



A045 FTAI, FTET and AI

### **Association of puberty induction protocol and timed-AI protocol in Nelore heifers**

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**Keywords:** Nelore heifers, puberty, time-AI.

The aim of this study was to evaluate protocols to induce puberty (IP) associated to timed-AI protocols in Nelore heifers. Four experiments were performed. Heifers with absence of CL in two ultrasonographic evaluations (US; Mindray – 2200VET DP) with seven days of interval were classified as prepubertal. In all experiments to IP were used a 4th use CIDR for 12 days (Control; C), and heifers were randomly assigned to the treatments at CIDR removal. The induction rates (IR; heifers with CL/treated heifers) were evaluated seven days after CIDR withdrawal by US. Exp1, evaluated the IR to the treatments C (n=450) and eCG (200 IU of eCG; im; n=446); Exp2, was evaluated the IR to the treatments C (n=79), eCG (n=160) and eCG+ECP (200 IU of eCG + 0.5 mg im of ECP®, Pfizer Animal Health, Brazil; n=162). Exp3 evaluated the IR to treatments C (n=235), eCG (n=234), eCG+ECP (n=234) and ECP (0.5 mg im of ECP; n=245), and the moment after the puberty induction protocol to initiate the TAI protocol. The induction protocol was initiated with a 2 days interval for TAI be performed at the same time, forming three groups with intervals of 10 (G10; n=256), 12 (G12; n=265) and 14 (G14; n=256) days between the device removal in puberty induction and the beginning of the TAI protocol, D0-US + BE (2.0 mg, Estrogin®, Farmavet, Brazil) + CIDR®; D9-device withdrawal + 0.5 mg ECP + PGF, (12.5 mg, im, Lutalyse®, Pfizer Animal Health); D11-TAI. In Exp4 prepubertal (n=1545) and pubertal (n=743) heifers were synchronized with the ECP+G12 protocol (pubertal heifers received PGF at the end of induction protocol), and a subgroup of pubertal heifers was synchronized only with the TAI protocol describe above, with PGF on D7 (C-TAI=575), to evaluate the effect of previously cyclicity in pregnancy. Only heifers with CL at begin of the TAI protocol were synchronized in experiments 3 and 4. The data were analyzed by PROC GLIMMIX, significance was considered when P<0.05. In Exp1 the IR was greater for heifers treated with eCG (72%) compared to C (53%); In Exp2 the IR was greater for heifers treated with eCG+ECP (90%<sup>a</sup>) than eCG (75%<sup>b</sup>) and C (46%<sup>c</sup>). In Exp3, heifers treated with eCG+ECP (85%) and eCG (85%) had greater IR compared to C (75%), and ECP (80%) was intermediate. There was no interaction between IP protocol and TAI. Heifers that received the protocol 12 days after the end of the puberty induction protocol (G12; 51%; 45%) had greater conception and pregnancy compared to G10 heifers (39%; 33%), and G14 heifers (46%; 40%) were intermediate, respectively. In Exp4 the IR was 66% and prepubertal heifers (46%) or pubertal (49%) synchronized with the ECP+G12 protocol had pregnancy rate to timed-AI similar to C-TAI heifers (47%). The CIDR + ECP and/or eCG protocol to IP and the beginning of the timed-AI protocol 12 days after had the same pregnancy in relation to previously cycling heifers (C-TAI), showing that it is possible to breed prepubertal heifers shortly after puberty induction.

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### **Effect of cryoprotectant removal and re-hydration on pregnancy rates of bovine embryos (*Bos indicus* vs *Bos taurus*) frozen in ethyleneglycol**

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**Keywords:** ethyleneglycol, re-hydration, removal.

Since the use of Ethyleneglycol (EG) as embryo cryoprotectant was described (Voelkel and Hu, Theriogenology: 37:687, 1992), direct transfer became the main alternative for commercial embryo transfer. However, pregnancy rates of *Bos indicus* embryos cryopreserved with EG by direct transfer have lower pregnancy rates compared to those obtained with *Bos taurus* embryos. The objective of this experiment was to compare the effect of EG removal or not after thawing in *Bos taurus* and *Bos indicus* embryos. A total of 116 *Bos taurus* (Angus, Hereford and Simmental) and 99 *Bos indicus* (Brahman) embryos frozen in 1.5 M EG (Vigro Ehtylene glycol®, Bioniche Animal Health, Pullman, USA) were used. All embryos were grade one and stage between morulae and blastocyst. Embryos were thawed in water bath at 32°C for 30 seconds and then were randomly divided to be transferred directly into recipients or were placed in Holding medium (Syngro Holding®; Bioniche Animal Health, Pullman, USA) at 37°C for 2 min and then loaded again in a straw and transferred into recipients. All transfers were done by the same operator and embryos were transferred in four replicates. Pregnancy rates were evaluated by ultrasonography on Day 45 after embryo transfer. Data was transformed by square root and then analyzed by ANOVA. Pregnancy rates in *Bos indicus* embryos thawed and re-hydrated in Holding medium were higher (21/43; 49%) than those transferred directly into the recipients (13/56; 23%; P=0.0074). However, pregnancy rates with *Bos taurus* embryos did not differ between those re-hydrated (30/60; 50%) and those transferred directly into recipients (20/56; 36%, P= 0.12). In conclusion, data suggest that re-hydration after thawing maybe a valuable alternative for the transfer of *Bos indicus* embryos frozen in EG.



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### **Study of the follicular population in zebu females subjected to a FTAI protocol: preliminary results**

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**Keywords:** antral follicles count, *Bos indicus*, FTAI.

This study aimed to characterize the follicular population in Zebu females subjected to a FTAI protocol and then compare that between different animal categories (CAT) and postpartum intervals (IPP). For that, 529 Nelore females, with body score of  $2.69 \pm 0.39$  (range 1-5), were synchronized according to Ferraz et al. (2010, *Acta scientiae veterinariae* 38, 728). In D4 protocol, females were evaluated by transrectal ultrasonography (Pie-Medical, 100 Falco, Sao Paulo, Brazil) using a linear transducer (8 Mhz) for determining the follicular population by counting the antral follicles  $\geq 3$  mm. For comparison of the follicular population between CATs, females were grouped into: CC (calved cows, n=351), NC (nonlactating cows, n=71) and NUL (nulliparous heifers, n=107). For comparison of the follicular population between the IPPs, the CC were subdivided into IPP1 (n=28), IPP2 (n=101) and IPP3 (n=160) presenting respectively,  $\leq 50$ , between 50 and 71 and  $\geq 71$  days postpartum. The characteristics of interest were analyzed using the ANOVA, with  $P < 0.05$ . The females showed an average follicular population of  $46.52 \pm 22.47$  follicles (FOL), verifying individual variability for this trait, ranging from 7 to 145 FOL/animal. According to this follicular variation and the greatest ovarian reserve intrinsically presented by the Zebu cattle, it was suggested a specific classification for this subspecies, which was considered low follicular population  $\leq 34$ , intermediate follicular population between 34 and 53 and high follicular population  $\geq 53$  FOL/animal. It was observed that of all females evaluated, 32.14% (170/529); 33.84% (179/529) and 34.03% (180/529) belonged to the categories of low, intermediate and high follicular population, respectively. The average follicular population for CC was  $46.75 \pm 23.50$  FOL not differing ( $P > 0.05$ ) from those obtained for the NC ( $47.87 \pm 22.41$  FOL) and NUL ( $44.88 \pm 18.84$  FOL). In respect to the IPP there was no difference ( $P > 0.05$ ) amongst the averages of follicular population for the IPP1, IPP2, IPP3 and being, respectively, of  $41.39 \pm 16.14$ ;  $49.06 \pm 22.94$  and  $48.66 \pm 25.31$  FOL. The results suggest that there is a great variation in the follicular population in Zebu females. The CAT and the IPP do not seem to affect the number of antral follicles observed for this subspecies, suggesting that the follicular population is an individual characteristic and that are compensatory mechanisms, which keep the number of follicles constant, independently of physiological status. Thus, future studies should provide a better understanding of the physiology behind antral follicle numbers in Zebu, as well as the effect of this characteristic on some reproductive parameters of fertility in cattle.



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### **Comparison of conception rates in suckled Nelore cows with uterine symmetry or asymmetry submitted a FTAI**

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**Keywords:** asymmetry, symmetry, uterus.

The FTAI in Nelore cows with 30 to 60 days postpartum is performed with the purpose of obtaining 12 month calving interval. However, some cows have uterine asymmetry, consistent with the late puerperal period. The aim of this study was to compare the conception rate of cows with uterine symmetry or asymmetry. Nelore cows (n=319) with 30 to 45 days postpartum and body condition score between 2.5 and 3.0 (range: 1 to 5), on pasture conditions from a farm at Congoinhas, Parana state. Animals were subjected to rectal palpation for the evaluation of uterine involution and presence of uterine asymmetry and transrectal ultrasonography for the presence of uterine fluid. Animals were divided into two experimental groups of cows with uterine symmetry (G-S, n=169) or uterine asymmetry (G-AS, n=150). All cows were submitted to the same synchronization protocol. They received an intravaginal device with 1.9 g of progesterone (CIDR, Pfizer, Brazil) and 2 mg intramuscular injection of estradiol benzoate (EB; Estrogin, Farmavet, Brasil). Seven days later 12.5 mg IM dinoprost (Lutalyse®, Pfizer, Brazil) was given. On the ninth day all the devices were withdrawn and the cows received 1 mg estradiol cypionate (ECP, Pfizer, Brazil) intramuscularly, plus 400 IU intramuscular injection of eCG (Novormon, MSD, Brazil). All cows were inseminated 48h after implant removal. The pregnancy diagnosis was made by transrectal ultrasonography 30 days after artificial insemination. Data were analyzed by ANOVA ( $P<0.05$ ). Conception rates for G-S and G-AS were 48.2% (82/169) and 61.3% (92/150), respectively ( $P<0.05$ ). The results showed that uterine asymmetry did not reduce conception rate of Nelore cows with 30-45 days postpartum.



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### **Effect of meloxicam on conception rates of high-producing Holstein cows submitted to ET, AI or FTAI during summer and winter**

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**Keywords:** conception rate, high-producing dairy cows, meloxicam.

The aim of this study was to evaluate the effect of meloxicam on conception rates at 30 (CR30) and 60 (CR60) days, and pregnancy loss (PL) of high-producing Holstein cows submitted to ET, AI or FTAI during summer and winter. Repeat breeder recipients (n=492) with 1.96±1.08 (average±SE) lactations, 320.73±6.55 DIM, 6.9±0.15 services and 24.93±0.30 kg milk/day were used for ET. For AI we used freshly calved cows (n=312) with 73.98±1.16 DIM, 33.44±0.35 kg milk/day and 2.06±0.06 lactations from natural estrus observation or induced by 500 µg of cloprostenol (PGF2α; Sincrocio®, Ourofino, Cravinhos, Brazil). For FTAI, freshly calved cows (n=336) with 67.30±0.83 DIM, 11.91±0.01 lactations and 34.12±0.02 kg milk/day were synchronized with 2 mg of EB and a P4 device (Sincrogest®, Ourofino); on D8 the device was removed and 400 IU of eCG (Folligon®, Intervet, Boxmeer, Netherland), 1 mg of estradiol cypionate (E.C.P®, Pfizer, Paulínia, Brazil) and 500 µg of cloprostenol (Sincrocio®, Ourofino) were administrated i.m. In all biotechnologies the cows were evaluated and allocated in one of two experimental groups: Control Group and Meloxicam Group. Cows from Meloxicam Group received a dose of 2% meloxicam (0.5 mg/kg of body weight; Maxicam®, Ourofino) on days 14, 15 and 16 of the possible pregnancy. Pregnancy diagnosis was done at 30 and 60 days of pregnancy. Statistical analysis was done by logistic regression using GLIMMIX of SAS procedure. There was no interaction (P>0.05) between treatment and season for ET and FTAI. In addition, there was no statistical difference (P>0.05) between Control group and Meloxicam group for CR30, CR60 and PL in all biotechnologies. For ET cows, there was an effect of season for CR30 [summer = 41.9% (102/243) and winter = 32.5% (81/249); P=0.03]. For FTAI cows, CR30 (P=0.01) and CR60 (P=0.01) were less in summer [19.6% (21/107) and 16.8% (18/127)] than winter [39.7% (91/229) and 34.9% (80/229)]. For AI cows, there was no effect of season for CR30 (P=0.15), CR60 (P=0.12), or PL (P=0.95). However, there was a tendency for an interaction between treatment and season (P=0.06) for PL [Control summer= 7.1% (1/14); Control winter= 21.2% (7/33); Meloxicam summer= 38.5% (5/13); Meloxicam winter= 14.3% (6/42)]. In conclusion, treatment with meloxicam on days 14, 15 and 16 of a possible pregnancy did not improve conception rates at 30 and 60 days and did not reduce pregnancy loss in high-producing Holstein cows submitted to ET, AI, or FTAI.

