ABSTRACTS FOR POSTER PRESENTATION

Artificial Insemination

7 UTILIZATION OF CELL PROFILING TO EVALUATE BOVINE SPERMATOZOA IN NORMAL AND SIMULATED MICROGRAVITY

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We developed a method to evaluate bovine sperm membranes in normal (1G) and simulated microgravity (Sim-μG). Bovine spermatozoa are used as a model system because they have cellular membranes analogous to those of other cell types, and yet are much simpler because they have no cytoplasm and do not participate in DNA transcription or mRNA translation. They can be cultured as single cells and are easily evaluated for membrane characteristics using flow cytometry. These features make the mammalian spermatozoon a useful model for exploring the principles of membrane structure/function in the presence of a variety of environmental challenges such as simulated microgravity. Cryopreserved, washed beef bull sperm ($4–8 \times 106\,mL^{-1}$) were incubated under non-capacitating conditions (modified glucose-free Tyrode's medium containing low bicarbonate, HEPES buffer, pyruvate and $3\,mg\,mL^{-1}$ BSA V; 23°C in air), and these spermatozoa remained alive for 24–48 h at 1G. To simulate μG, spermatozoa were incubated under the same conditions, in a HARV 10 rotating wall vessel (RWV, Synthecon, Inc, Houston,TX, USA) at 9 rpms. Spermatozoa were incubated in 1G and Sim-μG environments for 2.5–4.5 h and subsequently exposed to 0, 60 or $80\,\mu g\,mL^{-1}$ LC for 0, 4, 8, 12, 16 and 20 min. Three fluorochrome combinations were used as probes at each [LC]/time point: (1) propidium Iodide (dead status) + SYBR 14 (live status); (2) PI + FITC-PSA (acrosome reactions [ARs]); (3) PI + MitoTracker Deep Red (mitochondrial activity). Approximately 1 million spermatozoa from 3 bulls were evaluated over 4 days. Data were acquired on a FACSVantage SE flow cytometer, and initially analyzed (quality control) using the bundled FACSVantage SE software package (Cell Quest, BD BioSciences, San Jose, CA, USA). This provided graphics of simple cell relations (fluorescence v. LC exposure time). For further statistical analysis, and incorporation of non-parametric statistical tools (including pattern recognition using Support Vector Machines), the data were processed using a collection of Perl scripts and C programs. Results: Live/dead status: When Sim-$\mu G + 60\,\mu g\,mL^{-1}$ LC sperm were compared to $1G + 60\,\mu g\,mL^{-1}$ LC, and $80\,\mu g\,mL^{-1}$ LC sperm, their profiles were more similar to the 1G $80\,\mu g\,mL^{-1}$ LC profiles. AR status: the Sim-$\mu G + 60\,\mu g\,mL^{-1}$ LC profiles were similar to the $1G + 60\,\mu g\,mL^{-1}$ LC profiles. Mitochondrial Status: the Sim-$\mu G + 60\,\mu g\,mL^{-1}$ LC profiles were more similar to $1G + 80\,\mu g\,mL^{-1}$ LC profiles. Summary: although Sim-μG sperm lost their motility within 3 h, they were alive. Cell profiles indicate that Sim-μG sperm nuclear membranes are less stable and their mitochondria are less functional than the 1G controls, but their acrosomes are intact indicating that fertilizing potential may remain. Additional experiments are needed to determine the time course for Sim-μG, induced changes, and whether Sim-μG sperm can penetrate eggs. Funding: NASA (2002)-Stennis-24 and The University of New Orleans.

8 RESYNCHRONIZATION OF OVULATION AND TIMED INSEMINATION IN LACTATING DAIRY COWS USING THE OVSYNCH AND HEATSYNCH PROTOCOLS INITIATED 7 DAYS BEFORE PREGNANCY DIAGNOSIS ON DAY 30 BY ULTRASONOGRAPHY

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Rapid re-synchronization of ovulation and insemination in cows found nonpregnant to a previous service is important to maintain high reproductive efficiency. The objective was to compare pregnancy rate (PR) and pregnancy losses (PL) in cows subjected to Ovsynch or Heatsynch protocols 7 days before pregnancy diagnosis by ultrasonography (U/S) on Day 30. Initiation of both protocols included administration of GnRH on Day 23, which was considered the optimal time according to the distribution of interestrus intervals in cows previously inseminated. The study was conducted in a large dairy herd located in north central Florida from March to May, 2003. Of 593 cows treated with GnRH on Day 23 (±1) after insemination and found nonpregnant at U/S on Day 30 (±1; experimental Day 0), 75% (445) had a CL (diestrus) and were sequentially assigned to the two experimental groups. Cows in the Ovsynch Group ($n = 225$) received 25 mg (i.m.) $PGF_{2\alpha}$ (Lutalyse, Pharmacia, Kalamazoo, MI, USA) on Day 0, 100 μg (i.m.) GnRH (Cystorelin, Merial Limited, Iselin, NJ, USA) on Day 2, and were timed inseminated 16 h later. Cows in Heatsynch Group ($n = 220$) received 25 mg (i.m.) $PGF_2\alpha$ on Day 0, and 1 mg (i.m.) estradiol cypionate (ECP, Pharmacia, Kalamazoo, MI, USA) on Day 1, and were

 10.1071/RDv16n1Ab9

timed-inseminated 36 h later. Pregnancy at Day 30 was determined by U/S and at Day 55 by rectal examination of the genital tract. Pregnancy rate and PL were evaluated using multiple logistic regression. Pregnancy rate at Day 30 for Ovsynch (64/225, 28.4%) and Heatsynch (63/220, 28.6%) and PR at Day 55 for Ovsynch (58/225, 25.8%) and Heatsynch (54/220, 24.5%) were not different. In addition, PL between Days 30 and 55 for Ovsynch (6/64, 9.4%) and Heatsynch (9/63, 14.3%) were not different. There were no effects of parity, inseminator and days in milk on PR. However, PR at day 30 was higher in lots with cooling systems (46/141, 32.6%) and lots of first calf heifers (41/135, 30.4%) compared to lots of multiparous cows late in lactation without cooling systems (32/148, 21.6%). In conclusion, Heatsynch and Ovsynch initiated 7 days before pregnancy diagnosis on Day 30 had comparable PR of 25% at Day 55 in lactating dairy cows during the period of March–May.

