

Artificial Insemination

7 HETEROSPERMIC INSEMINATION AT TWO SPERM CONCENTRATIONS IN TIMED AI: CASA SEMEN PARAMETERS AND PREGNANCY RATES

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The latest entry in the field of semen evaluation is computer assisted semen analysis (CASA). Heterospermic insemination has been used to increase pregnancy rates from low fertile bulls. The objective of this study was to evaluate, with the aid of CASA, heterospermic semen characteristics and pregnancy rates using different concentrations of bull semen in a timed artificial insemination protocol. Semen was collected from two bulls of known fertility by artificial vagina and all CASA motility parameters were evaluated individually and combined. Straws were filled using a semi-defined semen extender (Andromed, Minitüb, Tiefenbach, Germany) as follows: single bull A and single bull B (12×10^6 of progressive motile cells after thawing); Mixed bull semen: A + B (12×10^6 of progressive motile cells after thawing) and Supermix bull semen: A + B (28×10^6 of progressive motile cells after thawing). All cows received a P4 intravaginal device (DIB, Syntex, Argentina) and 2 mg of EB i.m. (Syntex) on Day 0, 500 mg cloprostenol (Estroplan, Syntex) at the time of DIB removal (Day 8), and 1 mg EB i.m. on Day 9. Fixed-time insemination (FTAI) was performed at 52 to 56 h after DIB removal. A total of 249 cows were randomly allocated to be inseminated with bulls A and B ($n = 76$), with Mixed A + B ($n = 87$), and with Supermixed A+B at a high concentration ($n = 86$) by a single inseminator. Pregnancy rates were evaluated at 38 days after insemination by transrectal ultrasonography. Means and standard deviations of each characteristic were calculated, compared, and statistically analyzed. The following sperm motility parameters were determined with the Ceros 12.1 sperm analyzer (Hamilton Thorne Biosciences, Inc., Beverly, MA, USA) on at least 1000 spermatozoa: velocity average path (VAP), velocity straight line (VSL), curvilinear velocity (VCL), amplitude lateral head (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), and percentage of rapid or static cells. There were no significant differences ($P > 0.05$) in VAP, VSL, VCL, ALH, STR, or LIN. There was a numerically higher percentage of rapid cells in the Supermix semen. Pregnancy rate from bulls A and B was 61% and from Mixed A + B 60%, while that from Supermixed A + B was 69%. Results from the analysis indicate that semen concentration is an important element to be considered in a timed artificial insemination program. Numerically higher pregnancy rates were obtained with double semen concentration in the straw. More research is required to evaluate the interaction between different breeds within a timed artificial insemination program.

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8 EFFECT OF SUCKLING RESTRICTION AND eCG TREATMENT ON PREGNANCY RATES IN POSTPARTUM *BOS INDICUS* CROSSBRED COWS TREATED WITH PROGESTERONE VAGINAL DEVICES AND ESTRADIOL BENZOATE

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Previous studies have shown that the use of eCG given at the time of removal of a progesterone (P4) releasing device improved pregnancy rates to fixed-time AI (FTAI) in postpartum *Bos indicus* cows (Bo GA *et al.* 2004 Reprod. Fertil. Dev. 16, 127). Suckling restriction with the placement of nose tags in calves has also been shown to induce cyclicity and increase pregnancy rates in postpartum *Bos indicus* cows (Stahring RC 2003 Taurus 18, 21–23). An experiment was designed to compare the effect of eCG treatment and restricted suckling on pregnancy rates in postpartum cows in fair to poor body condition score (BCS). A secondary objective was to evaluate the effect of restricted suckling on weaning weights. Lactating primiparous crossbred *Bos indicus* cows ($n = 399$), 60 to 90 days postpartum with a BCS 2.0 (1 to 5 scale), were randomly allocated to 1 of 4 treatment groups, in a 2×2 factorial design. On Day 0, all cows received a P4 intravaginal device (DIB, Syntex, Argentina) and 2 mg estradiol benzoate (EB) i.m. (Syntex). On Day 8, DIB devices were removed and all cows received 150 µg D(+)-cloprostenol i.m. (Ciclose, Syntex) and were randomly divided to receive 400 IU eCG (Novormon, Syntex) or no treatment at the same time. On Day 9, all cows received 1 mg EB i.m. and were FTAI 52 to 56 h after DIB removal. Nose tags were placed in half of the calves from Day 0 to the time of FTAI, whereas the other half of the calves remained untreated. All cows were examined by ultrasonography 30 d after FTAI to determine pregnancy status. Pregnancy data were analyzed by logistic regression, and birth and weaning weights were analyzed by Student's *t*-test. Ovarian activity was estimated by rectal palpation on Day 0: there were 57/399 (14.3%) cows with a CL, 203/399 (50.8%) cows with palpable medium size follicles, and 139/399 (34.8%) cows with ovaries containing no detectable structures. There was no effect of ovarian status at the time of treatment ($P = 0.52$) or semen ($P = 0.89$) on pregnancy rates. Suckling restriction increased ($P = 0.03$) pregnancy rates (91/195, 46.6% and 81/204, 39.7% for cows not suckled or suckled during the treatment, respectively). However, there was no effect of eCG or eCG by weaning interaction ($P = 0.82$) on pregnancy rates (82/192, 42.7% and 90/207, 43.4% for cows treated or not treated with eCG, respectively). Although mean ($\pm$ SEM) birth weights were not different (29.4 ± 0.4 vs. 29.1 ± 0.4 ; $P = 0.6$), weaning weights were significantly affected ($P = 0.001$) by the suckling restriction (190.1 ± 1.9 vs. 200.4 ± 2.1 for calves treated or not treated with the nose tags, respectively). Although restricting suckling, by placement of a nose tag from Day 0 to FTAI, may increase pregnancy rates in primiparous *Bos indicus* cows in fair to poor BCS, the adverse effect of this treatment on weaning weights of the calves has to be considered. Furthermore, eCG did not increase pregnancy rates in the present study, as it has been previously reported.

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9 DOES STRAW CONCENTRATION AFFECT POST-THAW SPERM QUALITY IN AI BULL SIRES?