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### **Effect of hCG on day 5 after synchronization of ovulation on luteal tissue volume and circulating progesterone concentration in primiparous and multiparous lactating Holstein cows**

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**Keywords:** CL, hCG, progesterone.

In a previous study we found that hCG treatment on Day 5 after fixed time AI increased pregnancies per AI in primiparous (PP, +10%) although no change was found in multiparous (MP, -0.3%) cows. The aim of this study was to evaluate the changes in progesterone (P4) concentrations and total volume of the corpus luteum (CL) to determine if there were parity effects that might explain the enhancement of fertility after hCG. Lactating Holstein cows (n=79) were divided in PP (n=26) with no further treatment (Control-PP, n=12) or treatment with 2,000 IU of hCG im (hCG-PP, n=15) and MP (n=53) with no further treatment (Control-MP, n=26) or treatment with 2,000 IU of hCG im (hCG-MP, n=27). Transrectal ultrasound examinations were performed on all animals on Days (D) 5, 8, 12, 19 and 22 after synchronization of ovulation in order to measure the primary and any accessory CL after hCG treatment. Blood samples from coccygeal venipuncture on D 5, 8, 12, 15, 19 and 22 after ovulation were used to evaluate the P4 concentration by radioimmunoassay. The hCG injection induced ovulation of the dominant follicle on D 5 of the estrous cycle either in PP (13/15, 86.6%) or MP (23/27, 85.2%) cows. Positive correlations were detected between circulating P4 and total CL volume in PP ( $r^2=0.50$ ) and MP ( $r^2=0.38$ ) cows with a stronger relationship in hCG-treated ( $r^2=0.47$ ) than controls ( $r^2=0.25$ ). Circulating P4 and CL volume were compared using repeated measures (Proc Mixed, SAS). Treatment with hCG increased ( $P<0.05$ ) circulating P4 in both PP and MP cows on Days 12 (7d after hCG), 15, 19, and 22 and tended ( $P=0.08$ ) to increase P4 in PP on D 8. However, the increase in circulating P4 was more profound in PP than MP cows. For example, on D 19 hCG treatment increased P4 to 161% of control values in PP (7.70 vs. 4.78 ng/mL) but only 118% in MP cows (6.04 vs. 5.13 ng/mL). There were no parity differences in circulating P4 in control cows on any day. However, there were significantly greater P4 in hCG-treated PP than MP cows on D 8 and 19; as well as tendencies for differences on D 12, 15, and 22. In contrast, there was no parity effect on the increase in CL volume induced by hCG with significant ( $P < 0.01$ ) increases in CL volume due to hCG on D 12 (138% vs. 143%), 19 (166% vs. 172%), and 22 (212% vs. 169%) in both PP and MP cows, respectively. There were no effects of parity on CL volume when comparing PP to MP cows in control or hCG-treated cows. Thus, parity differences in the fertility response to hCG may be partially explained by a later and less profound increase in circulating P4 in MP than in PP cows. This parity effect in circulating P4 was not explained by differences in CL volume induced by hCG.



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## **Risks associated with the conception of the first artificial insemination in lactating Holstein cows postpartum**

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**Keywords:** conception, Holstein, postpartum.

The aim of present study was to evaluate the factors associated with conception rate (CR) at first insemination (AI) of postpartum lactating Holstein cows. It was considered: 1) milk yield first 100 days of lactation (MP100d); 2) parity (primiparous vs. multiparous), and 3) season of insemination (summer/autumn and winter/spring). The study was conducted in a commercial farm (Fazenda Santa Rita - Agrindus S/A, Descalvado-SP) and it was related to 6,149 AIs from 2007 to 2011. Statistical analysis was performed using the Proc GLIMMIX of SAS. The CR 30 days after AI was affected by parity (Primiparous = 36.1%; 891/2,468 vs. Multiparous = 24.3%, 882/3,624, P<0.0001) and by the calving season (winter/spring = 30.5%, 1,006/3,299 vs. summer/autumn = 27.3%, 752/2,753), P<0.0001). There was interaction (P<0.0001) between the MP100d and parity on the CR. Therefore, the data were analyzed separately. In primiparous, the increase of MP100d has reduced (P<0.0001) the CR [Q1 (1,500.0 to 2,646.6 L) = 40.9%<sup>a</sup>, 248/606 vs. Q2 (2,646.7 to 3,026.5 L) = 42.9%<sup>a</sup>, 261/608 vs. Q3 (3,026.6 to 3,399.4 L) = 34.8%<sup>b</sup>, 211/606 vs. Q4 (3,399.5 to 5,791.3 L) = 25.7%<sup>c</sup>, 156/608]. However, in multiparous, it was observed an interaction between MP100d and season of AI on the CR. During the winter/spring, CR was not affected (P>0.05) by MP100d [Q1 (1,500.0 to 2,710.2 L) = 22.7%, 100/441 vs. Q2 (2,710.1 to 3,197.2 L) = 27.1%, 147/543 vs. Q3 (3,197.3 to 3,667.5 L) = 28.8%, 169/586 vs. Q4 (3,667.6 to 5,699.0 L) = 26.9%, 146/543], however higher MP100d (Q4) reduced the CR in cows breed during the summer/autumn (Q1 = 23.4%<sup>a</sup>, 109/466 vs. Q2 = 21.8%<sup>a</sup>, 78/358 vs. Q3 = 25.3%<sup>a</sup>; 82/324 vs. Q4 = 14.1%<sup>b</sup>, 51/363, P<0.0001). Thus, it can be concluded that multiparous cows or cattle inseminated during summer/autumn are associated with lower risk to conceive to the first AI postpartum. Also MP100d affects CR of multiparous during summer/autumn, and affects CR of primiparous cows, regardless the season.

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### **Influence of endogenous progesterone in follicular growth and conception rates of zebu cows submitted to FTAI**

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**Keywords:** bovine, corpus luteum, FTAI.

The combination of estrogen and progesterone (P4) is used at the beginning of the protocol of fixed-time artificial insemination (FTAI) for inducing follicular atresia and the emergence of a new synchronized wave. However, some studies suggest that the synergism between the concentrations of P4 supplied by devices containing more than 1g of this hormone, when added to those produced by the corpus luteum (CL), could interfere on the frequency of LH pulses, affecting follicular growth and the conception rate. Thus, this work aimed to evaluate the effect of the presence of CL previously to the FTAI protocol in the diameter of the ovulatory follicle and the conception rate of zebu cows. We used 269 Nelore cows with average body condition score of  $2.82 \pm 0.6$  (scale 1-5) and average postpartum interval of  $82.4 \pm 8.0$  days. All cows were evaluated by transrectal ultrasonography, in order to detect the presence or absence of CL, and then divided into two experimental groups: Group with CL (n = 79) and the Group without CL (n = 190). After that, they were submitted to the following hormonal protocol: in a random stage of the estrous cycle (D0) all animals received an intravaginal device of P4 (Sincrogest®, Ourofino, São Paulo, Brazil) and an application of 2.0 mg of estradiol benzoate (Sincrodiol®, Ourofino), intramuscular (im). On D8, the device was removed and 500 µg sodium cloprostenol (Sincrocio®, Ourofino), 1.0 mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) and 300 IU of equine chorionic gonadotropin (Folligon®, MSD Saúde Animal, São Paulo, Brazil) were injected im. On D10, the measurement of diameter of the ovulatory follicle was performed by transrectal ultrasonography and all cows were inseminated. The pregnancy diagnosis was performed 30 days after insemination by transrectal ultrasonography using a 5.0 MHz linear transducer (Mindray, DP2200vet, São Paulo, Brazil). The data were analyzed by SPSS software ( $P < 0.05$ ) and the variables were compared by Chi-square test. The results found for the diameter of the ovulatory follicle did not differ ( $P = 0.22$ ) for cows in the group with CL ( $12.20 \pm 3.01$  mm) compared to females without CL ( $12.68 \pm 3.08$  mm). However, there was difference ( $P = 0.047$ ) for the conception rate of the group with CL (51.9%) compared to the cows without CL (64.7%). Thus, the presence of CL at the beginning of the protocol did not influence the diameter of ovulatory follicle; however, higher concentrations of progesterone, possibly displayed in these animals may have influenced events related to the ovulation to compromise conception rate.



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### **Timing of timed artificial insemination in the three-handling protocol using sex-sorted sperm in Nelore cows**

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**Keywords:** sex-sorted sperm, TAI, three handling.

Previous studies have shown that it is possible to obtain satisfactory conception rates at timed artificial insemination (TAI) when estradiol benzoate (EB) is used at the time of progesterone device removal (P4) using either non sex-sorted or sex-sorted sperm. The aim of this study was to compare two different moments of insemination after P4 device removal (42 vs. 50 h) in lactating Nelore cows inseminated with sex-sorted sperm. For this purpose, a protocol of synchronized ovulation with three managements using EB at the time of P4 device removal, was used. A total of 369 cows between 30 and 60 days postpartum were randomly assigned to two treatments (TAI 42 vs. 50 h). The cows were treated with P4 devices (DIB®, MSD Animal Health) and with 2 mg of EB i.m. (Gonadiol®, MSD Animal Health). Nine days later, the P4 devices were removed and 0.3975 mg of cloprostenol sodium (Ciosin®, MSD Animal Health), 300 IU of equine chorionic gonadotropin (eCG; Novormon®, MSD Animal Health) plus 1 mg of EB were administered i.m. The TAI was performed 42 (n = 187) or 50 h (n = 182) after removal of the P4 device using sex-sorted sperm (purity 85%, Sexing Technologies Brazil, Sertãozinho-SP) from two Nelore bulls. Pregnancy diagnosis was performed 30 days after TAI and the data analyzed by the GLIMMIX procedure of SAS. There was no difference (P = 0.77) on conception rate from females inseminated at 42 (32.6%, 61/187) or 50 hours (33.0%, 60/182) after removal of the P4 device. There was also no difference between bulls (Bull 1 = 35.7%, 65/182 vs. Bull 2 = 30.0%, 56/187, P = 0.24) and no interaction between treatment and bull (P > 0.05) was found. It was concluded that the TAI with sex-sorted sperm after the protocol of three managements using EB to induce ovulation may be performed either at 42 or at 50 hours after P4 device removal.