9 THE USE OF eCG TO INCREASE PREGNANCY RATES IN POSTPARTUM BEEF COWS FOLLOWING TREATMENT WITH PROGESTERONE VAGINAL DEVICES AND ESTRADIOL BENZOATE AND FIXED-TIME AI

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Treatments with progesterone (P4) releasing devices and estradiol benzoate (EB) have been extensively used in fixed-time AI (FTAI) programs in beef cattle. However, pregnancy rates in postpartum cows kept on pasture often have been lower than expected because of poor body condition score (BCS) and a high incidence of anestrous. A recent study (Cutaia L *et al*., 2003 Theriogenology 59, 216) suggested that the addition of eCG to a P4/EB FTAI program may improve pregnancy rates in cows with fair to poor BCS, but results were not conclusive. Therefore, this experiment was designed to further investigate the effect of eCG treatment on pregnancy rates in postpartum beef cows in those conditions. The experiment was performed at 2 locations with lactating Angus cows ($n = 93$), 60 to 90 d postpartum with a BCS 1.9 (1 to 5 scale; Location 1), and crossbred Zebu cows ($n = 290$), 60 to 90 d postpartum with a BCS 2.0 (Location 2). In each Location, cows were randomly allocated to 1 of the following treatment groups: Control, eCG Day 6, or eCG Day 8. All cows received a P4 intravaginal device (DIB, Syntex, Argentina) and 2 mg EB i.m. (Syntex) on Day 0, 500 mg cloprostenol (Estroplan, Syntex) at the time of DIB removal (Day 8), 1 mg EB i.m. on Day 9 and FTAI 52 to 56 h after DIB removal. Cows in the Control group received no further treatment, whereas cows in the eCG-treated groups received 400 IU eCG (Novormon 5000, Syntex) on Day 6 or Day 8. Cows were examined on Day 0 by rectal palpation (Location 1) or by ultrasonography (US; Location 2) and were classified as those with a CL or without a CL, with either large (>8 mm) or small (<8 mm) small follicles. Pregnancy was determined by US 45 d after FTAI. Data were analyzed by logistic regression and the effects of location, treatment, ovarian status, AI technician and semen were considered in the model. There was no effect of location ($P = 0.3$), AI technician ($P = 0.2$) or semen ($P = 0.8$) on pregnancy rates. However, there was an effect of treatment ($P = 0.02$), attributed to higher pregnancy rates in the eCG Groups than in the Control Group (Table). Furthermore, cows with a CL or without a CL but with large follicles on Day 0 had higher pregnancy rates than those with small follicles ($P = 0.04$). It was concluded that the use of eCG in a P4/EB FTAI program improved pregnancy rates in postpartum beef cows that were in fair to poor BCS.

Ovarian status	Group eCG Day 6	Group eCG Day 8	Control group	*P* values
CL	26/33 (78.8%)[a]	28/41 (68.3%)[a]	26/47 (55.3%)[b]	$P = 0.03$
Large follicles	33/49 (67.8%)[a]	34/59 (57.6%)[ab]	29/68 (42.6%)[b]	$P = 0.02$
Small follicles	13/27 (48.1%)	19/32 (59.4%)	11/27 (40.7%)	$P = 0.4$
Total	72/109 (66.1%)[a]	81/132 (61.4%)[a]	66/142 (46.5%)[b]	$P = 0.02$

[a,b]Percentages differed significantly.

10 CASA EVALUATION OF SEXED AND NON-SEXED FROZEN BULL SEMEN

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Flow citometry cell sorting has been proven successfully to separate X and Y sperm; however, the technology is still too stressfull for the viability of the sorted semen. The objective of this study was to evaluate nonsexed and sexed frozen sperm motility characteristics using a CASA technology. Ejaculates from 4 different bulls (3 Holstein and 1 Angus) were collected, and processed as split non-sexed and sexed semen samples using Tris egg yolk extenders. X and Y sperm were separated using a high-speed sorter (SX Moflo). Cryopreservation was done at the same time under appropiate conditions using a programmed cryochamber. Thawing procedure was done at 37°C for 30 s and a sample of each straw was placed in the evaluation chamber. The experiment was repeated twice and two chambers with 30 observations each were analyzed each time. Mean and standard deviation of each characteristic were calculated, compared and analyzed statistically. The sperm concentration was determined by means of a burker counting chamber. Sperm quality was determined at 0 h after thawing, and later at 1 h, 2 h and 3 h after incubation in a glass tube at 30°C. The following sperm motility parameters were determined with the Hamilton Thorne (HTM-ceros 12.1) on at least 1000 spermatozoa: velocity average path (VAP), velocity straight line (VSL), amplitude lateral head (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), and percentage of progressively motile spermatozoa (PMS). Linearity of nonsexed spermatozoa was 53 ± 3.5, 47 ± 0.8, 43 ± 7.8 and 42 ± 4.5 for the 0 h and the 3 test incubation times and 49.5 ± 3.7, 51.2 ± 3.7, 43.3 ± 7.8 and 44.5 ± 7.6, respectively, for sexed semen. There were no significant differences ($P > 0.05$) in the progressive velocity, track speed and linearity between sexed and nonsexed semen. The percentage of static cells was 33%, 30%,

