH at the beginning or EB or ECP at the end of the protocol provided same P/AI at 30 and 60 d after FTAI, despite that ECP, at the end of protocol, provided greater embryonic/fetal loss. Also, cows with less DIM had greater P/AI at 60 d. Thanks to CNPq, FAPESP, CAPES and VALLÉE.

Key Words: fertility, synchronization.

M241 The use of a modified 5-day CIDR-Cosynch protocol for resynchronization in lactating dairy cows. J. A. Spencer^{*1}, K. G. Carnahan¹, B. Shafii¹, C. Claypool¹, J. C. Dalton², and A. Ahmadza-deh¹, ¹University of Idaho, Moscow, ID, ²University of Idaho, Caldwell, ID.

Approximately 60% of lactating Holstein dairy cows fail to conceive to the first artificial insemination (AI) and are required reinsemination. Resynchronization facilitates timely reinsemination; however, pregnancy rates to the second AI (PR/AI) from resynchronization protocols have been inconsistent. The development of an effective resynchronization protocol is needed to improve PR/AI to second AI. The objective of this study was to determine the effect of the initial GnRH on PR/AI using a 5-d CIDR-Cosynch resynchronization protocol for second AI in lactating Holstein dairy cows. Approximately 37 d after first AI and upon non-pregnancy diagnosis, cows eligible for second AI (n = 429) were subjected into a modified 5-d CIDR-Cosynch protocol. On d 0, all cows received CIDR inserts and were assigned randomly to oe one of 2 treatments, initial GnRH (100 μ g; n = 226) or no GnRH (n = 203). On d 5, CIDR inserts were removed and all cows received one injection of $PGF_{2\alpha}$ (500 µg). From d 5 to 7, cows were monitored daily for estrus. Animals detected in estrus on d 6 or 7 received AI and were not given GnRH. On d 8, all cows not identified in estrus on d 6 or 7 were timed AI (TAI) and receieved GnRH. Blood samples were collected from cows (n = 184) on d 0 and analyzed for progesterone (P_4) concentration. Logistic

regression was used to examine the main effects of treatment, day of AI, sire, parity and all 2-way interactions with treatment on PR/AI. There were no effects of treatment (no GnRH, 27.1% vs. initial GnRH, 21.2%) on PR/AI (P > 0.10). There were also no effects of method of AI (based on estrus vs. TAI), sire (n = 10), or any 2-way interactions with treatment on overall PR/AI. There was a significant difference (P < 0.05) between primiparous (n = 119) and multiparous cows (n = 310) in PR/AI (31% vs. 21%). Progesterone concentrations (n = 184) were categorized into high (≥ 1 ng/mL) and low (<1 ng/mL) groups. Overall, PR/AI tended (P = 0.09) to be greater for high P₄ (n = 136) compared with low P₄ (n = 48) (26% vs. 16%). These results provide evidence that the initial GnRH injection in a 5-d CIDR-Cosynch resynchronization program may not be necessary to achieve a similar PR/AI following second AI.

Key Words: dairy cows, resynchronization, 5-d CIDR-Cosynch

M242 Assessment of an application for mobile devices developed to evaluate the AI procedure. Santiago Bas* and Gustavo M. Schuenemann, Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Improving reproductive performance in dairy cattle is paramount to maximize dairy productivity and sustainability. Artificial insemination (AI) is the most common breeding practice for dairy cattle in the US and worldwide. It is common to observe large within- and among-herd variation in conception rate and performance of AI technicians. The objective was to assess a user-friendly application (APP) for mobile devices developed to allow the evaluation of the AI procedure under field conditions. The APP was designed to collect information during the AI procedure to allow the identification of breeding practices (e.g., semen handling, breeding procedures) that may affect reproductive outcomes. In addition, the APP generates a database for benchmarking metrics associated with performance of AI technicians. Novel components of the APP include: (1) login screen for individual users, (2) capture of farm information (e.g., type of facility, herd size), (3) sire identification, (4) semen used (sexed or conventional), (5) breeding strategies (e.g., estrous detection, synchronization programs), (6) animals inseminated (e.g., cows, replacement heifers), and (7) recording of the following metrics: number of semen straws simultaneously thawed, thawing time, AI catheter loading time, time from loading of AI catheter to the cow, time to AI, and time elapsed for each round of insemination. In addition, information is summarized to generate an electronic report, and all captured data are uploaded into a cloud database. The APP features a user-friendly interface for entering and managing data, and accurately (100%) captured, summarized and integrated multiple events associated with the AI procedure under field conditions.