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### **Risk factors for a Holstein cow becoming a repeat breeder**

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**Keywords:** Holstein, milk production, repeat breeder.

The aim of the present study was to evaluate the risk factors associated with a Holstein cow becoming a repeat breeder (RB; > 3 services) in a single lactation. The analyzed variables were the milk yield during the first 100 days of lactation (MP100d), parity (primiparous and multiparous) and days in milk (DIM) at first service (DIM1: 45-63; DIM2: 64-72; DIM3: 73-88, and DIM4: 89-199 days). The analyzed data were obtained from Fazenda Santa Rita (Agrindus S/A, Descalvado - SP, Brazil) and were related to 5,732 lactations, totaling 24,185 artificial inseminations performed from 2007 to 2011. Only cows that received four or more services in a single lactation were considered RB. Statistical analysis was performed using the SAS GLIMMIX. There were no effects of year ( $P = 0.44$ ) or interaction of year and parity ( $P = 0.85$ ), year and MP100d ( $P = 0.36$ ) or year and DIM at first service ( $P = 0.21$ ) on the proportion of cows that became a RB. There was an effect of parity (primiparous = 41.9%; 964/2,299 vs. multiparous = 51.9%, 1,783/3,433,  $P < 0.0001$ ), but no effect of the interval between calving and first service (DIM1 = 47.9%, 792/1,653; DIM2 = 46.6%, 578/1,241; DIM3 = 47.3%, 673/1,422, and DIM4 = 49.7%, 704/1,416;  $P = 0.21$ ). Furthermore, no interactions were found between interval from calving to the first service and the parity ( $P = 0.28$ ). There was a significant interaction between parity and MP100d ( $P < 0.0001$ ) on the risk of cows becoming a RB. Thus, the effect of MP100d on the risk of a cow becoming a RB was analyzed separately for primiparous and multiparous cows. An increase in MP100d increased the risk of a cow becoming a RB either in primiparous (Q1: 1,500.0 to 2,649.2 = 20.4%<sup>d</sup>, 117/575; Q2: 2,649.3 to 3,092.7 = 32.4%<sup>c</sup>, 186/575; Q3: 3,092.8 to 3,530.2 = 45.5%<sup>b</sup>, 261/574, and Q4: 3,530.3 to 5,465.3 = 69.5%<sup>a</sup>, to 399/574,  $P < 0.0001$ ) or multiparous (Q1: 1,500.0 to 2,693.7 = 35.9%<sup>c</sup>, 308/859; Q2: 2,693.8 to 3,180.3 = 50.4%<sup>b</sup>, 433/859; Q3: 3,180.4 to 3,638.0 = 54.7%<sup>b</sup>, 469/857, and Q4: 3,638.1 to 5,699.0 = 66.8%<sup>a</sup>, 573/858,  $P < 0.0001$ ) cows. Thus, higher milk yield during the first 100 days of lactation and also later parity increase the risk for a Holstein cow becoming a RB during that lactation.

**Acknowledgments:** Agrindus S.A.



A055 FTAI, FTET and AI

### **Follicular growth and ovulation in ewes receiving hormone injections in the Bai Hui acupuncture point**

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**Keywords:** acupuncture, estrus, ewe.

This study aimed to evaluate follicular growth and ovulation of ewes subjected to synchronization protocols with application of PGF2 $\alpha$  and eCG at Bai Hui acupoint. Intravaginal sponges impregnated with of 60mg medroxyprogesterone acetate (Progespon®, Syntex, Argentina) were inserted in 30 Santa Ines ewes for 7 days. After this application, five treatments (T) were tested: T1: 0.125mg of cloprostenol (Ciosin®, Coopers, Brasil) (100% of dose) and 300IU of eCG (Folligon®, Intervet, Brasil) (100% of dose) injection intramuscularly (IM); T2: 0.0375mg of cloprostenol (30% of dose) at Bai Hui acupuncture point and 300IU of eCG (100% of dose) injection IM; T3: 0.125mg of cloprostenol (100% of dose) injection IM and 90IU of eCG (30% of dose) at Bai Hui acupuncture point, T4: 0.0375mg of cloprostenol (30% of dose) and 90IU of eCG (30% of dose) both in the Bai Hui acupuncture point and T5: 0.0375mg of cloprostenol (30% of dose) and 90IU of eCG (30% of dose) both at acupuncture false point (IM). The animals were monitored for follicular growth and ovulation every 12h. The parameters were assessed using the Variance Analysis, with 5% significance level. There was no difference ( $P>0.05$ ) for the interval between sponge removal to ovulation [T1 - 70 (14), T2 - 72 (7), T3 - 78 (12), T4 - 81 (13), and T5 - 67 (16h)]; interval between beginning of estrus and ovulation [T1 - 28 (14), T2 - 28 (6), T3 - 24 (7), T4 - 28 (10), and T5 - 24 (14h)]; ovulation number [T1 - 1.5 (0.5), T2 - 1.3 (0.5), T3 - 1.3 (0.5), T4 - 1.6 (0.5), and T5 - 1.2(0.4)]; follicular growth rate [T1 - 0.81 (0.26), T2 - 0.93 (0.36), T3 - 0.72 (0.28); T4 - 0.77 (0.27), and T5 - 0.95 (0.55mm/day)]; diameter of the largest follicle [T1 - 6.4 (0.7), T2 - 6.3 (0.6), T3 - 6.6 (0.7), T4 - 6.9 (0.8), and T5 - 7.2 (0.8mm)], and diameter of the second largest follicle [T1 - 5.3 (1.1), T2 - 5.6 (0.8), T3 - 5.3 (1.0), T4 - 5.2 (1.3), and T5 - 5.6 (0.9mm)]. Thus, ovulation was effectively synchronized with progestagen associated with low doses of PGF2 $\alpha$  (0.0375mg cloprostenol) and eCG (90IU) independent of application site (the Bai Hui acupuncture point or in the acupuncture false point).



A056 FTAI, FTET and AI

## Efficiency of treatment with different doses of eCG in dairy buffaloes during the non breeding season

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**Keywords:** eCG, synchronized ovulation, buffaloes.

The aim of this study was to evaluate the efficiency of two commercial brands of equine Chorionic Gonadotropin (eCG; Folligon®, MSD Saúde Animal, Brazil and SincroeCG®, Ourofino Agronegócio, Brazil) in different doses (300 and 400 IU), by synchronizing ovulation in dairy buffaloes during the non breeding season. For this purpose, 68 non-cyclic dairy buffaloes were assigned according to the number of calvings, days postpartum, body condition score and ovarian activity in four groups: Folligon® 300 IU (GF300; n=17); SincroeCG® 300 IU (GS300; n=18); Folligon® 400 IU (GF400; n=16); SincroeCG® 400 IU (GS400; n=17). At random stage of the estrous cycle (D0; pm), the buffaloes received an intravaginal progesterone device (P4; Sincrogest®, Ourofino Agronegócio) and 2.0 mg intramuscular (im) of Estradiol Benzoate (Sincrodiol®, Ourofino Agronegócio). On D9 (pm), females received im 0.53 mg of PGF2 $\alpha$  (Cloprostenol, Sincrocio®, Ourofino Agronegócio) and 300 (GF300 and GS300) or 400 (GF400 and GS400) IU of eCG (Folligon®, MSD Saúde Animal or SincroeCG®, Ourofino Agronegócio), followed by P4 removal. On D11 (pm) 10  $\mu$ g of GnRH (Sincroforte®, Ourofino Agronegócio) was administrated im. The ultrasound evaluation (Mindray DP2200Vet, China) was performed on D0 to check ovarian activity, on D9 to verify the follicular diameter and from D11 to D14 (12/12h per 60h) to determine the time of ovulation. The experimental design was a 2x2 factorial (two commercial brands and two doses of eCG) and the variables were analyzed using the GLIMMIX procedure of SAS®. No interaction was verified between doses of eCG and commercial brands treatments ( $P>0.05$ ). Moreover, there were no differences between experimental groups (GF300, GS300, GS400 and GF400) to dominant follicle diameter ( $\emptyset$ ) on D11 (14.4 $\pm$ 0.8 mm, 13.5 $\pm$ 0.4 mm, 13.8 $\pm$ 0.7 mm, 13.7 $\pm$ 0.5 mm), to ovulatory follicle  $\emptyset$  (16.5 $\pm$ 0.5 mm, 15.0 $\pm$ 0.4 mm, 16.0 $\pm$ 0.5 mm, 15.7 $\pm$ 0.6 mm), to follicular growth/day (1.7 $\pm$ 0.3 mm, 1.7 $\pm$ 0.2 mm, 1.4 $\pm$ 0.2 mm, 1.6 $\pm$ 0.2 mm), to interval from device removal to ovulation (71.5 $\pm$ 1.9 h, 72.5 $\pm$ 4.0 h, 69.0 $\pm$ 3.3 h, 75.2 $\pm$ 3.1 h) and to ovulation rate [64.7% (11/17), 72.2% (13/18), 75.0% (12/16), 76.5% (13/17)], respectively. We conclude that the two commercial brands of eCG (Folligon® and SincroeCG®) at doses of 300 or 400 IU have shown similar efficiency to synchronize and induce ovulation in dairy buffaloes during the non-breeding season.



A057 FTAI, FTET and AI

### **Follicular dynamics in Holstein heifers subjected to short protocols (5 days) for TAI**

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**Keywords:** Holstein, short protocols, TAI.

The objective of this study was to evaluate follicular dynamics in Holstein heifers treated with short protocols for synchronization of ovulation using a progesterone-releasing intravaginal device (DispP4; DIB®, Merck Animal Health) or a norgestomet ear implant (NORG; Crestar®, Merck Animal Health), with or without GnRH on the first day of the protocol (D0). In Experiment 1 (Exp. 1), 24 heifers received Disp4 and were homogeneously allocated [presence of CL and follicles (fol)  $\geq 10$  mm] to either receive (GnRH+DispP4) or not (DispP4) GnRH (0.1mg gonadorelin; Fertagyl®, Merck Animal Health) on D0. In Exp. 2, 36 heifers were treated with NORG with or without GnRH on D0: DispP4, NORG and NORG+GnRH. In both Exp, the P4 source was withdrawn and PGF2 $\alpha$  (0.53mg Sodium cloprostenol; Ciosin®, Merck Animal Health) was administered on D5. On D8 GnRH was administered. Ultrasonography exams were performed every 24h from D0 to D7 and 12h apart until D10 PM. Statistical analysis was performed using PROC GLIMMIX of SAS. In Exp. 1, GnRH administration on D0 did not alter the follicular regression (50.0 vs. 50.0%,  $P=1.0$ ), and the rates of emergence (83.3 vs. 91.7%;  $P=0.6$ ) and synchronization (75.0 vs. 83.3%;  $P=0.6$ ) of the new follicular wave, and ovulation rate of a synchronized follicle (88.9 vs. 80.0 %,  $P=0.6$ ). Moreover, the day of follicular wave emergence ( $2.2 \pm 0.4$  vs.  $2.9 \pm 0.4$  d,  $P=0.2$ ), diameter ( $\emptyset$ ) of the ovulatory follicle ( $12.4 \pm 0.6$  vs.  $13.4 \pm 0.5$  mm,  $P=0.2$ ) and time of ovulation ( $90.0 \pm 5.7$  vs.  $96.0 \pm 5.1$ ,  $P=0.4$ ) were not altered; DispP4 and DispP4 + GnRH, respectively. In Exp.2, the implant type (DispP4 or NORG) and the concomitant application of GnRH with the NORG insert did not affect the day of the new wave emergence ( $2.9 \pm 0.4$  vs.  $1.9 \pm 0.3$  vs.  $2.2 \pm 0.4$  d,  $P=0.2$ ) and the rates of follicular regression (30.8 vs. 18.2 vs. 41.7%;  $P=0.5$ ), synchronization (84.6 vs. 90.9 vs. 83.3%,  $P=0.9$ ) and ovulation of the synchronized fol (72.7 vs. 40 vs. 70%,  $P=0.3$ ); DispP4, NORG and NORG+GnRH, respectively. However, the  $\emptyset$  of the ovulatory follicle was greater in heifers treated with NORG ( $14.0 \pm 0.4^b$  and  $13.6 \pm 0.4^b$  mm) compared to DispP4 ( $12.3 \pm 0.4^a$ ;  $P = 0.01$ ). Also, when NORG was used, it was found that the GnRH administration provided an appropriated time of ovulation (Disp4 =  $98.5 \pm 4.1^b$ ; NORG =  $76.4 \pm 4.3^a$ ; NORG+GnRH =  $86.8 \pm 4.3^{ab}$ ;  $P = 0.003$ ). Although the usage of DispP4 in short protocols dispensed the use of GnRH, which is assessed when using NORG, heifers treated with norgestomet had greater  $\emptyset$  of the ovulatory fol. Thus, among the tested protocols, NORG+GnRH seem to be a good option in TAI programs with AI 72h (Cosynch), by associating the greater  $\emptyset$  of the ovulatory fol and appropriated time of ovulation. However, the Disp4 protocol cannot be discarded. More studies are required to test the efficiency of these protocols.