47% and 50% for the 0 h and the 3 test incubation periods; however, the percentage of static cells for the sexed semen was 53%, 71%, 77% and 82%, respectively. Results from the analysis indicate a significant increase ($P < 0.01$) in the number and the percentage of static cells with time. The lateral amplitude of sperm motility for nonsexed semen was 5.9 ± 0.5, 6.8 ± 0.8, 6.0 ± 0.4 and 5.1 ± 0.7, and for sexed semen 6.6 ± 0.7, 6.8 ± 0.4, 6.4 ± 0.4 and 5.5 ± 1.7, respectively. The percentage of progressively motile sperm was significantly different at 0 time 49.7 ± 4.9 and 23.1 ± 4.9 for nonsexed and sexed semen respectively. After 3 hours of incubation the percentage of progressively motile sperm was 38.7 ± 10.2 and 3.7 ± 3.2 for nonsexed and sexed semen, respectively. In conclusion, sexed frozen semen seems to have characteristics similar to those of normal nonsexed semen. However, a significant decrease in the percentage of progressively motile cells could affect pregnancy rates. More research needs to be done to detect differences between bulls and cryoprotectans. Research supported by Centro Genetico Bovino de EOLIA sa Argentina.

11 THE EFFECT OF PRESYNCHRONIZATION ON PREGNANCY RATE TO FIXED-TIME AI IN BEEF HEIFERS SUBJECTED TO A COSYNCH PROTOCOL

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The objective was to investigate the effect of presynchronization with PGF prior to a Cosynch protocol on estrus synchrony, CL and preovulatory follicle diameters and pregnancy rate following timed-AI (TAI) in beef heifers. Cycling beef heifers ($n = 148$) were treated with 100 μg GnRH i.m. (Cystorelin, Merial Canada Inc., Victoriaville, Quebec, Canada) on Day 0, 500 μg cloprostenol i.m. (PGF; Estrumate, Schering Plough Animal Health, Pointe-Claire, Quebec, Canada) on Day 7, and GnRH concurrent with TAI on Day 9 (54 h after PGF). Half of the heifers (Control) received the first GnRH treatment at random stages of the estrous cycle, while the other half (Presynch) received two injections of PGF 11 days apart, with the first injection of GnRH 11 days after the second injection of PGF. Estrus detection was done between the first GnRH and 12 h after PGF, and heifers detected in estrus were inseminated (and considered nonpregnant to TAI), while all other heifers were TAI. Heifers were examined by transrectal ultrasonography for CL and follicle development, and confirmation of pregnancy. Data were compared between groups using Student's *t*-test and chi-square procedures. The numbers of heifers in estrus early (after first GnRH and before TAI) was higher in the Control group than in the Presynch group (18/74 v. 2/74, respectively; $P < 0.0001$). Mean (±SD) diameters of the dominant follicle (12.1 ± 3.1 v. 14.2 ± 2.5 mm) and CL (17.3 ± 5.5 v. 20.5 ± 4.3 mm) at first GnRH injection were smaller ($P < 0.0001$) and more variable ($P < 0.03$) in Control than Presynch heifers, but diameters of the preovulatory follicle ($P = 0.3$) and CL ($P = 0.1$) at TAI did not differ. Although the diameter of the preovulatory follicle was more variable ($P < 0.004$) in Control (5 to 19 mm) than Presynch (8 to 17 mm) heifers, pregnancy rate to TAI did not differ ($P = 0.4$; 29.7 v. 36.5%, respectively). Overall pregnancy rates were 45.9 and 37.8% for Control and Presynch groups, respectively ($P = 0.3$). Pregnancy rate tended ($P < 0.08$) to be affected by diameter of the preovulatory follicle at the time of TAI (0, 23.1, 45.7, 41.4, and 60.0% pregnant for diameters of <9, 9–11, 12–14, 15–17, and >17 mm, respectively). Regardless of treatment, diameter of the preovulatory dominant follicle ($P < 0.02$) and CL ($P < 0.03$) 7 days after TAI was smaller, and CL diameter was more variable ($P < 0.004$), in open than in pregnant heifers (12.7 ± 2.6 v. 13.8 ± 2.1 mm, and 16.5 ± 4.4 v. 18.0 ± 3.0 mm, respectively). In summary, presynchronization with PGF prior to a Cosynch protocol reduced the proportion of heifers in estrus before TAI, suggesting that this approach may be useful in the successful application of Ovsynch or Cosynch programs in heifers. However, pregnancy rate to TAI did not differ between groups in this study. Diameter of the preovulatory follicle tended to positively affect pregnancy rate, regardless of treatment.

12 PREDICTION OF BULL FERTILITY BY COMPUTER ASSISTED SEMEN ANALYSIS

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Sperm motility is clearly essential for fertilization both in vivo and in vitro. Motility is necessary for successful sperm transport, a step that is bypassed with in vitro fertilization. Recently, increasing attention has been paid to the objective evaluation and characterization of sperm motility more than simply determining the total proportion of motile spermatozoa. The purpose of computer-assisted semen analysis (CASA) is to provide values for sperm concentration and sperm motility more rapidly and accurately than those obtained with traditional semen analyses methods. The objective of our experiment was to investigate the effect of specific aspects of sperm movement, such as the velocity of progression and the actual pattern of movement, to the fertilizing capability of sperm. Frozen semen samples of 10 HF breeding bulls were used in the study. For the motility analyses, Medealab CASA system (Medealab, Germany, Ver. 4.1) was used, and the velocity parameters of VCL (curvalinear velocity, $\mu m\,s^{-1}$), VSL (straight line velocity, $\mu m\,s^{-1}$), and VAP (average path velocity, $\mu m\,s^{-1}$) were evaluated and compared with the Day 30 and 75 non-return rates (NR30 and NR75). For every sample, a total of 10 fields were examined for 8 s using a disposable 20 micron capillary chamber (CellVision, USA) giving a total of 1165 to 2831 cells evaluated. Chi square analysis, analyses of variance and linear correlation coefficient was applied to the statistical evaluation and comparison of the results. Data are based on weighted values. From the same batch of the analyzed frozen semen, a total of 8099 females were inseminated in more than 100 farms with a total of 6590 animals being positive for pregnancy at Day 30 and 4525 animals at Day 75. Within the bulls, differences were found in the values of NR30 and NR75 ($P < 0.05$). Our data indicate very strong differences between the males' NR30 and NR75 values (NR30: $65.6\% \pm 13.04$ to $79.6\% \pm 11.17$; $P < 0.001$ and NR75: $37.8\% \pm 10.38$ to $58.3\% \pm 15.53$; $P < 0.001$) reflecting the individual differences in the fertilizing capability of the males. All velocity parameters show very high correlation with strong significance both