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There is increasing interest in decreasing the number of spermatozoa per AI dose, owing to the discrimination of threshold concentration and fertility, economical revenues and the use of sexed semen. This study evaluated the quality (as sperm viability, acrosome integrity, membrane stability and chromatin stability) post-thaw of semen collected from four elite AI sires and frozen at 15×10^6 (control) or 2×10^6 spermatozoa (spz) per dose to disclose eventual deleterious extension effects. Semen collected via a.v. from 4 elite AI sires was split-processed and frozen in 0.25 mL plastic straws under commercial conditions, at either 2×10^6 or 15×10^6 spz/straw (the latter as control). The semen parameters were within acceptable limits of normality. The post-thaw samples showed acceptable motility (above 50%). Sperm viability and stability were assessed post-thaw using flow cytometry of cells loaded with SYBR-14 and propidium iodide (PI) for sperm viability (membrane integrity), and with carboxy-SNARF-1 (SNARF; Invitrogen AB, Frolunda, Sweden), PI, and FITC-*Pisum sativum* agglutinin (PSA, triple stain) for acrosome status. Membrane stability status was measured with Annexin-V/PI, while sperm chromatin condensation and stability were assessed following *in situ* acid-induced DNA denaturation and staining with acridine orange. No significant differences were seen between the two concentrations regarding sperm motility, plasma membrane integrity (SYBR/PI, SNARF), or chromatin stability. The highly extended semen (2×10^6 spz/straw), however, showed a higher ($P < 0.05$) frequency of spermatozoa with translocated phosphatidyl serine (as detected with Annexin-V), indicating their plasma membranes had become unstable. Also, there were more spermatozoa showing acrosomal damage (PSA). In conclusion, low concentrations of spermatozoa do not properly sustain conventional cryopreservation, although damages appear restricted to the sperm membrane. These changes in the stability of the plasma membrane apparently did not affect the viability of the spermatozoa, although they may negatively affect their lifespan. These findings may have an impact on the care that must be taken when cryopreserving low concentrations of spermatozoa with conventional freezing protocols.

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10 TIMED ARTIFICIAL INSEMINATION IN CATTLE WITH REDUCED DOSAGE OF SPERMATOZOA

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Estrus detection and determination of time of insemination are very important factors in reproduction management of cattle. Therefore an estrus synchronization schedule in combination with induction of ovulation and a single insemination at a predetermined time in dairy cattle was established to achieve high pregnancy rates (Kanitz *et al.* 2002 Reprod. Nutr. Dev. 42, 587–599; Becker *et al.* 2004). The aim of the recent study was to investigate the influence of the number of spermatozoa per insemination dosage on embryo development and the interrelationship between number of accessory sperms per embryo and its development using this schedule. In total 116 German Holstein heifers received GnRH (i.m.; 0.05 mg Gonavet[®], Veyx, Schwarzenborn, Germany) 60 h after PGF2 α application (i.m.; 0.5 mg Cloprostenol forte[®], Jenapharm, Jena, Germany) administered between Days 8 and 14 of the estrous cycle. Artificial insemination was carried out 13 h after GnRH application. Three different dosages of spermatozoa (15×10^6 , 5×10^6 , and 1×10^6) from three ejaculates from four fertile bulls were used. Embryos and oocytes were flushed from the oviducts of animals ovulated ($n = 106$; ovulation rate 91.3%). Animals were slaughtered on Day 4 after insemination. The quality of the embryos and oocytes was evaluated by microscopic examination. Embryos were stained with Hoechst 33258 to verify the number of accessory sperm. The evaluation of the data was carried out with the GLM procedure of the statistics software package SAS[®] (SAS Institute, Inc., Cary, NC, USA). As a post-hoc test the Student's *t*-test was used. Significance was set at $P = 0.05$. After flushing of 106 animals, 85 embryos and oocytes was recovered (recovery rate 80.2%). Relative to the three sperm concentrations, there were no significant differences among fertilization rates (92.3, 96.2, and 78.8%) and among the portions of normal developed embryos (84.6, 80.7, and 75.8%, all respectively) between groups. Interestingly, significant differences were found according to the mean number of accessory sperm/embryo (29.6 ± 8.4 , 45.3 ± 8.6 , and 6.5 ± 7.2 , respectively) and in the portion of embryos without or with more than 10 accessory sperm/embryo. Results show that fixed-time insemination, independent of detection of onset of estrus can result in high fertilization rates. Insemination with dosages $< 5 \times 10^6$ spermatozoa can reduce fertilization rates. Likewise, significant differences regarding fertilization rate were found after insemination of reduced sperm dosages of individual bulls. The number of accessory sperms/embryo seems to be an irrelevant parameter for quality of embryos produced under described conditions.

11 TIME OF INSEMINATION RELATIVE TO OVULATION EXPLAINS FERTILITY VARIATIONS OF FROZEN-THAWED SPERMATOZOA BETWEEN FARMS

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Swine fertility after AI with frozen-thawed spermatozoa varies between trials. As thawed spermatozoa have an extremely limited life span in the female genital tract, fertility of frozen-thawed spermatozoa depends mainly on the time of insemination relative to ovulation. The objective of this study was to evaluate whether the time of insemination relative to ovulation could explain the farm differences in fertility when frozen-thawed spermatozoa are used. Pooled sperm-rich fractions collected from three mature Pietrain boars were diluted in lactose/egg-yolk/glycerol/Orvus-ES-Paste extender,

loaded in 0.5-mL straws (1×10^9 cells/mL), and frozen under controlled conditions (Carvajal *et al.* 2003 *J. Androl.* 25, 389–396). Thawing was conducted in a waterbath at 37°C for 20 s. Inseminations were performed using the deep intrauterine insemination technique (Martínez *et al.* 2002 *Reproduction* 123, 167–170) with 1×10^9 thawed spermatozoa (post-thaw motility > 50%) diluted in 5 mL of Beltsville thaw solution (BTS). Ninety-seven and 82 weaned sows (parity 2–7) in farms A and B, respectively, were twice inseminated at 30 and 36 h after onset of estrus (estrus detection was performed twice a day by allowing females nose-to-nose contact with a mature boar and by applying back pressure). At insemination time, both ovaries were checked for ovulation by transrectal ultrasonography and sows were classified into three groups: F sows (follicles visible during the two examinations), O sows (ovulation visible during at least one examination), and C sows (corpora lutea visible during both examinations). Data were analysed with ANOVA and chi-square test, and are reported as % or mean $\pm$ SEM. Overall farrowing rates differed ($P < 0.01$) between farms: 70.1% (68/97) and 51.22% (42/82) in farms A and B, respectively. Litter size did not differ ($P > 0.05$) between farms (9.18 ± 0.24 and 9 ± 0.39 in farms A and B, respectively). Distribution of sows among F, O, and C groups differed ($P < 0.05$) between farms. Seventeen (17.52%), 70 (72.16%), and 10 (10.31%) sows in farm A and 33 (40.24%), 24 (29.27%), and 25 (30.49%) sows in farm B were classified as F, O, and C, respectively. Fertility in F, O, or C sows did not differ ($P > 0.05$) between farms. Farrowing rates and litter size in O sows (82.98% and 9.45 ± 0.23) were higher ($P < 0.05$) than in F (48% and 8.67 ± 0.54) and C (48.57% and 7.55 ± 0.62) sows. We can conclude that time of insemination relative to ovulation explains fertility differences between farms when frozen-thawed spermatozoa are used.