A058 FTAI, FTET and AI

## Plasma profile of different doses of injectable progesterone in high yield dairy cows

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**Keywords:** cows of high production, plasmatic profile, progesterone.

We evaluated the plasma levels of progesterone (P4) in different doses of injectable P4 (Sincrogest Injectable, Ourofino Animal Health Ltda, Brazil) in cows of high milk production. After synchronization of ovulation, 29 lactating cows (producing  $30.9 \pm 6.8$  kg/day,  $41.0 \pm 12.3$  days in milk,  $1.4 \pm 1.3$  births and body condition score of  $2.8 \pm 0.5$  - scale 1-5) were selected for the study. Ten days after ovulation (ovulation identified by U.S.; D-1), cows received a norgestomet implant and 5 mg estradiol valerate (Crestar®, Intervet, Holland) and 500 µg cloprostenol (Sincrocio®, Ourofino Animal Health Ltda, Brazil). Blood samples were collected 0, 12 and 24 hours after implantation to identify the animals with lower levels of 1 ng/mL and only these were submitted to treatment 24 hours after the initial of the treatment (D0). At this time, the animals were distributed in four experimental groups according to the daily milk production, days in milk, number of births and body condition score: Control (n = 8) the cows received no treatment; G300 (n = 7) animals received 300 mg of P4 i.m.; G600 (n = 7) the cows received 600 mg of P4 i.m. and G900 (n = 7) animals received 900 mg of P4 i.m. Blood samples were collected serially from 24/24 hours until 216 hours after treatment to determine the plasmatic concentration of P4. The data were analyzed using the GLM and MIXED procedure of SAS. The concentrations obtained in the control group animals, G300, G600 and G900 were: 0h ( $0.40 \pm 0.98$ ,  $0.46 \pm 0.09$ ,  $0.44 \pm 0.10$ ,  $0.83 \pm 0.30$ ), 24 ( $0.16 \pm 0.03$ ,  $1.57 \pm 0.17$ ,  $2.23 \pm 0.13$ ,  $3.73 \pm 0.70$ ), 48h ( $0.33 \pm 0.09$ ,  $1.01 \pm 0.15$ ,  $1.5 \pm 0.18$ ,  $2.80 \pm 0.57$ ), 72h ( $0.11 \pm 0.10$ ,  $0.56 \pm 0.09$ ,  $0.97 \pm 0.30$ ,  $1.90 \pm 0.36$ ), 96h ( $0.00 \pm 0.0$ ,  $0.31 \pm 0.46$ ,  $0.51 \pm 0.11$ ,  $1.21 \pm 0.19$ ), 120h ( $0.03 \pm 0.02$ ,  $0.33 \pm 0.02$ ,  $0.71 \pm 0.10$ ,  $1.33 \pm 0.14$ ), 144h ( $0.06 \pm 0.5$ ,  $0.35 \pm 0.05$ ,  $0.70 \pm 0.16$ ,  $1.31 \pm 0.28$ ), 168h ( $0.02 \pm 0.02$ ,  $0.36 \pm 0.04$ ,  $0.87 \pm 0.15$ ,  $1.46 \pm 0.29$ ), 192h ( $0.03 \pm 0.03$ ,  $0.37 \pm 0.04$ ,  $0.67 \pm 0.11$ ,  $1.45 \pm 0.29$ ) and 216h ( $0.00 \pm 0.00$ ,  $0.23 \pm 0.04$ ,  $0.54 \pm 0.09$ ,  $1.29 \pm 0.34$ ). There was an interaction between treatment and time ( $P = 0.002$ ). The group G300 presented values higher than the control group only at the collection made 24 hours after administration. The groups G600 and G900 presented statistical difference from the Control group at 24 to 216 hours. We conclude that treatment with different doses of injectable P4 was effective in increasing plasmatic concentrations of P4 (24h after the treatment) and the circulating levels of P4 in the subsequent days are related to the dose administered that can persist until 9 days after treatment.



A059 FTAI, FTET and AI

### **Effect of eCG and/or temporary calf removal in pregnancy rate at TAI in Nelore suckled cows**

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**Keywords:** calf removal, cows, TAI.

The aim of this study was to evaluate the effects of temporary calf removal (TCR) and/or the use of eCG on follicular diameter ( $\emptyset$ ), ovulation rate (OR), pregnancy rate per insemination (PR/AI) and conception rate (CR) in Nelore suckled cows. The protocol for fixed-time artificial insemination (TAI) used was: day 0: estradiol benzoate (EB, 2 mg, im, Ciclovor®, Jofadel, BR) and insertion of progesterone device (P4) CIDR® (1.9 g, Pfizer Animal Health Animal BR); day 7: prostaglandin F2 $\alpha$  (PGF2 $\alpha$ , 12.5 mg, im, Lutalyse, Pfizer Animal Health, USA); day 9: estradiol cypionate, ECP® (0.5 mg, im, Pfizer Animal Health, BR); day 11: removal of P4 device and insemination (48 h after ECP®). The animals were randomized to receive on day 9 the following treatments: TCR for 48 hours, eCG (Novormon®, 300 IU, im, Intervet Schering-Plough, USA) or eCG + TCR. On day 18 blood samples were collected to determinate the concentration of P4. On day 11, the  $\emptyset$  was evaluated by ultrasound and 30 days after TAI the pregnancy diagnosis was made. The CR was calculated by the number of pregnant cows/ovulated (P4  $\geq$  1 ng/ml on day 18) and PR/AI by the number of pregnant cows inseminated. For analysis the P4 concentration and  $\emptyset$  only ovulated cows were considered. Data were analyzed by PROC GLIMMIX of SAS; differences were considered significant when P<0.05. In this study primiparous (n=258) and multiparous (n=1087) cows were used. The  $\emptyset$  in multiparous cows was higher (14.0  $\pm$  0.08 mm) than in primiparous (13.2  $\pm$  0.77 mm), no difference was observed between treatments (13.9  $\pm$  0.06 mm). The concentration of P4 in primiparous cows treated with eCG + TCR (5.25  $\pm$  0.29 ng/ml) was higher than in those with only TCR (3.78  $\pm$  0.29 ng/ml) or eCG (4.3  $\pm$  0.28 ng/ml), whereas no difference in multiparous cows was observed (4.98  $\pm$  0.07 ng/ml). The OR in primiparous cows treated by TCR was lower (81.6% [73/88]), than those treated by eCG (94.6% [78/83]) or by eCG + TCR (97.1% [84/87]). There was no difference in multiparous cows (95.7% [1041/1087]). In primiparous cows, the CR was lower in eCG group (47.5% [37/78]) than in TCR (61.7% [45/73]) or eCG + TCR group (61.6% [51/84]), and in multiparous cows it was lower for TCR + eCG (50.1% [162/331]) than in TCR only (58.8% [207/353]) or eCG only (56.6% [202/357]). The PR/AI was higher in primiparous cows treated by TCR + eCG (60.0% [51/87]) than in multiparous cows (47.9% [162/346]), and eCG and TCR were similar in primiparous (44.8% [37/83], 50.8% [45/88]) and multiparous cows (54.3% [202/373], 56.5% [207/368]), respectively. Primiparous have a lower  $\emptyset$ , possibly a larger input of gonadotropin is necessary for ovulation, whereas multiparous cows require a lower exogenous support; probably the TCR treatment with eCG overstimulated the LH concentration, so it affected the pregnancy rate.



A060 FTAI, FTET and AI

## Ovarian follicular dynamics in anestrous cows submitted to Tai protocol using injectable progesterone

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**Keywords:** follicular dynamics, injectable progesterone, TAI.

Two experiments were performed to compare the ovarian follicular dynamics in anestrous cows submitted to TAI protocol based on injectable P4 or intravaginal P4 device. In the experiment I (n=40) and II (n=22) multiparous Nelore cows with 72 to 84 months of age, BCS 2.5 to 3.5 and weight of 438.8±49.1 kg were used. The anestrus was considered at 45 to 65 days postpartum, without CL and no follicles ≥ 8 mm. In the experiment I, 20 cows of control group (CG) received an intravaginal device with 1g P4 (DIB®, Syntex, Argentina) and 2mg EB (Benzoato de Estradiol Syntex®, Syntex, Argentina) IM on D0. The device was removed in D8 and animals were injected with 500µg cloprostenol (Cyclase® DL, Syntex, Argentina), 300IU eCG (Novormon®, Syntex, Argentina) and 1mg EC (Cipiosyn®, Syntex, Argentina) IM. The TAI was performed on D10. Injectable P4 group (IG), 20 cows received 350mg injectable P4 SC and 2mg EB IM on D0. On D6, cows received 500µg cloprostenol, 300IU eCG and 1mg EC IM. The TAI was performed on D8. In experiment II the same protocols were used, but both the CG (n=11) and IG (n=11) cows received 1mg EB to induce ovulation. The time of protocol with injectable P4 was established due to results of previous studies. Ultrasonography evaluations were performed on D0, D4 and every 24h until TAI and then every 12h until ovulation and for 12 days after TAI for the measure of the CL. It was evaluated the presence of follicles > 5mm on D4, the diameter of preovulatory follicle, the ovulation rate and the size of CL. The data were analyzed in MINITAB 16, using ANOVA and T, Fisher and Chi-Square tests, with significance at P≤0.05. The number of cows with follicles > 5mm on D4 did not differ between the CG 65% (5.9±0.6mm) and IG 65% (6.5±0.7mm) in experiment I, and CG 54% (6.6±0.9mm) and IG 54% (6.8±0.8mm) in experiment II. There was not difference in the diameter of preovulatory follicle of CG (11.7±1.4mm) and IG (11.6±1.6mm) (P=0.88) in experiment I. In experiment II, the preovulatory follicle of CG (11.9±2.1mm) was greater than IG (9.8±2.0mm) (P=0.05). The ovulation rate within 36h after TAI was higher in CG 90% vs. IG 20% (P=0.001) in experiment I, and CG 100% vs. IG 45% (P=0.01) in experiment II. There was not difference in CL size between the CG (18.7±4.3mm) and IG (17.7±3.6mm) (P=0.58) in experiment I, as well as between the CG (17.4±3.3mm) and IG (15.3± 2.7mm) (P=0.17) in experiment II. We believe that the lower ovulation rate in IG is related to the time metabolism of injectable P4. It is possible that there is individual difference on P4 metabolism, resulting in variables levels of LH. The ovulation rate after injectable P4 was lower than the implant and further studies are necessary to better clarify the use of injectable P4 at TAI protocols.