non-return rates but the best values belong to VAP (NR30 and NR75; $P < 0.02$). Our data indicate that the bulls with lower VCL (25.51 ± 33.04 to 79.54 ± 58.03), VSL (11.35 ± 19.45 to 36.36 ± 35.71), and VAP (12.67 ± 19.06 to 41.75 ± 34.45) values showed lower fertilization rates both at NR30 and NR75. Computer and video technologies have advanced rapidly in recent years; thus the capability and accuracy of the latest versions of CASA systems are considerably better and they give more information about the different motion characteristics of spermatozoa. Because of the vital role of sperm motility in the reproductive process, such systems will enable us to move into a new era of diagnostic andrology and predict the fertilizing capability of semen. Supported by NKFP-Grants 4/040/2001 and 4/031/2001.

13 FOLLICLE TURNOVER DURING SYNCHRONIZATION TREATMENTS IN MEDITERRANEAN ITALIAN BUFFALOES (*BUBALUS BUBALIS*)

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The utilization of synchronization protocols for ovulation and AI in the buffaloes has not gained a widespread use among breeders due to the usually low conception rates achieved. The tendency to enter into postpartum anestrus in the period of the year characterized by increasing day length further affects the manipulation of their reproductive efficiency. This study, carried out in the months of february to may, was designed to test the hypothesis that newly growing dominant follicles towards the end of synchronization protocols for ovulation, are more competent for establishing pregnancies following AI in cycling and non cycling buffaloes. Animals were checked by ultrasound (7.5 MHz linear-array probe and SSD 500 Aloka monitor) for signs of ovarian activity and classified into cycling and not-cycling based on the presence of functional CLs and follicle turnover. Cycling buffalo heifers (CHE; $n = 30$), cycling mixed parity buffaloes (CMP; $n = 14$) and non-cycling mixed parity buffaloes (NCMP; $n = 17$) were selected for a direct comparison between two synchronization protocols. CHE and CMP received two GnRH administrations at Day 0 and 9 and a luteolytic dose of PG2a at Day 7, followed by a single AI at 16 h from last GnRH ('Ovsynch'). NCMP group received a PRID implant for 9 days and 1,000 IU of PMSG at Day 7 followed by two AI at 72 and 96 h from PRID removal ('PRID + PMSG'). Ultrasound monitoring for CMP and CHE of animals was performed at day 0, 2, 4, 7, 9, and day of AI. In NCMP, ultrasound monitoring was continued also at 48, 72, 96, and 120 h after PRID removal. Like superscripts (^{a,b}) after % signs indicate significant difference. A follicle >9 mm was present in 16/17 NCMP (94.1%^{a}), 14/14 CMP (100%^{a}), and 17/30 CHE (56.6%^{b}; $P < 0.05$) at the beginning of the synchronization protocol (day 0). Demise of the first large follicle recorded at day 0 and presence of a new large follicle at day 7 to 9 leading or not to ovulation, in the ovary ipsilateral or contralateral ('follicle shift') occurred in 15/17 (88.2%^{a}), 11/14 (78.5%^{a}), and 14/30 (46.6%^{b}; $P < 0.05$) for NCMP, CMP, and CHE respectively. Synchronized ovulations were recorded in 15/17 (88.2%^{a}), 12/14 (85.7%^{a}), and 26/30 (86.6%^{a}; $P > 0.05$) for NCMP, CMP, and CHE respectively. Conception rates (CR) by ultrasound examination at 25 to 30 days post-insemination were 12/17 (70.5%^{a}), 6/14 (42.8%^{ab}), and 11/30 (36.6%^{b}; $P < 0.05$) for NCMP, CMP, and CHE, respectively. Conceptions derived from follicle shift (either ipsilateral or contralateral) were 11/12 (91.6%^{a}), 5/6 (83.3%^{a}), and 7/11 (63.6%^{a}; $P > 0.05$) for NCMP, CMP, and CHE, respectively. In conclusion, both synchronization protocols in the three groups of buffaloes produced good ovulation and conception rates in the unfavourable period of the year. Most pregnancies resulted from fertilization of oocytes maturing in newly selected growing follicles toward the end of the synchronization protocol, suggesting a higher development competence compared to oocytes in large follicles already available at the beginning of the procedure, not regressing and leading to ovulation.