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12 CASA PARAMETERS OF FRESH BULL SEMEN COLLECTED BY ARTIFICIAL VAGINA OR ELECTROEJACULATION IN ARGENTINA

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The latest entry in the field of semen evaluation is computer assisted semen analysis (CASA). Its greatest advantages are elimination of the subjective nature of routine semen evaluation and the addition of detailed motion analysis unquantifiable by visual examination. The objective of this study was to evaluate CASA motility parameters of fresh bull semen collected by artificial vagina (AV) or electroejaculation (EE) from a total of 56 beef different bulls. Semen samples from a total of 45 beef bulls were collected by AV from winter to the end of spring (740 collections), and from 11 beef bulls by EE (120 collections) in the same period. First and second AV collections were analyzed as individual data. EE collection was performed only one. Means and standard deviations for each characteristic were calculated, compared, and statistically analyzed. A sample of the collection was diluted 1:20 in a semi-defined semen extender (Andromed, Minitüb, Tiefenbach, Germany) and held in a glass tube at 36°C for 5 min before analysis. The sample was loaded into 20- μ m chambers, and six microscope fields from each chamber were analyzed. The following sperm motility parameters were determined with the Ceros 12.1 sperm analyzer (Hamilton Thorne Biosciences, Inc., Beverly, MA, USA) on at least 1000 spermatozoa: concentration (CONC), velocity average path (VAP), velocity straight line (VSL), curvilinear velocity (VCL), amplitude lateral head (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), and percentage of rapid or static cells. There were no significant differences ($P > 0.05$) in VAP, VSL, VCL, ALH, STR, LIN, and the percentage of rapid and static cells of semen collected by AV or EE. The concentration (sperm/mL) of the AV-collected sperm was significantly higher than for the sperm collected by EE. Results from the analysis indicate that semen collected by artificial vagina have motility characteristics similar to those collected by electroejaculation. More research needs to be done to evaluate motility parameters of frozen/thawed semen collected by electroejaculation and by artificial vagina.

	CONC (sp/mL)	VAP (μ m/s)	VSL (μ m/s)	VCL (μ m/s)	ALH (μ m)	STR (Hz)	LIN (%)	Rapid (%)	Static (%)
AV	1067.1 ^a	122.3	100.1	213.2	7.82	80.9	48.9	67.4	13.8
EE	789.2 ^b	123.8	100.8	216.6	8.20	81.0	49.9	64.7	19.3

^{a,b} Values within a column with different superscripts differ significantly ($P < 0.05$).

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13 EFFECTS OF PRESYNCHRONIZATION WITH A USED CIDR, AND TREATMENT WITH eCG ON FERTILITY IN LACTATING COWS SUBJECTED TO A COSYNCH PROTOCOL

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The objectives were to investigate the effects of pretreatment with a used CIDR on follicle size and ovulation rate in cows after an injection of GnRH, and treatment with eCG at the time of PGF on preovulatory follicle size and fertility in cows subjected to a Cosynch protocol. Lactating crossbred beef cows ($n = 292$), 2 to 12 years of age were allocated to two groups to receive either a used CIDR (Bioniche Animal Health; Belleville, Ontario, Canada) for 15 days or no treatment (Control). At CIDR removal (Day 0), all cows received 100 μ g of GnRH i.m. (Cystorelin, Merial Canada Inc., Victoriaville, Quebec, Canada). On Day 7, all cows received 500 μ g of cloprostenol i.m. (PGF; Estrumate, Schering-Plough Animal

Health, Pointe-Claire, Quebec, Canada) and were further allocated to receive either 400 IU of eCG i.m. (Pregnacon; Bioniche Animal Health) or no treatment (2×2 factorial design; $n = 73$ per group). On Day 9 (54 h after PGF), cows were given 100 μg of GnRH i.m., concurrent with timed AI (TAI). Transrectal ultrasonographic examinations were done on a subset of approximately 40 cows in each group on Days -15, 0, 7, and 9 to assess ovarian structures, and on all cows on Day 37 to confirm pregnancy. Data were analyzed by SAS CATMOD and ANOVA. Overall, 53% of cows had a CL present on Day -15 ($P = 0.23$). At first GnRH (Day 0), fewer CIDR-treated cows than Control cows had a CL (15.2 vs. 85.0%, respectively; $P < 0.001$), while mean ($\pm$ SEM) diameters of the dominant follicle were larger in CIDR-treated cows (18.2 ± 0.4 vs. 13.8 ± 0.4 mm, respectively; $P < 0.001$). Moreover, the proportion of cows that ovulated following the first GnRH was higher ($P < 0.001$) in CIDR-treated (75.0%) than Control (48.7%) cows. Eight (10.0%) cows presynchronized with a CIDR did not ovulate and had a luteinized follicle (31.7 ± 1.9 mm) at the time of PGF. Although CIDR-treated cows had larger ($P < 0.002$) dominant follicles than Control cows on Day 9 (16.6 ± 0.3 vs. 15.2 ± 0.3 mm), presynchronization did not affect fertility (53.4 vs. 54.1%, respectively). However, diameter of the dominant follicle at TAI in cows that became pregnant was smaller in CIDR-treated vs. Control cows (15.3 ± 0.3 vs. 16.6 ± 0.3 ; $P < 0.005$). Treatment with eCG on Day 7 did not affect ($P = 0.17$) the diameter of the dominant follicle at TAI, but tended ($P = 0.06$) to increase pregnancy rate (58.9 vs. 48.6%). Furthermore, pregnancy rate tended to be higher ($P = 0.08$) in Control cows given eCG (47/73, 64.4%) than in the Control- (32/73, 43.8%), CIDR- (39/73, 53.4%) or CIDR/eCG- (39/73, 53.4%) treated cows. In addition, pregnancy rate was affected by parity; 2-yr-old cows had a lower ($P < 0.04$) pregnancy rate than older cows (42.9, 58.7, and 58.2% for 2, 3–4, and >5 years, respectively). Treatment with eCG increased pregnancy rate by 33% ($P < 0.03$) in 2-yr-old Control cows. In summary, presynchronization with a used CIDR prior to a Cosynch protocol increased the proportion of cows responding to the first GnRH. Although CIDR-treated cows had a smaller dominant follicle at TAI, pregnancy rate was not affected. Treatment with eCG increased fertility in Control cows; eCG may be useful in GnRH-based protocols in lactating beef cows.