A061 FTAI, FTET and AI

### **Effect of administration of eCG 7 days after FTAI on development of ovarian structures and pregnancy rates in beef cows**

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**Keywords:** corpus luteum, eCG, FTAI.

The early embryo development is affected by the progesterone concentration, especially on the first weeks after conception. The use of treatments to increase luteal function in different periods after FTAI has been tested recently to improve embryo survival. The objective of the experiment was to evaluate the effect of treatment with eCG 7 days after the FTAI on the corpus luteum (CL) development, follicular growth and pregnancy rates in beef cows. A hundred seventy nine Brangus cows were synchronized to FTAI using intravaginal implants containing 1g of progesterone (DIB, Schering-Plough, Brazil) for 8 days and injecting 2 mg of Estradiol Benzoate (EB) (Gonadiol, Schering-Plough, Brazil), on the first day of treatment. All cows received 150 mcg of D-Cloprostenol (Veteglan, Hertape Calier, Brazil), i.m., on Day 8 and 24 hours after, 1 mg of EB, i.m. The timed inseminations were 52-56 hours after the implant removal. Seven days after the insemination, the cows were randomly assigned in two different groups according the treatment: eCG (n=41) injection of 400 IU, i.m. of eCG (Novormon, Schering-Plough, Brazil) and Control (n=138) receiving saline solution, i.m. The ultrasonographic examinations (Chison 8300, linear transducer, 5MHz, China) were done on days 0, 7 and 12 to determine the presence and diameter of follicles and the area of CL on the subgroups eCG (n=26) and Control (n=18). The pregnancy diagnoses were done 30 days after FTAI. The statistical analyses were performed using the SPSS 18 for Windows, pregnancy rates were compared by chi-square test, other parameters by Student t test. The dominant follicle did not differ between the groups on the Day 0 (eCG=9.62±4.07 mm; Control=11.58±2.19 mm) (P=0.06). The measurement of the CL area (cm<sup>2</sup>) on D7 presented similar values (eCG=2.05±0.65; Control=2.31±0.89) (P=0.31). However, after the administration of eCG on D7, it was verified by ultrasonography that the mean CL area on D12 on this group was 3.06±1.22 cm<sup>2</sup>, while on Control group was 1.96±1.54 cm<sup>2</sup> (P<0.01). The measurement of dominant follicle diameter on D7, did not have statistical difference between groups (eCG=5.52±3.31 mm; Control=7.32±3.60 mm), but, on D12, eCG treated cows had significantly larger follicles (10.14±7.19 mm) in comparison with the saline treated (2.23±4.21 mm) (P=0.02). The pregnancy rates of FTAI were 60.98% (25/41) and 40.58% (56/138) to eCG and Control, respectively (P=0.02). These results indicate that treating cows with eCG 7 days after FTAI promotes development of CL, increased size of follicles on D12 and determined higher pregnancy rates when compared to the Control group.



A062 FTAI, FTET and AI

### **Size of the uterus and induction of ovarian cyclicity in prepubertal Nelore heifers treated with progesterone**

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**Keywords:** heifers, ovary, progesterone.

The aim of this study was to compare the size of the uterus and induction of ovarian cyclicity in prepubertal heifers. Fifty seven Nelore heifers with 22 and 24 months, in a body condition score between 3.0 and 3.5 (Range: 1.0 to 5.0) on grazing conditions from a farm at Congoinhas, Paraná state were used. The heifers were evaluated by transrectal ultrasound scanning (Mindray 2200, 7.5 MHz) to measure the uterine diameter, obtained from the three cross sections and averaging between dimensions. The cyclicity was evaluated prior to hormone treatment by the presence of CL in two ultrasound examinations ten days apart. The heifers were treated with intravaginal progesterone devices previously used for 32 days (CIDR, Pfizer, Brazil). Ten days later the devices were removed and were administered 1 mg of Estradiol benzoate (EB; Estrogen, Farmavet, Brazil), intramuscularly (IM). On the tenth day all the devices were withdrawn and the heifers received 1 mg intramuscular of EB. Seven days later ultrasound was performed to measure uterine size and early FTAI treatment. They received a progesterone intravaginal device previously used for 24 days (CIDR, Pfizer, Brazil) and 2 mg intramuscular injection of estradiol benzoate (EB; Estrogen). Seven days later it was given 12.5 mg IM dinoprost (Lutalyse®, Pfizer, Brazil). On the ninth day, all the devices were withdrawn and the heifers received 1 mg intramuscular of estradiol cypionate (ECP, Pfizer, Brasil), plus 400 IU intramuscular injection of equine chorionic gonadotropin (Novormon, MSD, Brasil). All animals were inseminated 48h after implants removal. The pregnancy diagnosis was made by transrectal ultrasonography after 35 days from artificial insemination. Data were analyzed by ANOVA ( $P < 0.05$ ). The uterine diameter was different ( $P < 0.05$ ) between measurements prior to and after treatment with progesterone ( $11.2 \pm 1.7$  vs.  $12.4 \pm 2.2$  mm). The induction of cyclicity occurred in 50.9% (29/57), and it was similar between heifers with CL ( $n = 29$ ) and without CL ( $n = 28$ ) ( $P < 0.05$ ). Size of the uterus was also similar ( $12.4 \pm 1.8$  vs.  $12.3 \pm 2.6$ , respectively). The conception rate was 47.4% (27/57). From preliminary results it is concluded that treatment with estradiol and progesterone previously to the hormone treatment for FTAI can induce cyclicity and increase in uterine diameter of prepubertal heifers.



A063 FTAI, FTET and AI

### Evaluation of ovulation synchronization protocols using FSH-p or eCG in beef cows

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**Keywords:** beef cattle, FSH-p, FTAI.

The present experiment aimed to evaluate follicular development and pregnancy rate in suckled beef cows treated with different doses of FSH-p (25 and 50 IU) or eCG (400 IU), without the temporary weaning of calves between implant removal and TAI. The experiment was conducted on two farms in Rio Grande do Sul, Brazil. Four-hundred and two Aberdeen Angus cows (n=121 at location 1 and n=280 at location 2) were synchronized with a protocol based on progesterone and estradiol. On day 0, intravaginal implants containing 1g of Progesterone (DIB, Shering-Plough, Brazil), were inserted and administered 2mg of Estradiol benzoate, BE, (Gonadiol, Shering-Plough, Brazil), i.m. On day 7, intravaginal implants were removed and 150µg of D-cloprostenol (Veteglan, Hertape Calier Saúde Animal, Brazil) were administered i.m. At this time (D7), four groups were assigned according to the treatments: Pluset25 (n = 98) 25 IU of FSH-p, i.m.; Pluset50 (n = 95) 50 IU of FSH-p, i.m. (Pluset, Hertape Calier Saúde Animal, Brazil); eCG (n = 106) 400 IU of eCG (Novormon, Shering-Plough, Brazil), i.m.; and Control (n = 103). On day 8, all cows were injected with 1 mg EB, i.m. On day 9, cows were inseminated in the period 52-56 hours after the implant removal. The ultrasonographic examinations of the ovaries (Chison 8300 Vet, transrectal transducer, 5 MHz, China) were done at the time of P4 device removal (D7) and at the TAI (D9) to measure follicular diameter. The pregnancy diagnosis was done 30 days after AI. Pregnancy rates were analyzed by chi-square test, ovarian parameters by ANOVA and the means compared by Tukey test and considered significant at 5%. The follicular diameter did not differ significantly between the groups: Pluset25 (D7 = 9.64±1.86 and D9 = 11.53±1.95 mm), Pluset50 (D7 = 9.85±2.75 and D9 = 11.82±2.74 mm), eCG (D7 = 10.07±2.07 and D9 = 12.73±2.51 mm) and Control (D7 = 10.36±2.29 and D9 = 12.57±1.97 mm). The daily follicular growth was greater in eCG group (1.33±0.56 mm/day) compared to Pluset25 group (0.94±0.53 mm/day) and similar to Pluset50 (1.11±0.48 mm/day) and Control (1.10±0.61 mm/day) groups. Pregnancy rates were similar in groups Pluset25 (52%), eCG (56.6%) and Control (50.4%) and higher when compared to Pluset50 (15.9%). Possibly, the higher dose of Pluset negatively affected the maturity of ovulatory follicle due to the level of LH in this product. The eCG and Pluset25 groups had higher frequency of cows with ovulatory follicles greater than 11mm, but treatments did not improve pregnancy rates.

**Acknowledgment:** Hertape Calier.



A064 FTAI, FTET and AI

### **Subclinical mastitis impact in the reproduction in lactating cows**

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**Keywords:** embryo transfer, mastitis, reproduction.

Studies indicate clinical and subclinical mastitis generate negative effects in reproductive performance (Hansen et al., 2004, American Journal of Reproductive Immunology 51:294–301; Santos et al., 2010, Animal Reproduction Science 80:31–45; Schrick et al., 2001, J. Dairy Sci.84:1407–1412). A study was conducted to evaluate the effects of subclinical mastitis on embryo survival in dairy cows. Milk samples were frozen and sent to a commercial lab (Vidavet, Botucatu – SP, Brazil) for culture and bacterial diagnostic (NMC, 1999, Laboratory Handbook on Bovine Mastitis, Madison WI). Data were used from 634 cows in lactation that received IVF embryos (DIM  $122 \pm 89$ , yielding  $17 \pm 7.5$  kg/milk/day). Before the timed embryo transfer (TET), composite milk samples (from the four teats) for microbiological culture were collect during the milking. The bacterias were classified in environmental Gram-positive bacteria (*Bacillus* spp., *Enterococcus* spp., *Streptococcus* spp.), environmental Gram-negative bacteria (*Coliformes*, *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp.), and contagious Gram-positive bacteria (*Corynebacterium bovis*, *Staphylococcus aureus*, *Streptococcus agalactiae*) and no bacterial growth. For statistical analysis PROC GLIMMIX of SAS was used. It was considered significant when  $P < 0.05$  and tendency when  $P < 0.10$ . Cows with growth of environmental Gram-negative bacteria showed less conception (19.35%,  $P=0.0014$ ) related to cows with no bacterial growth (34.69%,  $P<0.001$ ), environmental Gram-positive bacteria (37.84%,  $P<0.001$ ) and contagious Gram-positive bacteria (38.52%,  $P<0.001$ ). The lower pregnancy maintenance in cow that isolated Gram-negative bacteria can be due to the production of endotoxins that may have led to a pregnancy loss (Giri et al., 1990 Veterinary Microbiology, 21:211-231). It is concluded that subclinical infections by environmental Gram-negative bacteria may decrease the reproductive rate in dairy cows in lactation.



A065 FTAI, FTET and AI

## Use of bovine somatotropin (bST) associated with protocols of timed artificial insemination (TAI) in beef cows

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**Keywords:** beef cows, bST, TAI.