14 OVARIAN FOLLICULAR DYNAMICS IN COWS TREATED WITH A CIDR, ESTRADIOL AND PROGESTERONE LATE IN THE ESTROUS CYCLE

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The objective was to characterize ovarian follicular dynamics in beef cows treated with a CIDR (Bioniche Animal Health; Belleville, Ontario, Canada) and an injection of estradiol-17β (E2), with or without progesterone (P4), late in the estrous cycle. Previously synchronized, non-lactating, crossbred beef cows ($n = 36$) received a CIDR (Day 0) 16 to 18 days after ovulation and were randomly allocated to one of three treatment groups: no further treatment (Control, $n = 12$), an injection of 5 mg E2 (E2, $n = 12$), or 5 mg E2 plus 100 mg P4 (E2P4, $n = 12$; both from Sigma Chemical Co., St. Louis, MO, USA) i.m. in 2 mL canola oil. On Day 7, CIDR were removed and cows received 500 μg i.m. of cloprostenol (Estrumate, Schering Plough Animal Health, Pointe-Claire, Quebec, Canada). Ovaries were examined once daily by transrectal ultrasonography to detect ovarian follicle growth profiles, and determine the time of ovulation. Blood samples were taken daily for progesterone determination. Data were analyzed by ANOVA (LSD and Bartlett's tests), Student's *t*-test and chi-square procedures. Diameter of the CL and the dominant follicle, and progesterone concentration on Day 0 did not differ among groups ($P = 0.6$; overall mean (±SD), 16.8 ± 2.7 mm, 14.1 ± 2.0 mm, and 1.5 ± 1.9 ng mL, respectively). Thirteen cows ovulated within 3 days of treatment (50% of E2- and E2P4-treated cows and 8.3% of Control cows; $P = 0.05$); cows that ovulated had smaller CL diameters (15.2 ± 1.7 v. 17.7 ± 2.7 mm; $P < 0.004$) and lower progesterone concentrations (0.4 ± 0.2 v. 2.1 ± 2.2 ng mL; $P < 0.001$) at the time of treatment. Follicular wave emergence occurred within 7 days in 4/12 Control cows, 10/12 E2-treated cows, and 10/12 E2P4-treated cows ($P < 0.01$). Although the interval from treatment to wave emergence did not differ among treatments ($P = 0.8$; overall, 3.4 ± 1.5 days), follicular wave emergence was more synchronous ($P < 0.004$) in the E2 group than in the Control or E2P4 groups. At CIDR removal, dominant follicle diameter was larger ($P < 0.02$) in the Control group (15.9 ± 5.5 mm) than in the E2 (11.9 ± 1.8 mm) or E2P4 (11.5 ± 3.4 mm) groups, but dominant follicle diameter

was less variable ($P < 0.003$) in the E2 group than in the other two groups. Three cows did not ovulate after CIDR removal; two in the Control group and one in the E2P4 group. Interval to ovulation was shorter ($P < 0.05$) in the Control group (70.8 ± 10.5 h) than in the E2 (87.0 ± 9.0 h) or E2P4 (86.2 ± 7.2 h) groups, and the intervals to ovulation in cows that ovulated following treatment (91.0 ± 8.0 h) was longer ($P < 0.001$) than in those that did not (76.6 ± 9.6 h). In summary, treatment of cows with an estradiol-progesterone protocol late in the estrous cycle resulted in ovulation (50.0%), atresia (33.3%) or persistence (16.6%) of the dominant follicle present at that time. As length of follicular dominance and timing of ovulation were affected, fertility may be impaired following AI.

15 EFFECT OF DOSE OF ESTRADIOL VALERATE ON OVARIAN FOLLICULAR DYNAMICS IN CIDR-TREATED BEEF COWS

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The objective was to investigate the effect of dose of estradiol valerate (EV) on ovarian follicular growth profiles, intervals to follicular wave emergence and, following CIDR removal, estrus and ovulation in beef cows. On Day 0, 43 non-lactating, crossbred beef cows, 3 to 9 yr of age and at random stages of the estrous cycle, received a CIDR (Bioniche Animal Health; Belleville, Ontario, Canada) and were randomly allocated to one of four groups to receive no further treatment (Control; $n = 10$), or an injection of 1 mg ($n = 11$), 2 mg ($n = 10$), or 5 mg ($n = 12$) i.m. of EV (Sigma Chemical Co, St. Louis, MO, USA) in 2 mL canola oil. On Day 7, CIDR were removed and cows received 500 μg i.m. of cloprostenol (Estrumate, Schering Plough Animal Health, Pointe-Claire, Quebec, Canada). Ovaries were examined by transrectal ultrasonography once daily until 48 h after CIDR removal to detect ovarian follicle growth profiles, and twice daily thereafter to detect ovulation. Data were analyzed by ANOVA (LSD and Bartlett's tests) and chi-square procedures. One cow (5 mg EV group) lost the CIDR and was removed from all analyses. There was an effect of day ($P < 0.0001$) on CL diameter, but the effects of treatment ($P = 0.3$), and the treatment-by-day interaction ($P = 0.1$), were not significant. Follicular wave emergence occurred within 7 d in 7/10 (70%) Control cows and 31/32 (97%; $P < 0.04$) EV-treated cows (one cow in late diestrus at the time of treatment did not respond to 1 mg EV). Mean (±SD) interval from treatment to wave emergence was longer ($P < 0.03$) in cows treated with 5 mg EV (4.8 ± 1.2 d) than in those treated with 1 mg (3.2 ± 0.9 days) or 2 mg EV (3.4 ± 0.8 days), while Control cows were intermediate (3.8 ± 2.0 days). Although follicular wave emergence tended ($P < 0.09$) to be more synchronous in cows receiving EV, intervals from CIDR removal to estrus ($P = 0.7$) and ovulation ($P = 0.8$) did not differ among groups. Diameter of the dominant follicle was smaller ($P < 0.04$) at CIDR removal and tended to be smaller ($P < 0.08$) just prior to ovulation in the 5 mg EV group (8.5 ± 2.2 and 13.2 ± 0.6 mm, respectively) than in the Control (11.8 ± 4.6 and 15.5 ± 2.9 mm, respectively) or 1 mg EV (11.7 ± 2.5 and 15.1 ± 2.2 mm, respectively) groups, with the 2 mg EV group (10.7 ± 1.5 and 14.3 ± 1.7 mm, respectively) intermediate. Diameter of the dominant follicle at CIDR removal was less variable ($P < 0.01$) in the 2 and 5 mg EV groups than in the Control group and intermediate in the 1 mg EV group. In summary, dose of EV affected follicular dynamics, interval to and synchrony of follicular wave emergence, and dominant follicle diameter at CIDR removal and just prior to ovulation in CIDR-treated cows. However, interval from CIDR removal to estrus and ovulation was not affected by treatment. Results suggest that a dose of 2 mg EV may be most efficacious in synchronizing follicular wave emergence in CIDR-treated cows.