14 EFFECT OF TEMPORARY WEANING AND ECG TREATMENT ON PREGNANCY RATES IN POSTPARTUM *BOS INDICUS* COWS FOLLOWING TREATMENT WITH PROGESTERONE VAGINAL DEVICES AND ESTRADIOL BENZOATE

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Although treatments with progesterone (P4)-releasing devices and estradiol benzoate (EB) have been extensively used in fixed-time AI (FTAI) programs in beef cattle, pregnancy rates in postpartum *Bos indicus* cows kept on pasture often have been lower than expected because of poor body condition score (BCS) and a high incidence of anestrus. Temporary weaning and eCG treatment have been shown to increase pregnancy rates in suckled beef cows. Therefore, this experiment was designed to compare the effect of eCG treatment and temporary weaning on pregnancy rates in postpartum cows in fair to poor BCS. Lactating multiparous crossbred *Bos indicus* cows ($n = 393$), 60 to 90 d postpartum with a BCS 2.0 (1 to 5 scale) were randomly allocated to 1 of 4 treatment groups, in a 2×2 factorial design. At the beginning of the experiment (Day 0), all cows received a P4 intravaginal device (DIB, Syntex, Argentina) and 2 mg EB i.m. (Syntex). On Day 8, DIB devices were removed and all cows received 150 μg D (+) cloprostenol i.m. (Ciclast, Syntex) and were randomly divided to receive 400 IU eCG (Novormon 5000, Syntex) or no treatment at the same time. Furthermore, half of the cows in each treatment group have their calves weaned temporarily, from the time of DIB removal until the end of the FTAI, whereas the other half remained with their calves all the time. Finally, all cows received 1 mg EB i.m. on Day 9 and were FTAI 52 to 56 h after DIB removal. Cows were examined by ultrasonography 42 d after FTAI to determine pregnancy status. Data were analyzed by logistic regression and the effects of treatment and semen used were considered in the model. Ovarian activity was estimated by rectal palpation on Day 0: there were 72/393 (18.2%) cows with a CL, 140/393 (35.6%) cows with palpable medium size follicles, and 181/393 (46.1%) cows with ovaries containing no detectable structures. There was no effect of ovarian status at the time of treatment ($P = 0.91$) or semen ($P = 0.91$) on pregnancy rates. Treatment with eCG tended ($P = 0.08$) to increase pregnancy rates (94/192, 48.9% and 79/201, 39.3% for cows treated or not treated with eCG, respectively). However, there was no effect of weaning or eCG by weaning interaction ($P = 0.98$) on pregnancy rates (86/191, 45.1% and 87/202, 43.1% for cows that have their calves weaned or not weaned for 56 h, respectively). Results confirm those of previous studies that demonstrated that the use of eCG in a P4/EB/FTAI program improved pregnancy rates in postpartum *Bos indicus* crossbred cows that were in fair-to-poor BCS. However, no improvement in pregnancy rates was observed after temporary weaning in the present study.

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15 EFFECT OF SEMEN THAW METHOD ON CONCEPTION RATE IN DAIRY HEIFER HERDS

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Semen processed with procedures permitting a flexible thaw method is used to breed millions of cows yearly. "Pocket thawing" is widely used as an alternative to warm-water thawing with such semen. To pocket thaw, a straw is retrieved from cryostorage, immediately wrapped in a folded paper towel, and moved to a thermally protected pocket for 2 to 3 min of thawing within the pocket before AI gun loading. Published field data are lacking for comparisons of such a thaw method with those for semen prepared to permit flexible-thawing. We measured the effect of warm-water or pocket thaw on conception rate in four dairy heifer herds using semen prepared with methods previously optimized for flexible-thawing success. Semen processing (Anderson S *et al.* 1994 J. Dairy Sci. 77, 2302–2307) includes two-step whole-milk extension, static vapor tank freezing (0.5-mL straws),

and IMV Digitcool mechanical freezing (0.25-mL straws). It is unclear which specific processing steps permit flexible thawing. These procedures have been developed using breeding results from decades of field trials by professional inseminators using both pocket and warm-water thaw. Semen prepared from each of 12 sires produced equal straw units at 10 and 15 million total sperm per straw, in both 0.5- and 0.25-mL straw packages. Professional inseminators used each combination evenly over 16 months. Additional commercial semen (55% of total) from the same source was used. The thaw methods alternated weekly. Thaw effect on conception status, from 70 day non-return data for 11,215 services (67.6% conception rate), was estimated by a generalized linear mixed model. Neither thaw method nor total sperm per straw significantly affected conception rate ($P = 0.658, 0.769$, respectively). Bull, herd, inseminator within herd, year, season, and straw size did significantly affect conception rate ($P < 0.05$). No thaw method interactions with herd, sperm number, season, and straw package size were significant ($P = 0.297, 0.526, 0.365, 0.723$, respectively). This suggests that if semen has been prepared with procedures specific to flexible-thawing, it can be either pocket thawed or warm-water thawed within a range of herdsman or inseminator practices, season, or straw packaging choices. Even at 10 million, the lowest total sperm per straw, pocket thaw was equally as successful as warm-water thaw. We generally observe that *in vitro* sperm quality, as expected, is maximal for rapidly thawed straws, with slower thawing resulting in lower values. However, while it appears that conventional measures of *in vitro* semen quality are improved with fast thaw rates, these measures do not appear to correspond to higher *in vivo* fertility for semen prepared intentionally to be flexibly thawed. We conclude that, for semen prepared with procedures that permit flexible thawing, the thaw method, whether pocket or warm-water thaw, does not affect conception under commercial conditions and with routine semen handling methods.