Use of bST in dairy cow TAI protocols is associated with increased pregnancy rates (Moreira et al., 2001, J. Dairy Sci. 84, 1646-1659). However, few studies have evaluated the effect of bST in beef cow on pregnancy when associated in a TAI protocol. Two experiments were conducted to evaluate the effect of bST (Lactotropin®, Elanco Animal Health, São Paulo, Brazil) on pregnancy rate when incorporated in a TAI protocol for suckled Nelore cows: D0 - BE im (2.0mg, Ciclovar®, Jofadel, São Paulo, Brazil) + intravaginal device of P4 (CIDR®, 1.9g of P4, Pfizer Animal Health, São Paulo, Brazil); D7 - PGF2 $\alpha$  im (12.5mg, Lutalyse®, Pfizer Animal Health, São Paulo, Brazil); D9 - CIDR® out + ECP (0.5mg, ECP®, Pfizer Animal Health, São Paulo, Brazil) + 300IU of eCG (Novormon®, MSD Animal Health, São Paulo, Brazil); D11 - TAI. In EXP1, the treatments were randomly assigned to 896 cows (371 primiparous; 525 multiparous): no bST (Control; n = 294), 167mg (n = 304) or 333mg (n = 298) of bST. In EXP2, the treatments were randomly assigned to 290 cows (81 primiparous; 209 multiparous): no bST (n = 179) or two injections of 333mg of bST (D0 and D11; n = 111). The bST dose was reduced because there is no GHR decrease detected in postpartum Angus cows (Jiang et al., 2005, J. Dairy Sci. 88, 1370-1377). In both experiments, bST injections were subcutaneous. Data were analyzed by PROC GLIMMIX of SAS. Model effects included parity, body condition score (BCS), treatment and interactions. Significance level was defined as P<0.05. In both experiments a parity effect was observed. Primiparous cows (EXP1=33.9%; 371; EXP2=27.2%; 81) had lower pregnancy rate relative to multiparous cows (EXP1=49.3%; 525; EXP2=53.1%; 209), but no effect was observed between parity and bST dose. In EXP1, cows treated with bST on D11 had higher pregnancy rate than control cows (167mg of bST = 45.4%; 304; 333mg of bST = 46.0%; 298; Control = 37.4%; 294), and no dose effect was observed. In EXP2, bST had no effect, 333mg bST administered on D0 and D11 (42.3%; 111) and Control (48.0%; 179). A single bST injection at the time of AI (D11), independent of dose, was associated with an increase in pregnancy rate. One possible explanation is increase IGF-I, with a positive impact on embryonic development. In EXP2, repeat bST injections may have increased nutritional requirements due to increased milk yield (Armstrong et al., 95, J. of Animal Sci. 73, 3051-3061). Alternatively, excessive increase of IGF-I may have resulted in asynchrony between uterine environment and embryo size (Bilby et al., 2004, J. Dairy Sci. 87, 3256-3267). Finally, hyperstimulation of plasma insulin may have resulted in negative impact on oocyte viability (Armstrong et al., 2003, Reprod. Suppl. 61, 403-414). Further studies are needed to investigate mechanisms associated with use of bST on beef cow reproductive performance.



A066 FTAI, FTET and AI

## **Identification of bacteria in Santa Ines ram semen under different farming systems**

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**Keywords:** bacteria, farming system, ram semen.

Several bacteria are identified in ram semen showing often negative effects on quality. The objective of this study was to identify and quantify the microbiota of fresh ram semen, evaluate the use of antiseptic Kilol-L® before the semen collection and test the sensitivity of bacterial strains to the antibiogram. The experiment was conducted in the experimental farm Sucupira at EMBRAPA and in the Laboratory of Veterinary Microbiology at UNB, Brasília-DF. Twenty-four rams were selected and divided into two groups, one housed in feedlot and another in pasture. Semen was collected by artificial vagina. Each ram was collected five times with a total of 120 samples. In all collections an external cleaning of the foreskin using soap was done. In the first two was used 0.9% saline intra preputial, and the last three collections were done with Kilol-L® at a concentration of 1:250 (Quinabra, SP, Brazil). Parameters of volume, motility, vigor and concentration were evaluated. For bacterial counts, the samples were added to the standard agar and incubated at 37°C for 24h. For bacterial culture, an aliquot of semen was inoculated on 5% sheep's blood agar and incubated for 24 - 48 h. Antibiogram was done by disk diffusion method using 9 antibiotics. The statistical analysis were analysis of variance (ANOVA, SAS version 9.0, 2002) considering statistical difference when  $P < 0.05$ . Of the 120 ejaculates, 99 had bacterial growth representing 82.5% of the total samples and 132 bacterial strains were isolated. The most isolated bacteria were *Staphylococcus* spp., *Bacillus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Listeria* sp. and *Escherichia coli*. Among the nine antibiotics tested *in vitro* against the 132 bacterial strains the Ceftiofur was the more effective (98.48%), followed of Gentamicin (93.18%) and the less effective was the Penicillin (55.30%). The use of antiseptic Kilol-L® reduced the number of colony forming units (CFU/mL) of ejaculate without detriment in semen quality. There was difference ( $P < 0.05$ ) in the presence of *Bacillus* spp., *Listeria* spp. and *E. coli* in the feedlot group when compared with pasture group. There was also difference ( $P < 0.05$ ) in the total bacterial count when Kilol-L® was used prior to semen collection. There was no difference between groups and type of cleaning in the parameters of motility, vigor and concentration. Results indicate that the use of antiseptic Kilol-L® is recommended before semen collection, to reduce the amount of bacterial in the ejaculate. Of the antibiotics, Ceftiofur and Gentamicin were the most effective in face of the isolated bacterial strains, showing that they can be used in the semen diluent to reduce bacterial growth in the ejaculate.



A067 FTAI, FTET and AI

### **Estrous behavior, ovulatory dynamics, and cervical mucus discharge of Toggenburg goats submitted to induction of estrus with different gonadotropins**

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**Keywords:** cervical mucus, estrous induction, goat.

This study evaluated the effects of different gonadotrophins on the estrous behavior, ovulatory dynamic and the cervical mucus discharge characteristics in anestrus Toggenburg goats. Twenty-four goats were assigned to three treatments. All animals received intravaginal sponges containing 60 mg of medroxyprogesterone acetate for 6 days plus 30µg d-cloprostenol latero-vulvar and 20 IU of FSH or 200 IU of eCG or 250 IU of hCG i.m. 24 hours before sponge removal when estrus was observed and ovaries were monitored by transrectal ultrasonography every 12 hours until confirmation of ovulation. In the same period, with the aid of a vaginal speculum, the discharge of cervical mucus was observed and classified into a scale of 1 (crystalline), 2 (crystalline/striated), 3 (striated), 4 (striated/caseous), and 5 (caseous). Estrus was successfully induced ( $P>0.05$ ) in FSH: 50%, eCG: 100%, and hCG: 87.5% of animals. The interval to estrus was greater ( $P<0.05$ ) in goats treated with FSH ( $56.0\pm 19.6$ h) than in goats treated with hCG ( $30.9\pm 8.6$ h), and both treatments were similar ( $P>0.05$ ) to eCG ( $43.0\pm 20.5$  h). The estrous duration was similar ( $P>0.05$ ) among animals of the treatments (FSH:  $32.0\pm 28.5$ ; eCG:  $19.0\pm 9.0$ ; hCG:  $32.0\pm 18.5$ h). The reproductive parameters measured by ultrasonography did not differ ( $P>0.05$ ) among the animals of treatments and showed the following overall averages:  $1.6\pm 0.7$  ovulation, interval from sponge removal to ovulation of  $73.5\pm 23.7$ h; interval from estrus to ovulation of  $32.3\pm 9.8$ h; mean diameter(s) of the ovulatory(s) follicle(s) of  $7.2\pm 0.8$ mm; and diameter of the subordinate follicles of  $4.6\pm 0.8$ mm. A positive correlation ( $r=0.6$ ,  $P<0.05$ ) was detected between estrous duration and ovulation time. The largest follicles diameters were higher ( $P<0.05$ ) of goats with mucus 1 ( $6.7\pm 1.4$ mm), 2 ( $7.2\pm 1.1$ mm) and 3 ( $7.3\pm 1.3$ mm) than in ones with mucus 4 ( $5.3\pm 1.4$ mm), and 5 ( $4.4\pm 1.1$ mm). Ovulation occurred in presence of mucous 4. The FSH and hCG can be considered to induce estrus in goats during the seasonal anestrus. The type of cervical mucus can be used to verify the proximity of ovulation and may be an additional criterion to determine the best time for artificial insemination.

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A068 FTAI, FTET and AI

### **Effect of phase of the estrous cycle on fertility in dairy cows inseminated with E2/P4 protocol**

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**Keywords:** fertility, phase of the estrous cycle, TAI.

Two experiments were conducted to assess if phase of the estrous cycle that begins the E2/P4 TAI protocol, and the presence or absence of CL at the beginning of the protocol or at the time of PGF impact the fertility in lactating Holstein cows. The binomial data were analyzed using PROC GLIMMIX and continuous using PROC MIXED of SAS. Effect was considered significant when  $P < 0.05$ . In Exp.1 cows that were detected in estrus ( $n = 220$ ) were inseminated with the following TAI protocol: D0–2mg im EB (2.0mL, Estrogin, Farmavet, Sao Paulo, Brazil) + intravaginal P4 device (CIDR, 1.9g of P4, Pfizer Animal Health, Sao Paulo, Brazil); D7- PGF im (5.0 mL, Lutalyse, Pfizer Animal Health, Sao Paulo, Brazil) D8-CIDR removal + 1mg ECP (0.5mL, Pfizer Animal Health, Sao Paulo, Brazil); D10- TAI. The estrous cycle days were divided into 1-4d, 5-9d, 10-16d, 17-21d, according to Vasconcelos et al. (Theriogenology 52:1067-1078,1999). The P4 at the beginning of the protocol (D0) differed between groups (1-4d =  $1.42 \pm 0.5$  ng/mL, 5-9d =  $2.75 \pm 0.5$  ng/mL, 10-16d =  $4.27 \pm 0.5$  ng/mL, 17-21d =  $2.30 \pm 0.6$  ng/mL). Cows submitted to TAI 5-9d after estrus had higher pregnancy rate per insemination (P/AI) at 30d [60% ( $n = 55$ )] compared to cows submitted to TAI 1-4d after estrus (38% [ $n = 54$ ]), but there was no difference within cows that were submitted to TAI between 10-16d (49% [ $n = 90$ ]) and 17-21d after estrus (41% [ $n = 21$ ]). At 60d cows that were submitted to TAI 5-9d after estrus had higher P/IA (60% [ $n = 55$ ]) than cows submitted to TAI 1-4d (36% [ $n = 54$ ]), 10-16d (44% [ $n = 90$ ]) and 17-21d after estrus (41% [ $n = 21$ ]). There was no difference in phase of the estrous cycle on ovulatory follicle diameter, in the incidence of double ovulation and in the P4 concentration 7 days after AI. In Exp.02 ( $n = 593$ ) cows were submitted to TAI with the same protocol described in Exp.01 to evaluate if the presence or absence of CL on D0 and D7 (PGF) of the protocol impact fertility. Cows were classified as 0-0 (without CL on D0 and D7), 0-1 (without CL on D0 and with CL on D7), 1-0 (with CL on D0 and without CL on D7) and 1-1 (with CL on D0 and D7). Cows 0-0 had lower P/AI at 30 and 60d (13% and 12% [ $n = 135$ ]) than cows 1-1 (26% and 23% [ $n = 170$ ]) however there was no difference with groups 0-1 (19% and 19% [ $n = 87$ ]) and 1-0 (20% and 16% [ $n = 201$ ]). Phase of the estrous cycle and/or the presence of CL can impact fertility of cows submitted to E2/P4 based protocols, and better P/IA is obtained when the protocol starts between days 5-9 of the estrous cycle, which corresponds with the presence of CL at the beginning of the protocol and at the time of PGF.



A069 FTAI, FTET and AI

### **Influence of recombinant bovine somatotropin (rbST) in different doses on the fertility of goats**

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**Keywords:** fertility, goat, rbST.