16 FERTILIZING POTENTIAL OF STORED TURKEY SPERM

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Considerable interest exists in prolonging the viability and duration of the fertilizing capacity of turkey sperm. Commercially available extenders for turkey semen are unable to maintain sperm survival for longer than a few hours and attempts to develop a suitable cryopreservation medium have met with limited success. The objective of the present study was to investigate the use of andrology products designed and optimized for turkey sperm (NidaCon International). In an insemination trial on a commercial turkey farm, semen was collected by abdominal massage and was immediately extended in 4 mL of either Turkey Semen Extend (NidaCon International, Gothenburg, Sweden) or Beltsville (Continental Plastic Corp, Delavan, WI). The extended semen (approximately 20 μL, representing a dose of approximately 70 million sperm) was used immediately for artificial insemination, with each bird being inseminated once weekly. The two groups of birds were housed in different buildings, with different semen donors for each. Eggs were candled after 5 days. Preliminary results showed that fertilization and hatching rates were similar in the two groups: Group 1 (Turkey Semen Extend) 966 eggs, 84.4% fertilized, 78.7% hatched; Group 2 (Beltsville) 966 eggs, 86.6% fertilized, 79% hatched. A second trial over a longer period showed similar results: Group 3 (Turkey Semen Extend) 18,450 eggs, 93.3% fertilized, 84.6% hatched; Group 4 (Beltsville) 40,873 eggs, 92% fertilized, 84.2% hatched. In a second experiment, turkey semen, extended as described above, was transported to the laboratory in the dark at ca. 34°C. Sperm motility was assessed subjectively at several time points. After 2 hours' storage, at least 50% of the sperm in Turkey Semen Extend were still motile, compared to only approximately 5% of the sperm in Beltsville medium. Aliquots of semen in Turkey Semen Extend were processed by discontinuous density gradient centrifugation on Turkey Gradient (NidaCon International). At least 40% motility was observed after 24 hours' storage at room temperature. In conclusion, the new Turkey Semen Extender and Turkey Density Gradient offer exciting possibilities for improving the viability of stored turkey sperm for insemination. Future studies will assess the fertility of both unprocessed stored turkey sperm in Turkey Semen Extend, and gradient-prepared stored turkey sperm.

17 PREVENTION OF ANESTRUS USING AN INTRAVAGINAL PROGESTAGEN DEVICE COMBINED WITH 17β-ESTRADIOL, GnRH, AND $PGF_{2\alpha}$ AT 60 DAYS POSTPARTUM IN DUAL PURPOSE CROSSBRED ZEBU COWS

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In order to study the effect of an intravaginal device (sponge) impregnated with Medroxyprogesterone acetate (MAP) on estrus induction and fertility, an assay was carried out on a commercial farm located in Zulia state, Venezuela. Eighty-four noncyclic (milk progesterone level <0.5 ng/mL) suckled crossbred cows (*B. taurus* × *B. indicus*) at 60 days postpartum and 3–3.5 body condition score (scale 1–5) were randomly allotted to one of the following treatments: T1 ($n = 22$), intravaginal device impregnated with 250 mg of MAP during 7 days + 50 mg of MAP and 5 mg of 17β-estradiol (17β-E) i.m. on the day of device insertion (Day 0) and 1.5 mg of 17β-E i.m. on Day 8 (Pregnaheat-E®; VIATECA,Venezuela); T2 ($n = 28$), 17β-E given at Day 0 was replaced by 20 μg of busereline (Conceptal® Hoescht, A. Germany) + injection of 25 mg of dinoprost (Lutalyse® Pharmacia Upjohn, Kalamazoo, MI, USA) on Day 7 and 1.5 mg of 17β-E 12 h after device removal (Day 8); T3 (control group; $n = 34$), without treatment. Animals detected in heat were inseminated in the uterine body 9 to 12 hours later in routine AM-PM. Pregnancy diagnose was carried out at 60 days after AI by transrectal examination. Studied variables were: estrus rate (ER), pregnancy rate (PR), anoestrus rate (AR), and partum-conception interval (PCI). Variables were analyzed by frequency procedures and chi-square test of Statistical Analysis System (SAS). PCI was analyzed by variance model (PROC GLM) and compared by Ls means. Results are shown in Table 1. T1 and T2 showed higher ER and PR ($P < 0.05$) than T3. AR was lower ($P < 0.05$) in T1 and T2 than T3. PCI was significantly reduced ($P < 0.05$) in T1, but not in T2 versus T3. The cows treated with MAP showed greater estrus and pregnancy rate, shorter partum-conception interval and lower anoestrus rate than the control group. Treatment with the intravaginal device was effective to prevent postpartum anoestrus and to improve reproductive performance in dual purpose zebu crossbred suckled cows.