Key Words: dairy, app, artificial insemination

M243 Estradiol and GnRH on ovulation induction in crossbred cows estrus synchronized. Fabrício Albani Oliveira, Jurandy Mauro Penitente Filho, Erly Luisana Triana Carrascal, Carlos Thiago Oliveira, Adriana Moreira Zolini, Italo Augusto da Costa Soares, and Ciro Alexandre Alves Torres*, *Federal University of Viçosa, Viçosa, MG, Brazil.*

Three different ovulation inductors, estradiol benzoate (EB), estradiol cypionate (EC) and gonadotropin releasing hormone (GnRH) were evaluated in timed artificial insemination (TAI) of lactating crossbred cows. Two hundred and 16 (216) cows were used in 2 experiments. In experiment I (n = 45), follicular dynamics and in the experiment II (n = 45)

= 171) pregnancy per AI were evaluated. All cows were submitted to a synchronization protocol: d 0 (D0) insertion of a progesterone-release intravaginal device (PRID) plus IM injection of EB 2 mg; D8, PRID removal plus IM injection of PGF_{2a} (0.150 mg) and eCG (400 IU). The cows were then randomly assigned into 1 of 3 groups: (1) EB9 (n = 61): IM injection of EB (1 mg) on D9, and AI 52 h after PRID removal; (2) EC (n = 58): IM injection of EC (1.0 mg) on D8 and AI 48 h after PRID removal and (3) GnRH (n = 52): IM injection of GnRH (100 µg) on D10 and AI 52 h after PRID removal. Ultrasonographic examinations of the ovaries were performed on d 0, 8, and on the day of TAI to determine follicular diameter and ovulation. Follicular data were analyzed using ANOVA and pregnancy per AI with Chi-squared test at 5% probability. No interaction was found between treatment and observation day on number of follicles or on the follicular classification (P > 0.05). The intervals from TAI to ovulation were 12.4, 12.8 and 14.8 for EB, EC and GnRH, respectively (P > 0.05). No interaction between treatment and day of observation was detected for diameter of the largest follicle (11.4, 11.0 and 10.7 mm for EB, EC and GnRH, respectively; P > 0.05). The rate of follicle growth was slower in the EC cows than the other treatments (EB = 1.5, EC = 0.7 and GnRH = 1.9 mm; P < 0.05). There were no effects (P > 0.05) of treatment on ovulation rate (EB = 100, EC = 93.3, GnRH = 93.3%) and pregnancy per AI (EB = 62.3, EC = 37.9, GnRH = 51.9%). Sample size might have limited the ability to detect statistical differences among treatments. The use of EB, EC, and GnRH as ovulation inductors in TAI protocols in crossbred cows resulted in no differences in ovulatory responses or pregnancy per AI.

Key Words: ovulation, crossbred cows, timed AI

M244 Effect of estrous synchronization program, season, body condition score and ovarian status on pregnancy rate to fixed-time AI in beef cows. Federico Randi*^{1,2}, Mervyn Parr², Peter Doolan², Michael G. Diskin², Alessio Valenza³, Pedro Rodriguez³, Pat Lonergan¹, and David A. Kenny², ¹University College Dublin, Dublin, Ireland, ²AGRIC Teagasc Grange, Dunsany, Meath, Ireland, ³CEVA Sante Animale, Libourne, France.