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16 A CIDR-BASED TIMED AI PROTOCOL RESULTED IN A HIGHLY ACCEPTABLE THERAPEUTIC OUTCOME ON OVARIAN FOLLICULAR CYSTS IN LACTATING DAIRY COWS

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Ovarian follicular cysts are a major reproductive failure in lactating dairy cows, prolonging the calving-to-conception interval and resulting in great economic loss. Treatment with GnRH is currently the most common therapy used for ovarian follicular cysts in dairy cows, but definitive results remain inconclusive. Recently, the Ovsynch protocol has been introduced as a therapeutic strategy for ovarian cysts in dairy cows (Bartolome *et al.* 2000 *Theriogenology* 53, 815–825). The objective of the present study was to evaluate the therapeutic effect of a CIDR-based timed AI (TAI) protocol on ovarian follicular cysts in lactating Holstein cows. Lactating Holstein cows with ovarian follicular cysts were randomly assigned to two treatments: (1) insertion of a CIDR intravaginal progesterone device (CIDRTM, InterAg, Hamilton, New Zealand) with an injection of 100 µg GnRH (Concertal[®], Dongbang Co., Seoul, Korea) on Day 0, an injection of PGF_{2α} (Lutalyse[®], Pharmacia & Upjohn, Puurs, Belgium) and removal of the device on Day 7, an injection of GnRH on Day 9, and TAI 16 h after the GnRH injection (CIDR+GnRH-PGF_{2α}-GnRH group, $n = 30$); and (2) an injection of 100 µg GnRH on Day 0, and AI at estrus (AIE) within 2 months after treatment with GnRH (GnRH group, $n = 72$). Day 0 was the day of initiation of the experiment. Pregnancy diagnosis was determined at 60 days after AI using both ultrasonography and rectal palpation. Pregnancy rates between groups were compared by chi-square analysis. Treatment-to-conception interval for cows that conceived by 150 days post-treatment was compared by Student's *t*-test. The pregnancy rate after TAI following the CIDR+GnRH-PGF_{2α}-GnRH protocol (46.7%) was higher ($P < 0.05$) than that after AIE following GnRH injection (25.0%). The treatment-to-conception interval (mean ± SEM) was shorter ($P < 0.01$) in the CIDR+GnRH-PGF_{2α}-GnRH group (46 ± 12 days) than in the GnRH group (88 ± 10 days), representing 21.9% more cows being pregnant by 150 days after treatment. These results indicate that the CIDR-based TAI protocol can be used as an efficient therapeutic tool for ovarian follicular cysts in lactating dairy cows. Follicular dynamics and endocrine changes during the CIDR+GnRH-PGF_{2α}-GnRH protocol is being determined to clarify the beneficial outcome in this study.

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17 COMPARISON OF FOLLICULAR WAVE EMERGENCE AND FOLLICULAR DEVELOPMENT FOLLOWING ESTRADIOL BENZOATE PLUS PROGESTERONE OR GnRH AT THE FIRST FOLLICULAR WAVE IN A CIDR-TREATED, LACTATING HOLSTEIN COWS

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Treatment with GnRH in a controlled internal drug release (CIDR)-based timed AI protocol induced synchronized follicular wave emergence, a large ovulatory follicle, and synchronous ovulation following a second injection of GnRH, while follicular wave emergence was relatively asynchronous in the estradiol benzoate (EB)-treated cows resulting in ovulation of smaller ovulatory follicles (Kim *et al.* 2004 *Theriogenology*, in press). In this study, we compared new follicular wave emergence and follicular development following treatment with EB plus progesterone (P4) or GnRH at the first follicular wave in CIDR-treated, lactating Holstein cows. Previously synchronized, lactating Holstein cows ($n = 20$) received a CIDR (CIDRTM, InterAg, Hamilton, New Zealand, Day 0) 3 to 8 days after ovulation and were randomly assigned to two treatments: cows in the GnRH group ($n = 10$) received 100 µg ferteirelin acetate i.m. (GnRH, Concertal, Dongbang Co., Seoul, Korea) and those in the E/P group ($n = 10$) received 2 mg EB (SY Esrone, Samyang, Seoul, Korea) and 50 mg P4 i.m. (SY Ovaron, Samyang, Seoul, Korea) at that time. Thereafter, all animals received

PGF_{2α} (Lutalyse, Pharmacia & Upjohn, Puurs, Belgium) at the time of CIDR removal on Day 7, and GnRH on Day 9. Ovaries of each cow were examined by transrectal ultrasonography (Sonoace 600 with 5.0 MHz linear-array transducer; Medison Co., Ltd., Seoul, Korea) every 24 h from Days 0 to 9, on Day 11, and on Day 14 of treatment to observe changes in ovarian structures (follicles and CL). Data were analyzed by Fisher's exact test, Wilcoxon Rank-Sum test and Student's *t*-test using an SAS program. Follicular wave emergence occurred within 7 days in 9/10 GnRH- and 8/10 E/P-treated cows ($P > 0.05$). The interval to wave emergence was longer ($P < 0.01$) in the E/P group (5.9 ± 0.5 days) than in the GnRH group (2.9 ± 0.4 days). While diameters of dominant follicles on Day 0 were not different ($P > 0.05$) between groups, diameters on Day 7 and Day 9 in the E/P group (9.4 ± 0.4 and 13.1 ± 0.7 mm, respectively) were significantly smaller ($P < 0.01$) than those in the GnRH group (12.9 ± 0.5 and 15.8 ± 0.7 mm, respectively). Growth rates of preovulatory follicles in the E/P group (2.1 ± 0.2 mm/day) were greater ($P < 0.01$) than in the GnRH group (1.2 ± 0.1 mm/day), while the duration of dominance of the preovulatory follicle was longer ($P < 0.01$) in the GnRH group (6.3 ± 0.4 days) than in the E/P group (3.3 ± 0.5 days). Synchronized ovulation by 40 h after the GnRH injection (Day 11) occurred in 10/10 GnRH- and 9/10 E/P-treated cows ($P > 0.05$). Results suggest that estradiol benzoate plus progesterone administered at the first follicular wave in CIDR-treated, lactating Holstein cows delays new follicular wave emergence, resulting in ovulation of smaller ovulatory follicles than GnRH. Endocrine changes during treatment and fertility following timed AI has been investigated in this study.