Table 1. Effect of an intravaginal progestagen device on reproductive performance in crossbred Zebu cows

Treatments (n)	Estrus rate % (n)	Pregnancy rate % (n)	Anoestrus rate % (n)	PCI* days
T1 (22)	59.1[a] (13)	45.5[a] (10)	31.8[a] (7)	132.5 ± 16.8[a]
T2 (28)	64.3[a] (18)	39.3[a] (11)	17.9[a] (5)	140.4 ± 14.4[ab]
T3 (34)	26.5[b] (9)	17.6[b] (6)	58.8[b] (20)	178.9 ± 14.7[b]

Values in the same column with different superscripts differ. ([a,b] $P < 0.05$). * Means ± S.E.

18 OVARIAN SUPPRESSION WITH THE PROGESTIN LEVONORGESTREL IMPROVES OVULATION INDUCTION FOR ARTIFICIAL INSEMINATION IN THE DOMESTIC CAT

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Ovarian response to gonadotropin stimulation for artificial insemination (AI) is variable in the domestic cat. After ovulation induction with gonadotropins, a secondary wave of corpora lutea (CL) is often produced that alters endocrine profiles. This study assessed the impact of ovarian suppression with the progestin, levonorgestrel, before ovarian stimulation with equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) on ovarian response in the cat. Queens were assigned randomly to: 1) levonorgestrel (LNG), 6 Norplant® rods implanted for 37 d + eCG/hCG ($n = 6$ cats); and 2) Control, eCG/hCG alone ($n = 6$). Ovarian response was graded (scale 1–4; 1 = excellent, 4 = ovulation failure) 36–40 h post hCG (Day 5) using laparoscopy. Ovariohysterectomy (OVH) was performed on Day 23 and CL number and CL progesterone (P) content determined. Fecal samples were collected daily and metabolites of estradiol (E) and P quantified from >60 d before inhibition through OVH. Estrus and luteal activity were defined as fecal E and P concentrations greater than two or three times baseline, respectively. Time data were analyzed using repeated measures analysis and remaining data were analyzed using ANOVA. LNG abolished ovarian activity in all cats. No E peaks were observed during LNG inhibition compared with pre-inhibition (mean ± SEM; 1.8 ± 0.3 peaks/37 days). In contrast, number of E peaks pre- v. during inhibition was similar ($P > 0.05$) in control (pre, 2.2 ± 0.3; during, 2.0 ± 0.0) cats. All LNG cats had baseline E and P concentrations at eCG administration. Conversely, three of six control cats had elevated E and two cats had elevated P concentrations when eCG was given. Ovarian grade was higher ($P < 0.05$) in LNG (1.3 ± 0.2) v. control (2.9 ± 0.4) cats. All LNG cats had ≤Grade 2 responses, whereas two control cats failed to ovulate (Grade 4) or had mature CL (Grade 3) at laparoscopy. For both LNG and control cats, mean peak E (overall mean, 117.4 ± 14.4 ng g^{-1} feces) was higher ($P < 0.05$) and duration of estrus (6.8 ± 0.9 d) was longer ($P < 0.05$) after eCG/hCG v. pre-inhibition values (81.4 ± 5.3 ng g^{-1} feces and 3.9 ± 0.3 d, respectively). However, P concentrations/luteal phase were higher ($P < 0.05$) after eCG/hCG v. pre-treatment CL in control but not LNG cats. In cats with an ovarian grade of ≤2, control cats had more ($P < 0.05$) CL at Day 23 (14.0 ± 2.9 CL/cat) compared to Day 5 (4.5 ± 0.5). LNG cats showed no ($P > 0.05$) accessory CL development on Day 23 (9.2 ± 1.9 CL/cat) compared to Day 5 (6.5 ± 1.8). CL P content was not different ($P > 0.05$) across treatments (overall mean, 90.8 ± 18.7 ng CL). Results show that inhibition of ovarian activity with levonorgestrel before eCG/hCG improves ovarian response and alleviates accessory CL development in the domestic cat.

19 POSTPARTUM ANESTROUS TREATMENT WITH INTRA-VAGINAL PROGESTERONE DEVICE OR CALF REMOVAL FOR 120 HOURS IN SUCKLED CROSSBRED DUAL PURPOSE COWS

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Postpartum anestrus is the main reproductive problem that affects crossbred cattle production in Venezuela. (Soto *et al.*, 2002 Theriogenology 57, 1503–1510; Soto *et al.*, 2000 Revista Científica FCV-LUZ 10, 391–398). In order to reduce the postpartum anestrus and lower the calving to conception interval, 167 crossbred (*Bos indicus* × *Bos taurus*) anestrous cows between 90 and 130 days postpartum with body condition score 3 (scale 1–5) were randomly allotted to one the following treatments: 1) PH ($n = 59$), intravaginal sponge (IS) impregnated with 250 mg of medroxy-progesterone acetate (MAP) during 7 days + 50 mg of MAP and 5 mg of 17β-estradiol (17β-E) i.m. at the day of sponge insertion (Day 0), plus 500 IU of eCG i.m. at Day 5 and 1.5 mg of 17β-E i.m. at Day 8 (PREGNAHEAT-E, VIATECA, Villa del Rosario, Venezuela); 2) CR ($n = 57$), 120-h temporary calf removal; and 3) CG ($n = 20$), control group. Work was performed in a commercial farm located in a subhumid tropical forrest region. Estrus, first service conception and pregnancy rate were analyzed by chi-square analysis. The intervals to conception were analyzed using analysis of variance (GLM) and the means was compared by least square means method. To evaluate the treatment effect, the interval to conception of each pregnant cow during the first 100 days were considered for the analysis. This interval (days) was significantly ($P \leq 0.05$) higher in the PH treatment (155.9 ± 7.3) compared to control (174.4 ± 8.1) but no differences were shown between PH and CR (161.0 ± 7.8). Other results are shown in Table 1. PH treatment improved the estrus and pregnancy rates and shorten the interval to conception indicating that this is a promising anestrous treatment for suckled crossbred dual purpose cows under tropical conditions.