Recombinant bovine somatotropin (rbST) has been used as a tool to increase reproductive rates in species of economical interest. The present study had as objective to evaluate the effects of rbST associated with fixed-time artificial insemination on the pregnancy rates of goats raised in a semi-intensive system. Females without defined racial standard, 122 adults, selected according to their reproductive history and health status, body condition score varying from 2.5 to 3.5 (scale 1 to 5), kept in native grasses and cultivated pastures, receiving mineral salt *ad libitum* were used. For estrous synchronization protocol, on D0, intravaginal sponges impregnated with 60 mg of medroxyprogesterone - MAP (Progespon®, Syntex, Argentina), were inserted during a period of 11 days. On D6, the animals were randomly divided in three experimental groups: G-I (n=42) received 250 mg of rbST (Boostin®, Schering Plough/Intervet, Brazil), subcutaneously in a single dose, G-II (n=39) received 125 mg of rbST and G-III (n=41) received saline solution (control). On D9 all females received intramuscular injections of 300 IU of Equine Chorionic Gonadotropin - eCG (Novormon®, Sintex, Argentina) and 75µg of Sodium Cloprostenol -PGF2α (Ciosin®, Coopers, Brazil). On the last day of hormonal treatment (D11), the sponges were removed and approximately 36±2 hours afterward the goats were inseminated at a fixed time by transcervical artificial insemination, with frozen/thawed semen, from a single buck, selected through andrological exam and known fertility soundness. The pregnancy diagnosis was done via ultrasound scanning using a Chisson D600 VET® (Chisson Medical Imaging Co. Ltd., China) equipped with a 3.5-5.0 MHz linear array transducer 30 days after the fixed-time AI. Data for pregnancy rate was assessed by  $\chi^2$  (chi-square test -  $P < 0.05$ ), in which the results were: GI - 57.14%, GII - 43.58%, and GIII - 56.09%. The rbST, in this experiment, was not able to influence the pregnancy rate in goats, because there was no statistical difference between groups.



A070 FTAI, FTET and AI

### **Effect of FSH and eCG on follicular dynamic and pregnancy rate in fixed-time AI protocol in primiparous and multiparous beef cows**

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**Keywords:** bovine, follicular growth, gonadotropin.

The objective of this study was to evaluate the effect of FSH and eCG on follicular growth and pregnancy rate of primiparous and multiparous beef cows (*Bos indicus*) submitted to fixed time artificial insemination (TAI). For the follicular growth study, 420 lactating cows (162 primiparous and 258 multiparous; 30 to 60 days postpartum) with body condition score (BCS) of  $2.73 \pm 0.01$  and  $3.23 \pm 0.02$  (1 to 5 point scale), respectively, were used. In a random day of the estrous cycle (D0), all animals received 2 mg of estradiol benzoate (RIC BE®, Tecnopec, Brazil) and an intravaginal progesterone device (Primer®, Tecnopec, Brazil). On D8, with the P4 device removal, all cows were submitted to an ultrasound (US) evaluation (8100 Vet, Chison, Brazil) to measure the diameter of the largest follicle. The cows were homogenously allocated in one of three treatment groups: Control, FSH and eCG according to size of the largest follicles. Still on D8, the animals received 150µg of Cloprostenol (Prolise®, Tecnopec, Brazil), 1mg of estradiol cypionate (ECP®, Pfizer, Brazil) and according to each group, 300IU of eCG (Novormon®, MSD, Brazil), 10µg of FSH (Folltropin®, Bionich, Canada) and no treatment. On D10, another US was accomplished to evaluate the follicular growth from D8 to D10. To analyze the pregnancy rate, 790 lactating cows (179 primiparous and 611 multiparous; 30 to 60 days postpartum) with body condition score (BCS) of  $2.73 \pm 0.01$  and  $3.15 \pm 0.01$  (1 to 5 point scale), respectively, were used. The cows were inseminated 48 h (TAI) after the P4 device withdrawal. Statistical analysis was performed using the SAS GLIMMIX. In the follicular growth study, no interaction was found between treatment and category (primiparous and multiparous) to follicular growth ( $P=0.31$ ). However, treated (FSH and eCG) and multiparous cows presented a larger follicular growth per day from D8 to D10 (Control =  $0.62 \pm 0.07^b$ , FSH =  $0.77 \pm 0.07^b$  and eCG =  $1.13 \pm 0.07^a$ ;  $P=0.002$  and multiparous =  $0.91 \pm 0.04$  and primiparous =  $0.74 \pm 0.08$ ;  $P=0.02$ ). For pregnancy rate, an interaction between treatment and category ( $P=0.008$ ) was found. The eCG treatment increased the pregnancy rate in primiparous cows [Control = 13.2% (9/68)<sup>b</sup>, FSH = 7.7% (3/39)<sup>b</sup> and eCG = 41.7% (30/72)<sup>a</sup>;  $P=0.0002$ ] and multiparous cows [Control = 41.3% (45/109)<sup>b</sup>, FSH = 51.0% (127/249)<sup>ab</sup> and eCG = 57.3% (145/253)<sup>a</sup>;  $P=0.06$ ]. However, the difference among treatments was most evident in primiparous cows. We conclude that unlike the eCG group, the treatment with FSH did not increase the follicular growth and pregnancy rate in lactating *Bos indicus* beef cows (primiparous or multiparous) submitted to a TAI protocol.



A071 FTAI, FTET and AI

## Recovery rate of structures in Santa Ines ewes after a superovulation protocol using pFSH

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**Keywords:** ewes, superovulation, embryos

The aim of this study was to determine the structures recovery rate in ewes submitted to a superovulation (SOV) protocol. The trial was done in July and February in Cachoeiras de Macacu – Rio de Janeiro (22° 27'S, 43° 39'W). Fifteen Santa Ines ewes, aging 2-4 years, weighing  $47.8 \pm 6.3$  kg and body condition score between 2.5 and 3.0 were allocated in two groups (GBR and GAI) in a cross-over design, in which each ewe was submitted to both treatments: In GBR the ewes were bred by a sexually mature ram and in GAI the ewes were artificially inseminated by laparoscopy. In both groups, estrus was synchronized using intravaginal sponges (60 mg of medroxyprogesterone acetate; Progespon®, Schering Plough, São Paulo, Brazil), maintained for 6 days. Twenty four hours before the sponge withdrawal the ewes were treated with 300 IU of eCG (Novormon®, Schering Plough Animal Health, São Paulo, Brazil) and 0.0375 mg of cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) i.m. Twelve hours after the sponge withdrawal ewes received 0.025 mg of gonadorelin acetate (Gestran®, Tecnopec, São Paulo, Brazil) i.m. The SOV treatment started 60 h after sponge removal and consisted of 5 IU/kg of pFSH (Pluset®, Hertape Calier, Minas Gerais, Brazil) i.m. in six decreasing doses: 1st: 25% of the full dose; 2nd: 25%; 3rd: 15%; 4th: 15%; 5th: 10% e 6th: 10% every 12 h. At the first pFSH dose new sponges were inserted. At the fifth dose 0,0375mg of cloprostenol was administered i.m. and the sponges were removed. Animals of the GBR were randomly separated in two groups and each group was bred by a sexually mature ram twice a day until the end of the estrus. The females of the GAI were inseminated with frozen semen, 24 and 36h after the end of SOV protocol. Prior to embryo collection, the number of CL present on both ovaries were determined by ultrasonography (8.0 MHz Pie Medical®, Aquila Vet, Tokyo, Japan) and further by laparoscopy (LAP). Ewes with four or more CL (15/30) were submitted to embryo collection by laparotomy and evaluated for the structures recovery rate (RR) [(n° of collected structures/ n° of CL at LAP) X 100]. The recovery rate in the animals with 4 to 5 (2 of 15 ewes), 6-10 (9/15) and more than 10 (4/15) CL was 44.4%, 63.6% and 67.3%, respectively. The total RR was 63.8%, and was lower compared to a study using the same surgical method that obtained 81% of RR (Cordeiro et al., Small Ruminant Research, 46, 19-23). The recovery rate on the first group was low and on groups 2 and 3 the RR was in agreement with those described by Lymberopoulos et al. (Theriogenology, 55, 1855-1862) and lower than those reported previously (Ramon-Ugalde et al., Czech. J. Anim. Sci. 53, 145-151). The present study demonstrated that the lower the number of CL is, the lower is the recovery rate, suggesting that the embryo recovery in ewes with 4 to 5 CL it is not viable in commercial herds.



A072 FTAI, FTET and AI

### **The effect of increasing the dose of estradiol benzoate on conception rate of Nelore cows with low body condition score submitted to FTAI program**

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**Keywords:** body condition score, ovulation inductor, postpartum cows.

Suckled cows with low body condition have low reproductive efficiency under fixed-timed artificial insemination (FTAI), especially at the Pantanal, where the environmental conditions do not provide the proper treatment of nutritional requirements. Therefore, the aim of this study was to evaluate the effect of increase estradiol benzoate (EB) dose, as ovulation inductor on the conception rate in FTAI program. It was used in 318 lactating Nelore cows (multiparous with 45 to 60 days post-partum), with low body condition score (BCS  $2.25 \leq 3.0$ ; 1 to 5 scales), and managed under native pastures on brazilian south pantanal. All animals were treated with 2 mg of EB (RIC-BE®, Tecnopec-Agener União, Brazil) and an intravaginal progesterone device (Primer®, Tecnopec-Agener União, Brazil), in random day of estral cycle (Day 0). On Day 8 at 10AM (D8.5), the moment of the intravaginal progesterone device withdrawal, the cows received 150µg of d-cloprostenol (Prolise®, Arsa, Argentina) and 10mg of FSHp (Folltropin-V®, Bioniche, Canada) and were allocated in one of two treatments: Group EB1 - with 157 cows and BCS  $2.65 \pm 0.16$  (mean±SD) and Group EB1.5 – with 161 cows and BCS  $2.65 \pm 0.17$ . Each group received 1mg and 1.5mg of the EB, respectively. On Day 10, 44 hours after device withdrawal the animals were inseminated. The pregnancy diagnosis was performed by ultrasonographic exam 40 days after FTAI (DP-2200 Vet®, Mindray, China). Data were analyzed by GLIMMIX procedure from SAS. The explanatory variables were body condition score, bull, semen straw and technician. There was no interaction between treatments and explanatory variables. The conception rates were 46.50% (73/157) for EB1 group and 42.24% (68/161) for EB1.5 group. There was no difference between the treatments ( $P > 0.05$ ). We concluded that increasing the EB dose presented similar results as ovulation inducers in suckling Nelore cows and with low body condition submitted to the protocol of synchronization for FTAI.

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A073 FTAI, FTET and AI

## **Use of intravaginal progesterone devices of first, second and third uses for the synchronization of ovulation and TAI of lactating buffaloes during the non breeding season**

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**Keywords:** buffalo, progesterone device, TAI.