18 STRATEGIES TO IMPROVE FERTILITY WITH COSYNCH-CIDR PROTOCOLS IN BEEF CATTLE

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Two experiments were designed to evaluate strategies to improve fertility with Cosynch-CIDR protocols in cattle. The first experiment investigated the effect of low levels of progesterone prior to a Cosynch-CIDR protocol. On Day 0, lactating beef cows ($n = 34$) and heifers ($n = 37$) were placed in two groups to receive 500 µg cloprostenol (PGF; Estrumate, Schering-Plough Animal Health, Pointe-Claire, Quebec, Canada) or a twice-used CIDR (Bioniche Animal Health, Belleville, Ontario, Canada) for 5 days (Pretreatment) or no treatment (Control). On Day 5, used CIDRs were removed and all cattle received a new CIDR (Pfizer Animal Health, Montreal, Quebec, Canada) and 100 µg GnRH (Cystorelin, Merial Canada Inc, Victoriaville, Quebec, Canada). On Day 12, CIDR were removed and PGF was given. A second GnRH was given concurrent with timed AI (TAI) on Day 14 (54–56 h after PGF). Cattle were examined by transrectal ultrasonography for CL and follicle development, and for confirmation of pregnancy (Days 42 to 49). Diameter of the dominant follicle on Day 5 was larger and more variable in cows than in heifers (15.5 ± 5.6 vs. 11.4 ± 3.5 mm, respectively; means, $P < 0.001$; variance, $P < 0.003$), and tended to be larger in the Pretreatment group (14.3 ± 4.9 vs. 12.6 ± 5.2 mm; $P = 0.13$). More Pretreated (60.0%) than Control (36.1%) cattle ($P < 0.005$), and more cows (64.7%) than heifers (32.4%; $P < 0.03$) ovulated following the first GnRH. At the time of TAI, 5 (13.5%) heifers had already ovulated ($P < 0.03$). Diameter of the preovulatory follicle was affected by parity ($P < 0.001$), but not Pretreatment ($P = 0.4$), and tended to be larger in heifers that became pregnant ($P = 0.13$). Pregnancy rate was not affected by parity ($P = 0.71$), or pretreatment ($P = 0.34$); pretreatment tended to increase pregnancy rate in heifers (63.2 vs. 38.9%; $P = 0.19$). The second experiment evaluated the use of eCG in a Cosynch-CIDR protocol in beef heifers. Beef heifers ($n = 127$) were fed 0.5 mg/head/day of MGA (Pfizer Animal Health) for 15 d; 12 d after the last feeding (designated as Day 0) heifers received a CIDR and 100 µg GnRH. On Day 7, CIDR were removed, and heifers received PGF, and were randomly placed in 2 groups to receive 300 IU of eCG (Pregnaol; Bioniche Animal Health) or no treatment (Control). On Day 9 (54–56 h after PGF), all heifers received 100 µg GnRH, concurrent with TAI. Ultrasonographic examinations were done as in the first experiment. Overall, 79.5% of the heifers had a CL, and 9.4% had a luteinized follicle on Day 0. Seventy-eight heifers (61.4%) ovulated following the first GnRH, and those that ovulated had a less variable preovulatory follicle size than those that did not (13.7 ± 1.7 vs. 13.8 ± 2.3 mm; means, $P = 0.76$; variance, $P < 0.01$). However, there was no difference in preovulatory follicle size ($P = 0.63$), or pregnancy rate (49.2 vs. 53.1%; $P = 0.7$) for eCG-treated vs. Control heifers. In summary, pretreatment with a twice-used CIDR plus PGF increased the proportion of cattle that ovulated to the first GnRH, but not preovulatory follicle size or fertility in cows; fertility tended to be improved in heifers. Treatment with eCG did not increase preovulatory follicle size or fertility in heifers subjected to an Cosynch-CIDR protocol.

19 LOW-DOSE DEEP INTRAUTERINE INSEMINATION IN SOWS UNDER CONDITIONS: INCIDENCE OF UNILATERAL FERTILIZATIONS

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A new procedure for nonsurgical deep intrauterine insemination (DUI) in non-sedated sows has recently been reported (Martinez *et al.* 2002 Reproduction 123, 163–170). In comparison to traditional artificial insemination (AI), using this procedure, a 20-fold reduction in the number of spermatozoa inseminated can be used without a decrease in fertility when hormonally treated post-weaning estrous sows are used. The aim of the present study was to evaluate the effectiveness of DUI under field conditions. In Experiment 1, crossbred sows (2–6 parity) were weaned at 20.75 ± 0.06 days. Estrous detection was performed once per day, beginning 3 days after weaning. Sows with a weaning to estrus interval of 4–5 days were selected to be inseminated. A total of 190 sows were inseminated at 12, 24, and 36 h after onset of estrus using one of the following two regimes: (1) DUI with 150×10^6 fresh spermatozoa in 5 mL of BTS ($n = 95$) and (2) Traditional AI with 3×10^9 fresh spermatozoa in 100 mL of BTS ($n = 95$) prepared from the same semen samples used for the DUI group. Farrowing rates (FR) and litter sizes (LTS; mean $\pm$ SEM) from both groups were compared using chi-squared test and ANOVA, respectively. There was no significant difference in the FR between groups (83.2 and 86.3% for DUI and AI groups, respectively). However, a decrease ($P < 0.001$) in the LTS was observed in sows inseminated by the DUI procedure

(9.8 ± 0.29 and 10.9 ± 0.17 , respectively). In Experiment 2, seventy one natural post-weaning estrus sows were used. Fifty-five sows were DUI inseminated three times with 150 ($n = 17$), 300 ($n = 19$), or 600 ($n = 19$) $\times 10^6$ spermatozoa in 5, 10, or 20 mL of BTS, respectively. The remaining sows ($n = 16$) were traditionally inseminated. On Day 6 after estrus, sows were subjected to laparotomy and the tips of both uterine horns were flushed in order to evaluate pregnancy rate (PR: percentage of sows with at least 4 viable embryos) and fertilization rate (ratio of viable embryos to the total number of embryos and oocytes). PR was similar in all the groups, ranging from 84.2% (DUI 300×10^6 spermatozoa group) to 94.7% (DUI 600×10^6 spermatozoa group). Fertilization rate and the percentage of bilateral fertilization after DUI with 600×10^6 spermatozoa did not differ from those of the AI group (97.8 and 100% vs. 98.4 and 100%, respectively), but a significant decrease in both parameters ($P < 0.05$; chi-square test) was observed in sows inseminated with 300 (94.3 and 87.5%) or 150 (84.4 and 66.7%) $\times 10^6$ spermatozoa. In conclusion, DUI with 150×10^6 spermatozoa offers similar FR but a lower LTS in sows with natural estrus in comparison with those parameters obtained when traditional AI is used. The lower litter size could be related to the low percentage of bilateral fertilization observed in that group.