Table 1. Reproductive performance of postpartum anestrous suckled cows treated with intravaginal progesterone or temporary calf removal

Treatments	Estrus rate *n* (%)	First service conception rate *n* (%)	Pregnancy rate *n* (%)
PH	47 (79.6)[a]	23 (48.9)[a]	23 (39.0)[a]
CR	30 (52.6)[b]	14 (46.6)[a]	14 (24.5)[a,b]
CG	14 (27.4)[c]	6 (42.8)[a]	6 (11.7)[b]

Values with different superscripts differ ([a,b,c] $P < 0.01$).

20 EFFECTS OF BOVINE SOMATOTROPIN TREATMENT ON AI PREGNANCY RATE IN DAIRY HEIFERS

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Treatment of superovulated cows with bovine somatotropin (bST) at the time of insemination has been reported to decrease the number of unfertilized oocytes, while increasing the development rate and morphological quality of embryos (Thatcher WW *et al.*, 2001 Theriogenology 55, 75–89). These findings suggest that bST treatment might be used to improve pregnancy rates in inseminated cattle. The objective of this study was to further investigate the effects of bST (Posilac; Monsanto Co., St. Louis, MO, USA) treatment at or after insemination on subsequent pregnancy rate in diary heifers. Crossbred dairy heifers ($n = 73$) between 14 to 15 months of age and averaging 331 ± 3.6 kg, were used for the study. Estrus was induced by a single or repeated (at 14-day intervals) injections of 25 mg of $PGF_{2\alpha}$. Heifers were observed at least twice daily and artificially inseminated about 12 h after detection of estrus. At estrus, heifers were randomly assigned across treatments. Treatments were bST (Posilac) injection (500 mg, s.c.) at the time of insemination (Day 0), on Day 14, or both at insemination and on Day 14. Untreated animals served as controls. On Day 45 after estrus, ultrasonography was used to determine pregnancy status and measure fetal-crown rump length. Chi-square analysis was used to evaluate the effect of treatment on pregnancy rate. Analysis of variance was used to compare fetal crown-rump length among treatments. Results are presented in the table below. Treatment of heifers with bST at insemination had no effect ($P = 0.306$) on pregnancy rate when compared with the control group. However, bST treatment on Day 14, or on both Days 0 and 14, reduced ($P = 0.009$) pregnancy rate, when compared with the control and Day 0 bST treatment groups. Pregnancy rate was similar ($P = 0.729$) for heifers receiving bST on Day 14 v. Days 0 and 14. Fetal growth, as measured by crown-rump length, was unaffected by treatment ($P = 0.603$). Results indicate the timing of bST treatment after the onset of estrus can influence the subsequent pregnancy rate in dairy heifers.

Treatment	No. of animals	No. preg (%)	C-R length (mm)
Control	18	17 (94.4)[a]	25.5 ± 2.0
bST, Day 0	19	16 (84.2)[a]	26.1 ± 4.1
bST, Day 14	18	12 (66.7)[b]	27.4 ± 0.9
bST, Days 0 and 14	18	11 (61.1)[b]	28.1 ± 0.9

Values with different superscripts differ significantly ([a,b] $P = 0.009$).

21 DEEP UTERO-TUBAL SEMEN DEPOSITION IN MEDITERRANEAN ITALIAN BUFFALOES USING A NEW ARTIFICIAL INSEMINATION DEVICE

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The use of AI in the buffalo species is still marginal due to traditional lower conception rates when compared to cattle. However recently a number of studies in this field have revealed a promising increase in the efficiency of synchronization protocols for AI linked to more acceptable pregnancy rates. The possibility of using lower spermatozoa concentration of high quality buffalo bulls for AI without reduction in pregnancy outcome can be an additional offset, especially if such spermatozoa can be sexed and used for better reproductive management in buffalo farms. Within this conceptual framework, a new artificial insemination device for semen deposition near the utero-tubal junction (UTJ) in cattle (Ghent device), developed at the University of Ghent (Belgium), has been used in this study. The Ghent device is made of disposable materials and consists of 2 hollow plastic tubes, wherein a catheter filled with semen is introduced. The outer tube is completely rigid, while the inner tube consists of a rigid caudal end and a flexible cranial tip. The outer plastic tube can move independently from the inner tube. Once the insemination device is introduced into the uterine body, the inner tube with its flexible tip is moved forward. The flexible tip makes it possible to follow the curvature of the contractile uterus of the estrous cow, but excludes the use of Cassou straws. The semen is then expelled from the catheter by means of 0.1 mL of air followed by 0.6 mL of physiological saline solution. To assess the efficacy of the new Ghent device, 67 buffalo cows (*Bubalus bubalis*) were inseminated during a field trial. Two different insemination methods were used: (1) insemination with the conventional insemination device in the uterine body, and (2) insemination with the Ghent device near the utero-tubal junction ipsi-lateral to the site of ovulation. Artificial insemination was performed twice at 72 and 96 hrs after administration of prostaglandins to buffaloes bearing a functional corpus luteum as recorded during ultrasound monitoring. Conventional inseminations were performed with full (16–20 × 106) and half (8–10 × 106) insemination doses of frozen-thawed semen, while UTJ-inseminations were performed with full, half and quarter (4–5 × 106) insemination doses. When inseminations were performed with the conventional insemination device, halving the insemination dose resulted in a non-significant decrease of conception rates from 53% (8/15) to 42% (8/19). However, no difference in conception rates was observed when UTJ-inseminations were performed with a full, half or quarter standard insemination dose: 50% (6/12), 45% (5/11) and 50% (5/10), respectively. Despite the limited number of inseminations performed, this preliminary field trial demonstrates that the Ghent device is suitable for the insemination of buffaloes under field conditions, and that UTJ inseminations can be performed with only one-fourth of the standard insemination dose without a reduction in conception rates.