Reproductive efficiency in the Irish beef cow population is challenged by age at first calving, long calving intervals and limited AI usage. Low average herd size and often part-time nature of beef production increases the challenge of detecting cows in estrus. The objective was to establish an estrous synchronization regimen to facilitate fixed-time AI (TAI) in beef cows under Irish conditions. A total of 1410 suckled cows located on 61 farms were enrolled across 2 replicates (Spring, n = 703 and Autumn, n = 707). Presence of a corpus luteum (CL) and body condition score (BCS) were recorded at treatment initiation. Following a voluntary waiting period of 35 d postpartum, cows were assigned to (1)receive progesterone-releasing intravaginal device (PRID Delta) on d 0 and PRID removal on d 7 with an 25 mg injection of $PF2\alpha$ (Enzaprost) (Group 1); (2) same as Group 1, but cows received a 100 ug of GnRH analog (Ovarelin) on d 0 (Group 2); (3) same as Group 2, but cows received 400 IU equine chorionic gonadotropin (eCG) (Syncrostim) on d7; (Group 3). All cows received GnRH at TAI 72 h after PRID removal with pregnancy diagnosis by transrectal ultrasonography 35-40 d later. Data were analyzed using the GENMOD procedure of SAS. Presence of a CL (Spring 50.6%; Autumn 70.0%) increased pregnancy rate independent of treatment and season (P = 0.03). Mean BCS in Spring and Autumn was not different but BCS positively affected pregnancy rate independent of treatment or season (P = 0.003). There was a significant treatment \times season interaction for pregnancy rate (P = 0.0002). In spring, overall pregnancy rate was 59.1% (416/703) and was affected by treatment (49.6 vs. 59.3 vs. 68.5%, for Groups 1, 2 and 3, respectively P <

0.05). In contrast, in autumn, overall pregnancy rate (52.6%, 364/707) was unaffected by treatment (53.7 v 52.0 v 48.7%, respectively). In conclusion, GnRH at the initiation of a TAI synchronization protocol and eCG both increased pregnancy rate in spring-calving suckled beef cows. This effect was not evident in autumn-calving cows. Seasonal differences in outcome may reflect differences in management (grazing vs. confinement), days postpartum or breed type and remain to be elucidated.

Key Words: bovine, fertility, synchronization

M245 Body weight loss of cows early postpartum is associated with negative effects on estrous expression. Tracy A. Burnett*, Muhammad A. Khan, Marina A. G. von Keyserlingk, and Ronaldo L. A. Cerri, *University of British Columbia, Vancouver, BC, Canada.*

The aim of this study was to determine the association of weight loss during the transition period with the expression of estrus. Sixty-seven Holstein cows were enrolled onto the study 2 wk before parturition. Body weight (BW) was determined 1 and 2 wk before expected calving date and again 5 wk postpartum. Beta-hydroxybutyrate (BHBA) and glucose concentrations were analyzed 2 wk postpartum. Cows were equipped with collar-mounted activity monitors (Heatime, SCR Engineering, Israel). Estrous expression was measured by an automated activity monitor (AAM) and quantified using 2 parameters: 1) peak activity and 2) duration of the estrus episode. Peak activity was defined as the maximum activity index during an estrus episode; the threshold activity was set at an index of 35 (an 80% increase relative to baseline). The duration of an estrus episode was calculated as the amount of consecutive time whereby the index level was greater than 35. Loss of BW from 2 wk prepartum until 5 wk postpartum was determined for all cows and used to retrospectively group them: (1) those that lost less than or equal to 100 kg of BW (Low; n = 46), and (2) BW loss more than 100 kg (High; n = 21). Data were analyzed by ANOVA using the MIXED procedure of SAS. Increased BW loss was associated with decreased estrous expression as measured with an AAM. Animals within the High group had decreased peak activity (63.6 ± 3.0 vs. 74.0 ± 3.9 peak activity index; P = 0.04) and decreased duration of estrus (8.4 ± 0.7 vs. 11.5 ± 0.9 h; P <0.01). Ovulatory follicle diameter and estradiol concentration in plasma were unaffected. Additionally, cow within the High group had increased BHBA concentrations $(0.57 \pm 0.12 \text{ and } 0.90 \pm 0.10 \text{ mmol/L}; P = 0.03)$ and reduced glucose concentrations $(48.3 \pm 1.5 \text{ vs. } 53.8 \pm 1.6 \text{ mmol/L};$ P = 0.02) at 2 wk postpartum. Also, the High group had increased days open in comparison to the Low group $(163 \pm 13 \text{ vs. } 113 \pm 17 \text{ d open}; P$ = 0.03). In conclusion, excessive BW loss early postpartum has negative effects on estrous expression as measured by an AAM and is associated with increased days open.