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20 EFFECTS OF DOSE OF ESTRADIOL BENZOATE AND PROGESTERONE IN PROSTAGLANDIN-TREATED BEEF HEIFERS

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Estradiol and progesterone have been used to synchronize follicular wave emergence and ovulation in a two-dose prostaglandin (PGF)-based synchronization program (Martínez *et al.* 2004 *Theriogenology* 62, 363–372). However, it was observed that some heifers displayed estrus prior to the second PGF, suggesting that premature luteolysis may have occurred. An experiment was designed to determine the effects of dose of estradiol benzoate (EB) and/or progesterone (P) on follicular and luteal dynamics in a two dose PGF-based protocol in beef heifers. In two replicates, beef heifers ($n = 28$; Simmental, Hereford, and Charolais crosses, 350 to 450 kg) received 500 µg cloprostenol (Schering-Plough Animal Health, Pointe-Claire, PQ, Canada) on Day -7. On Day 0, heifers were randomly allocated to nine treatment groups to receive 0, 1, or 2 mg of EB and 0, 50, or 100 mg of P i.m. in canola oil in a 3×3 factorial design. A second PGF treatment was administered on Day 14. Ultrasonography was done once daily from Days -5 to 9, and every 12 h thereafter until ovulation. Blood samples were collected at 12-hour intervals from Day 0 to 5 for estradiol and FSH concentrations, and every 24 h for progesterone. The effects of EB and P and their interaction on corpus luteum (CL), follicles, and hormone profiles were analyzed by analysis of variance, and means were compared by LSD or Tukey's test. All variables were normally distributed (Wilk-Shapiro test and rankit plots). The day of follicular wave at the time of treatment tended to vary among groups ($P = 0.08$) and the diameter of the dominant follicle also differed ($P < 0.05$). The interval from treatment to wave emergence was shorter ($P < 0.05$) in heifers that received 2 mg EB (4.6 ± 0.3 d) than in those that did not receive EB (5.9 ± 0.6 d), while the 1 mg EB group (5.1 ± 0.6 d) was intermediate. The interval to wave emergence in the 2 mg EB group was the least variable ($P < 0.05$). There was no effect of EB ($P = 0.72$) on the diameter of the CL at the time of the second PGF, but there was an effect of P treatment ($P = 0.01$). The variability of the interval from the second PGF to ovulation may have been influenced by treatment group but was statistically not significant ($P < 0.1$). There was an effect of time ($P < 0.01$) on plasma progesterone concentrations and a $P \times$ time interaction ($P = 0.06$) can also be assumed. Estradiol concentrations were affected by EB dose, time, and EB $\times$ time interaction (all $P < 0.01$). FSH concentrations were modified by time ($P < 0.01$). In summary, treatment with EB 7 days after a single injection of PGF affected follicular development, while P treatment at that time appeared to influence CL function.

21 EFFECTS OF HETEROLOGOUS SEMEN PLASMA AND SEMEN EXTENDERS ON PROGRESSIVE MOTILITY OF FROZEN-THAWED RAM SPERM

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Frozen-thawed ram semen crosses the cervix poorly, necessitating laparoscopic insemination. Acceptable fertility can be achieved with frozen-thawed ram semen deposited at the external cervical opening if ram semen plasma (SP) is added (McPhie *et al.* 2000 14th ICAR 2, 78 abstr). Homologous SP improves the fertility of frozen-thawed sperm of boars and dogs. Heterologous SP may have effects as well; the addition of bovine SP increased the ability of buffalo sperm (*Syncerus caffer*) to fertilize bovine oocytes *in vitro* (de Haas *et al.* 2003 *Theriogenology* 59, 392). The aim of the current study was to compare the effects of SP of rams (SPR), bulls (SPB), and dogs (SPD), protein-free TALP, Triladyl (Minitüb, Tiefenbach, Germany), and skim milk upon longevity and percentage of progressively motile frozen-thawed ram sperm. Three ejaculates from each of six rams (2 Dorpers, 2 Döhrne merinos, and 2 merinos), aged 2–4 years, were extended in Triladyl, pooled and frozen as a single batch per ram at 200×10^6 /mL in 0.25-mL straws. SPR was obtained from the same rams and SPB from 5 bulls by centrifugation, while the post-sperm fractions were collected from 5 dogs (SPD). Within a species, the SP from different donors was pooled and frozen in aliquots at -18°C . The 108 straws (6 rams, 6 diluents, 3 replicates) were thawed in random order. Once thawed, a straw was emptied into a tube with 0.85 mL of the appropriate fluid at 37°C and kept for 6 h. Percentage of progressively motile sperm was estimated at $\times 200$ magnification immediately and 2, 4 and 6 h after thawing. One person thawed the semen and prepared motility specimens, while another performed all motility evaluations. Data were evaluated by means of repeated-measures ANOVA, with rams as subjects and time and fluid as fixed effects. Non-significant interactions were removed from the model. Means were compared by means

of Bonferroni's test ($P < 0.05$). The model included ram, time, fluid, and ram $\times$ fluid, and time $\times$ fluid interactions, which were all significant ($P < 0.01$). Mean motility decreased from each time to the next and were 39.0% (0 h), 26.0% (2 h), 19.6% (4 h) and 12.6% (6 h), SEM 1.38%, $n = 108$. Mean motility was higher for skim milk (39.9%) than for all other fluids except Triladyl (27.7%), which was better than SPB (13.0%), whereas TALP (20.5%) and SPR (21.9%) were similar to Triladyl and SPB ($n = 72$, SEM 2.85%). The interactions (ram $\times$ fluid or time $\times$ fluid) were mainly due to SPD, SPR, Triladyl, and TALP, while milk resulted in the best and SPB in the lowest motility. This study shows that heat-treated skim milk maintains progressive motility of frozen-thawed ram sperm better than the SP of various species and protein-free TALP. In contrast to SPR, skim milk is known to result in poor fertility of frozen-thawed ram semen after cervical insemination. It would thus appear that maintenance of progressive motility *in vitro* may be a poor indicator of fertility after cervical insemination.