Key Words: activity monitor, body weight loss, estrus

M246 Automated detection of estrus using multiple commercial precision dairy farming technologies in synchronized dairy cows. L. M. Mayo*, W. J. Silvia, G. Heersche, I. C. Tsai, B. A. Wadsworth, A. E. Stone, and J. M. Bewley, *Department of Animal* and Food Sciences, University of Kentucky, Lexington, KY.

The objective of this study was to evaluate precision dairy farming technologies (PDFT) for estrous detection. Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus) beginning 45–85 DIM. Resumption of ovarian cyclicity at enrollment, presence of a corpus luteum (CL) on the day of the final injection (designated experimental d 0), regression of the CL by d 5, and presence of

a new CL on d 11 were verified by transrectal ultrasonography. Cows were observed for estrous behaviors for 30 min, $4 \times$ per day, on d 2 to 5. Blood samples were collected on d -2, -1, 0, 1, 2, 5, 7, 9, and 11 to quantify progesterone to verify luteal regression and ovulation. Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/mL on days -2, -1 and 0, <1.0 ng/mL on d 2 and > 1.0 ng/ml on d 9 and 11). Eighteen cows followed this pattern. Cows that failed to follow the pattern served as negative controls (n = 6). Detection of estrus by PDFTs, an estrous behavioral scoring system, and by visual observation of standing estrus were compared with the gold standard (Table 1). Sensitivity and specificity for detection of estrus was similar among all PDFT. Only 56% of cows that ovulated were observed standing by visual estrous detection. All systems tested are capable of detecting estrus at least as effectively as visual observation.

Table 1 (Abstr. M246). Detection of estrus using alerts generated by PDFT¹ and visual observation (n = 24)

Detection method	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)
Afimilk AfiAct Pedometer Plus	16	0	6	2	89	100
GEA CowScout (leg version)	15	0	6	3	83	100
ENGS Track A Cow	14	0	6	4	78	100
Agis SensoOr	14	0	6	4	78	100
Estrus behavioral score ²	12	1	5	6	67	83
Standing behavior	10	0	6	8	56	100

¹Sensitivity = TP/(TP + FN) and specificity = TN/(TN + FP); where TP = true positive, TN = true negative, FP = false positive, and FN = false negative. ²Scoring system as defined by Van Eerdenburg et al. (1996).

Key Words: automated estrus detection, precision dairy technology, estrous behavior

M247 Using the Draminski Estrous Detector in the dry cow to measure electrical resistance of vaginal mucus as it relates to animal factors pre- and postcalving. Caitlin L. Widener*, William M. Graves, and Jillian F. Bohlen, *University of Georgia, Athens, GA*.

Poor uterine health can be detrimental to a fresh cow's ability to efficiently re-breed. This 2-phase study aimed to establish changes in vaginal mucus resistance as it relates to calving date and uterine health post-calving. In phase one, nulliparous heifers (n = 11) and multiparous cows (n = 21) were examined approximately 2 weeks before and the week of calving with a device designed to measure changes in electrical resistance in ohms (Ω) of cervical/cranial vaginal mucus (Draminski Estrous Detector, Poland). Three consecutive readings were taken at each time point with the average being utilized for analysis. At time of Draminski readings, urine pH, rectal temperatures, body condition scores and udder edema levels were recorded. Increasing blood-flow and uterine contractions associated before calving as the result of estrogen may change the deposition of circulating minerals in mucus, as indicated by electrical resistance, which may have an impact on the uterine environment post-calving. In phase 2, 10 heifers and 14 cows from the original groups were assessed for incidence of metritis on d 7 and 14 (\pm 3 d) post-calving. Vaginal mucus was visually examined using the Metricheck (Simcro, New Zealand) and subjectively scored by one individual (0-clear, 1-up to 25% white or off white pus, 2-25-75% pus, 3 - 75% pus). Draminski readings averaged $183 \pm 28.8\Omega$ and $184 \pm 42.8\Omega$ for the first and second readings, respectively. The Draminski readings did not consistently increase (n = 12) or decrease (n = 15) relative to calving date. Precalving data were analyzed using the PROC GLM procedure of SAS for their relationship to postcalving mucous scores. The first model included overall increase or decrease in Draminski readings relative to calving, rectal temperature and body condition score with group (heifer or cow) held constant. The second model included the first or second Draminski reading with group held constant. There was no association between actual Draminski readings (P > 0.05) or changes in Draminski readings over time (P > 0.05) and the Metricheck scores postcalving. The lack of statistical associations was likely the result of high variability in Draminski readings.