22 INFLUENCE OF SIRE AND SIRE BREED (GIR VS. HOLSTEIN) ON EARLY PREGNANCY AND EARLY EMBRYONIC LOSS RATES IN HOLSTEIN COWS DURING SUMMER HEAT STRESS

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Heat stress has negative effects on pregnancy rates of lactating dairy cattle. There are genetic differences in tolerance to heat stress, and it has been shown that *Bos indicus* cattle are more resistant to high temperatures than *Bos taurus*. In the present work, effect of sire and sire breed on conception rate of Holstein cows during the Brazilian summer (January and February, 2003 and 2004) was determined. Lactating Holstein cows ($n = 1113$), producing an average of 25.2 kg milk/day and at 247 ± 136 days postpartum, were AI approximately 12 h after estrus detection ($n = 433$) or fixed-time AI (FTAI) after hormonal induction of ovulation ($n = 680$), using semen from 4 Gir (*Bos indicus* dairy bull, 657 AI) and 3 Holstein sires (*Bos taurus*, 456 AI). Pregnancy diagnosis was performed by ultrasonography or rectal palpation at 28–45 and 70–84 days after AI. Cows diagnosed pregnant at the first examination and open at the time of the second were considered as having lost their embryo. The results were analyzed by logistic regression using PROC GENMOD: the model considered the effect of sire, sire breed, inseminators, milk production 7 days before AI, period of lactation, and AI type (FTAI vs. AI after estrus detection). There was no significant effect of sire breed on early pregnancy rate (EPR: Gir = 78/657, 11.9%; and Holstein = 30/456, 6.6%, $P = 0.34$) or early pregnancy loss (EPL: Gir = 25/78, 32%; and Holstein = 10/30, 33.3%, $P = 0.73$). However, there was a sire effect on pregnancy loss (43.7, 35.3, 10, and 40% for Gir bulls 1, 2, 3, and 4, respectively) and (50, 18.2, and 38.5% for Holstein bulls 5, 6, and 7, respectively), with bull 3 having the lowest rate of pregnancy loss ($P = 0.03$). There was no effect of the AI type on EPR (FTAI = 65/680, 9.5%; and AI after estrus = 43/433, 9.9%; $P = 0.8$). Surprisingly, there were higher pregnancy losses in cows AI after estrus detection (17/43, 39.5%) as compared to FTAI cows (18/65, 27.7%; $P = 0.03$). The variables days at postpartum, milk production 7 days before AI, and inseminator did not significantly influence EPR or EPL. It is concluded that use of *Bos indicus* sires did not improve EPR of lactating Holstein cows during summer as compared to use of *Bos taurus* sires. However, some bulls, either *indicus* or *taurus*, were more effective in decreasing EPL and selection of bulls by this criterion may result in higher parturition rates in lactating Holstein cows.

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23 PREGNANCY RATE IN NELORE COWS AFTER TEMPORARY CALF REMOVAL, AND USE OF HORMONAL PROTOCOLS WITH eCG

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Both temporary calf removal (TCR) and administration of eCG are potentially useful to improve pregnancy rates in animals treated with fixed-time artificial insemination (FTAI) protocols. In the present work, two experiments were performed to compare the efficiency of eCG and/or TCR in FTAI protocols, with or without exogenous progesterone. In experiment 1, lactating Nelore cows (40 to 70 days post-partum, $n = 220$) were allocated to two Groups. At a random stage of the estrous cycle (Day 0), animals from Group GPE (GnRH/PGF2 α /Estradiol)/eCG were treated with GnRH (50 mg lecoreline, i.m.; Gestran Plus[®]; Tecnopec, Sao Paulo, Brazil). Seven days later (Day 7) they received PGF2 α (150 mg D-cloprostenol, i.m.; Prolise[®], Tecnopec) and eCG (300 UI, i.m.; Novormon[®], Syntex). On Day 8 estradiol benzoate (EB, 1 mg, Estrogin[®], Tecnopec) was administered, and 30–36 h later the animals were inseminated (FTAI). In Group DIB (bovine intravaginal device)/eCG an intravaginal progesterone-releasing device (1.0 g, DIB[®], Syntex, Buenos Aires, Argentina; Day 0) was inserted into the vagina of cows and EB (2.5 mg, i.m.) was given in parallel. Eight days later (Day 8), eCG (300 UI, i.m.) and D-cloprostenol (150 mg) were administered. Then the DIB was removed. Twenty-four hour after DIB removal, cows were treated with EB (1.0 mg, i.m.), and 30–36 h later the animals were inseminated (FTAI). Ultrasonographic evaluation of ovaries was performed in all experiments 10 days before and at the beginning of the treatments or TCR, in order to detect the presence of CL. Cows from Group DIB/eCG showed higher pregnancy rates than those from Group GPE/eCG (58%, 72/124 vs. 39.5%, 38/96, respectively, $P < 0.01$). Furthermore, only in Group GPE/eCG were pregnancy rates higher in animals with CL (47.6%, 20/42) when compared to those without CL (33%, 18/54, $P < 0.05$). In a second experiment, a possibly beneficial effect of TCR on GPE/eCG protocol was tested in lactating Nelore cows (40 to 70 days postpartum, $n = 140$). Animals of Group GPE/eCG (control) were treated as described above, whereas calves were removed for 48 h from cows in Group RTB/GPE/eCG prior to hormonal treatments. Lactating Nelore cows having their calves removed showed a significant increase in pregnancy rates compared to those without TCR (51.2%, 34/66 v. 28.4%, 21/74, respectively, $P < 0.01$), in both situations: animals with CL (54.8%, 17/31 v. 33.3%, 11/33, respectively, RTB/GPE/eCG v. GPE/eCG) or without CL (48.5%, 17/35 v. 24.3%, 10/41, respectively, RTB/GPE/eCG v. GPE/eCG). In conclusion, these results indicate that addition of eCG to the GPE protocol was not efficient enough to produce comparable results to those obtained with DIB/eCG protocol. However, calf removal before the GPE/eCG treatment increased pregnancy rates in cycling or anestrus (without CL) lactating Nelore cows.