Key Words: electrical resistance, metritis, periparturient

M248 Estrus lying behavior of Holstein cows: Risk factors for estrus expression, ovulation risk and pregnancy per AI. Bruna F. Silper*¹, Augusto M. L. Madureira², Liam B. Polsky¹, Eraldo L. Drago Filho², José L. M. Vasconcelos², and Ronaldo L. A. Cerri¹, ¹Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, ²Faculdade de Medicina Veterinária e Zootecnia, UNESP, Botucatu, SP, Brazil.

Objectives were (1) to quantify lying behavior (LB) changes around estrus, and (2) to assess risk factors for reduced changes in LB during estrus, ovulation risk and pregnancy per AI (P/AI). Holstein cows (n = 1,039; 45.6 \pm 10.7 kg milk/d) were fitted with pedometers; 1,179 estrus episodes were evaluated. LB measurements were bout frequency (BOUT), total lying time/d (TOTAL), average lying bout duration (AVG), and ratio of lying/standing time (RATIO). Relative changes from d-7 to d-1 (d of estrus) and from d-7 to d+1 were calculated. Thresholds for high intensity estrus were set as a decrease in BOUT >25% and in TOTAL and RATIO >22%. AVG did not change with estrus. Descriptive statistics and logistic regressions were used to analyze the data. At d -1 BOUT, TOTAL, and RATIO were, respectively, $19 \pm 34\%$ $(-3 \pm 5 \text{ bouts/d})$, $20 \pm 30\%$ $(-145 \pm 190 \text{ min/d})$, and $21 \pm 26\%$ $(-11 \pm 100 \text{ min/d})$ 21%) lower than at d -7. Major risk factors associated with reduced estrus expression were d 0 progesterone (P4) concentration, season and parity. Cows with P4 < 0.2 ng/mL were twice as likely to have high intensity estrus (large decrease in LB from d-7 to d-1). High intensity estrus increased ovulation rate (OR = 2.7 [BOUT], 4.6 [TOTAL], 4.8 [RATIO]). Cold season also increased ovulation rate (OR = 1.8 [BOUT] and 1.9 [TOTAL and RATIO]). BOUT, TOTAL, and RATIO tended to affect P/AI at 32 and 60 d, but parity, BCS, and season were more important predictors. Interestingly, TOTAL, RATIO, and AVG were greater at d +1 than at d -7 by $8 \pm 30\%$ (TOTAL), $10 \pm 21\%$ (RATIO), and $13 \pm 36\%$ (AVG). When d +1 TOTAL and RATIO were $\geq 10\%$ d -7values, there was greater likelihood of ovulation (OR = 2.6 [TOTAL] and 2.4 [RATIO]; 92% vs. 81%). P/AI at 32 d was 1.5 and 1.6 times greater for episodes followed by high d +1 TOTAL and RATIO (38% vs. 27%). BOUT, TOTAL and RATIO could improve the use of information from activity monitors (e.g.: increased estrus detection, fertility prediction). Relationships between P4 at AI, estrus behavior, ovulation and conception should be further investigated.

Key Words: estrus, lying behavior, progesterone

M249 Characterization of ovarian function in nonpregnant previously inseminated lactating dairy cows. Robert Wijma*, Matias L. Stangaferro, and Julio O. Giordano, *Department of Animal Science, Cornell University, Ithaca, NY.*

Our objective was to characterize ovarian dynamics in lactating dairy cows diagnosed nonpregnant after AI focusing on time points when resynchronization programs are initiated or PGF2 α can be used to induce estrus expression. After synchronization with the Presynch-Ovsynch

(PGF-14d-PGF-12d-GnRH-7d-PGF56h-GnRH-16h-TAI) protocol cows (n = 64) received timed-AI. Blood was collected every 48 h from 14 to 42 d after AI to determine concentrations of progesterone (P4) and estradiol (E2). Daily, ovarian ultrasound (US) was used to determine size and location of all ovarian structures. Retrospectively, cows were classified as pregnant (n = 25; PG) when a viable embryo was observed (excluded from analysis) or nonpregnant (n = 39; NP) when no viable embryo was observed. A cow was considered to have an active CL (ACL) when P4 > 1 ng/mL and \geq 1 CL was observed. Also, a CL was considered mature (MCL) starting 7 d after ovulation based on the timing when the CL becomes sensitive to PGF2a. Follicles were considered active (AFOL) when reached 10 mm until 2 consecutive days of reduction in diameter. Periods evaluated were: 24(D24), 32(D32), and 38(D38) d after AI. Parameters evaluated were cows with ACL, MCL, and an AFOL by day, E2 concentrations and E2 to P4 ratio. Quantitative data were analyzed by ANOVA using PROC MIXED whereas binary data were analyzed by logistical regression using PROC GLIMMIX of SAS. More (P < 0.01) cows had an ACL on D32 (82.1%) and D38 (84.2%) than on D24 (35.9%). The greatest (P < 0.01) proportion of cows with a MCL was for D38 (84.6%) followed by D32 (56.4%) and least for D24 (28.2%). The proportion of cows with an AFOL tended (P = 0.06) to be affected by day with 79.5, 92.3, and 97.4% of cows with an AFOL for D24, D32, and D38, respectively. Concentrations of E2 were greater (P = 0.05) for cows with an AFOL $(1.1 \pm 0.3 \text{ pg/mL})$ vs cows without AFOL $(0.5 \pm 0.1 \text{ pg/mL})$ across all days. Concentrations of E2 were affected by day because on D24 (1.3 \pm 0.2 pg/mL) E2 was greater (P < 0.05) than for D32 (0.7 \pm 0.2 pg/mL) whereas on D38 (1.0 \pm 0.2 pg/ mL) E2 was intermediate. We conclude that there is more variation in ovarian function than expected based on the number of days after AI in nonpregnant previously inseminated cows. Supported by Hatch project NYC-127813.

Key Words: corpus luteum, follicle, dairy cow

M250 The effects of ovulatory status of the dominant follicle and spatial relationship of the corpus luteum on diameter and average growth rate of that dominant follicle. Ashleigh M. Muth-Spurlock*, Garrett F. Cline, Caleb O. Lemley, and Jamie E. Larson, *Mississippi State University, Mississippi State, MS.*

The aim of this study was to investigate the effects of ovulatory status of the dominant follicle (whether the follicle eventually ovulated or not) and spatial relationship of the corpus luteum (CL; contralateral or ipsilateral to the dominant follicle) on the diameter and average growth rate of that dominant follicle. Sixteen non-pregnant, multiparous beef cows were observed daily for estrus. Cows were randomly assigned to 1 of 2 treatments, daily ultrasound of: (1) the dominant anovulatory (AN) follicle (1st follicular wave), or (2) the ovulatory (OV) follicle (2nd or 3rd follicular wave). Ultrasonography exams were initiated on d 1 (AN treatment group) or on d 7 (OV treatment group) of the estrous cycle. Exams ended after 5 d of follicular dominance. Follicle diameter and whether the CL was ipsilateral or contralateral to the dominant follicle were recorded. The MIXED procedure of SAS was used for data analysis, with d of dominance (0 to 4) as a repeated measure; LSMeans and pooled SEM are reported. There was a treatment × spatial relationship interaction (P = 0.056) when evaluating average growth rate of the dominant follicle. Anovulatory follicles contralateral to the CL grew faster $(1.137 \pm 0.045 \text{ mm/d})$ than both ovulatory follicles contralateral to the CL $(0.954 \pm 0.036 \text{ mm/d})$ or ovulatory follicles ipsilateral to the CL $(0.996 \pm 0.029 \text{ mm/d})$. However, average growth rate of the dominant follicle was less (P = 0.041) in cows in the OV group compared with cows in the AN group $(0.975 \pm 0.023 \text{ and } 1.07 \pm 0.040 \text{ mm/d}, \text{ respec-}$

tively). When evaluating diameter of the dominant follicle over the 5 d of dominance, treatment, spatial relationship of the CL, d of dominance, and treatment \times spatial relationship all affected diameter of the dominant follicle. However, when only evaluating the last d of dominance, these parameters did not significantly affect diameter of the dominant

follicle. In conclusion, additional research is necessary to understand the interactions between future ovulatory status of a dominant follicle and spatial relationship of the CL.

Key Words: corpus luteum, follicle diameter, follicle growth rate