The present study aimed to compare the efficiency of the progesterone intravaginal devices according to the number of uses, for the synchronization of ovulation and TAI of lactating buffalo cows during the non breeding season. In the study, 486 buffaloes were distributed according to the body condition score, number of parturitions, postpartum period and ovarian activity in one of three groups (G1x, n=163; G2x, n=166; G3x, n=157). At random stages of the estrous cycle (Day 0 = D0), in the afternoon, the females received an intravaginal P4 device (Sincrogest®, Ourofino Agro business) of first (G1x), second (G2x) and third (G3x) uses and 2.0 mg of estradiol benzoate intramuscularly (i.m., Sincrodiol®, Ourofino). At D9 (afternoon), the females received 0.53 mg of PGF2 $\alpha$  (i.m., Sodium cloprostenol, Sincrocio®, Ourofino) and 400 IU of eCG (Novormon®, MSD Animal Health), followed by removal of P4. Two days after (D11, afternoon), it was administered 10  $\mu$ g of GnRH (i.m., Sincroforte®, Ourofino). Part of the animals (G1x, n=10; G2x, n=11; G3x, n=10) was randomly selected for the ultrasonographic examinations (Mindray 2200Vet, 7.5 MHz linear-array transducer) performed on D0 to evaluate the ovarian activity, on D9 to measure the follicular diameter and from D11 up to D14 (12 h apart for 60 h) to establish the moment of the ovulation. All animals were subjected to TAI 64 h after the P4 removal and, 30 days later to the pregnancy diagnosis (D42; Mindray DP2200Vet). The statistical analyses were performed by SAS program using the GLIMMIX procedure. There were no differences between experimental groups (G1x; G2x e G3x; P>0.05) for the diameter ( $\emptyset$ ) of the dominant follicle on D9 (9.0 $\pm$ 0.8 mm; 10.1 $\pm$ 0.9 mm; 8.6 $\pm$ 0.8 mm),  $\emptyset$  of the ovulatory follicle (12.8 $\pm$ 1.0 mm; 12.9 $\pm$ 0.8 mm; 11.9 $\pm$ 0.9 mm), the interval between GnRH administration and ovulation (31.5 $\pm$ 4.5 h; 29.3 $\pm$ 4.7 h; 26.0 $\pm$ 4.4 h), the ovulation rate [80.0% (8/10); 81.8% (9/11); 60.0% (6/10)] and the pregnancy rate [55.2% (90/163); 56.0% (93/166); 49.0% (77/157)], respectively. The results of the present study showed that Sincrogest® of first, second or third uses enables efficient synchronization of the follicular growth and ovulation, and satisfactory pregnancy rate, confirming previous results for first or second uses and purposes for second or third uses. The use of Sincrogest® in buffaloes for up to three times allows the reduction of cost of the ovulation synchronization protocol for TAI in the non breeding season.



A074 FTAI, FTET and AI

### **Estrus behavior of ewes submitted to synchronization by Bai Hui acupuncture point**

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**Keywords:** acupuncture, ovine, synchronization.

This study aimed to evaluate the estrous behavior of ewes subjected to synchronization protocols with application of PGF2 $\alpha$  and eCG at Bai Hui acupoint. Intravaginal sponges impregnated with 60mg of medroxy-progesterone acetate (Progespon®, Syntex, Argentina) were inserted in 30 Santa Ines ewes. After seven days sponges were removed, five different treatments (T) were used: T1: 0.125mg of cloprostenol (Ciosin®, Coopers, Brazil) (100% of dose) and 300 IU of eCG (Folligon®, Intervet, Brasil) (100% of dose) injection intramuscularly (IM); T2: 0.0375mg of cloprostenol (30% of dose) at Bai Hui acupuncture point and 300IU of eCG (100% of dose) injection IM; T3: 0.125mg of cloprostenol (100% of dose) injection IM and 90IU of eCG (30% of dose) at Bai Hui acupuncture point, T4: 0.0375mg of cloprostenol (30% of dose) and 90IU of eCG (30% of dose) both in the Bai Hui acupuncture point and T5: 0.0375mg of cloprostenol (30% of dose) and 90IU of eCG (30% of dose) both at acupuncture false point (IM). The animals were monitored for estrus detection every 12h. The parameters were assessed using the Variance Analysis, with 5% significance level. There was no difference ( $P>0.05$ ) between treatments for the percentage of ewes that exhibited estrus after sponge removal. It was observed 100% of estrus in the groups T1, T2 and T3, and 80% for T4 and T5. There was no difference ( $P>0.05$ ) in the interval between sponge removal and the onset of estrus (T1 - 42 $\pm$ 14; T2 - 44 $\pm$ 9; T3 - 54 $\pm$ 6; T4 - 55 $\pm$ 13 and T5 - 45 $\pm$ 10h); or for interval between sponge removal and the end of estrus (T1 - 62 $\pm$ 4; T2 - 72 $\pm$ 7; T3 - 68 $\pm$ 9; T4 - 76 $\pm$ 10 and T5 - 69 $\pm$ 10h), and for duration of the estrus (T1 - 21 $\pm$ 4; T2 - 30 $\pm$ 8; T3 - 18 $\pm$ 10; T4 - 24 $\pm$ 12 and T5 - 27 $\pm$ 6h). Thus, 0.0375mg cloprostenol and 90IU eCG both in the Bai Hui acupuncture point or in the acupuncture false point, were effective in synchronization of estrus in ewes.



A075 FTAI, FTET and AI

### **Luteolytic efficacy of a cloprostenol based product in cows**

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**Keywords:** cloprostenol, cows, luteolytic.

The objective of this experiment was to verify the effectiveness of a cloprostenol based product (Estron - Tecnopec/União Química, Embu Guaçu, Brazil) to induce luteolysis and synchronize estrus in cows at different stages of the estrous cycle. Sixty crossbreed cows and heifers, 24 to 48 months and weighing between 318 and 460 kg, were randomly assigned to four groups (n = 15) according to the estrous cycle: between 6 and 8 days after estrus (group 1), 9 and 11 days (group 2), 12 and 14 days (group 3) and 15 and 17 days (group 4). In each group, 12 animals received the test product (CLOPR) at a dose of 2 mL (equivalent to 0.5 mg of cloprostenol) and three received 2 mL of saline (CONTR) intramuscularly. Blood samples were collected immediately prior to application of the products and 36 hours after to determine progesterone (P4) concentration using commercial kit (Coat-A-Count CPS - Siemens, Los Angeles, USA). There was no difference between the concentrations of P4 from animals of CLOPR and CONTR groups before the application of products. The average concentration of P4 in CLOPR animals of all groups decreased from 2.58 to 0.14 ng/mL after treatment, which represented a 94% drop (P<0.05 - ANOVA). In each study group, the concentrations of P4 in the animals of CLOPR treatment suffered a significant decrease: 1.77 and 0.12 ng/mL, 2.34 and 0.16 ng/mL, 2.57 and 0.23 ng/mL and 3.75 and 0.15 ng/mL, respectively for the groups 1, 2, 3 and 4 (Fischer's test), and all groups had a 100% luteolysis rate. The average concentration of P4 in the CONTR animals of all groups before and after treatment were 2.21 ng/mL and 2.08 ng/mL, with no significant statistically difference. It could be concluded that the tested product is efficient to induce luteolysis in cattle, regardless of stage of estrous cycle.



A076 FTAI, FTET and AI

### **Comparative performance of two protocols for fixed-time artificial insemination (FTAI) in a herd of cows housed in the Southern Pantanal of Mato Grosso**

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**Keywords:** eCG, FSH, FTAI.

The objective of this experiment was to compare the performance of two FTAI protocols commonly used by field technicians and detect which one has better results when applied to multiparous Nelore cows, housed in the Pantanal floodplain, in the city of Rio Verde do Mato Grosso, state of Mato Grosso do Sul. For this experiment, 319 multiparous and lactating Nelore cows with body condition score ranging from 2-4 (scale 1-5) and a mean age of 45 days postpartum, were divided into two treatment groups equalized by the body condition score and age postpartum. In Group 1 (n = 155), cows received at baseline (Day 0) an intravaginal progesterone device (Primer, Tecnopec/União Química, Embu-Guaçu, Brazil) and 2 mg of estradiol benzoate (RIC-BE, Tecnopec/União Química, Embu-Guaçu, Brazil). Eight days (Day 8) after, the devices were removed and it was administered 112.5 µg of D-cloprostenol (1.5 mL of Prolise, Tecnopec/União Química, ARSA, Argentina), 1 mg of estradiol benzoate and 10 mg FSHp (Folltropin V Tecnopec/União Química, Bioniche, Canada). These cows were inseminated at 10 days between 44-50 hours after removal of the devices. In Group 2 (n=164), the animals received at the beginning of treatment (Day 0) an intravaginal progesterone device and 2 mg estradiol benzoate. Eight days after (Day 8) the devices were removed and it was administered 112.5 µg of D-cloprostenol, 1 mg of estradiol cypionate (ECP, Pfizer) and 300 IU eCG (Folligon, MSD). Cows were inseminated on day 10, 48 to 54 hours after removal of the devices. All animals were inseminated with semen from a single bull. The pregnancy results in the treatments were compared by Chi-square test. There was no significant difference between pregnancy rates in Groups 1 (50.9%, 79/155) and 2 (43.3%, 71/164) (P = 0.16). It can be concluded that the two protocols used were equally efficient at the conditions of this experiment.



A077 FTAI, FTET and AI

## **Comparison between estradiol benzoate and leirelin as ovulation inducers in protocols of fixed-time artificial insemination (FTAI) in heifers pre-synchronized with progesterone and estrogen**

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**Keywords:** FTAI, heifers, leirelin.

The objective of this study was to compare the effectiveness of two different protocols of FTAI, using estradiol benzoate (EB) or a synthetic analogue of GnRH (leirelin) to induce ovulation in zebu Nelore heifers. A total of 409 heifers belonging to three different lots were used. All heifers were raised and maintained under field conditions (grazing and mineral supplementation) in the region of Alta Floresta, northern Mato Grosso State. The animals were aged between 18 and 36 months and weighing  $316 \pm 33$  kg. At the beginning of the experiment, the heifers were evaluated by ultrasonography and classified according to the ovarian condition as pubertal (presence of corpus luteum) or prepubertal (absence of corpus luteum). All animals underwent a protocol of induction of puberty, in which on Day 0 received an intravaginal progesterone device previously used for 24 days (Primer, Tecnopec/União Química, Embu-Guaçu, Brazil) and an application of 2 mg of estradiol benzoate (RIC-BE, Tecnopec/União Química, Embu-Guaçu, Brazil) at the time of device placement. Moreover, the animals received 1 mg of estradiol benzoate at the device removal eight days later. The animals were evaluated by ultrasonography 12 days after the treatment to determine the degree of cyclicity, and simultaneously it was started the FTAI protocol. Heifers in group GnRH (n = 195) received an intravaginal progesterone device (Primer previously used by 8 days) and 2 mg estradiol benzoate. On day 9, the devices were removed and the animals received at the same time 112.5 µg of D-cloprostenol (Prolise, Tecnopec/União Química, ARSA, Argentina), 0.5 mg of estradiol benzoate and 5 mg of FSHp (Folltropin V, Tecnopec/União Química, Bioniche, Canada). The animals were inseminated in 11 days, between 44 and 50 hours after removal of the devices and, at the time of insemination, 25 mg of leirelin (Gestran Plus, Tecnopec/União Química, ARSA, Argentina) were administered. Animals in Group EB (n = 214) received an intravaginal progesterone device (Primer previously used by 8 days) associated to 2 mg of estradiol benzoate. On day 9, the devices were removed and the animals received at the same time 112.5 µg of D-cloprostenol, 1 mg of estradiol benzoate and 5 mg FSHp. The animals were inseminated in 11 days, between 44 and 50 hours after removal of the devices. The division of the heifers in both groups was done by keeping the balance of cyclicity, age and weight of the animals (less than 300 kg, 301 to 350 kg 350 kg and above). Two bulls were used and only one person was responsible for inseminations. The cyclicity and pregnancy data were analyzed by Chi-square test. The rate of cyclicity before puberty induction protocol was 38.7%, rising to 69.5% 12 days after the treatment. There was no effect of the bull and the lot on the pregnancy rate. There was no significant difference between pregnancy rates (P = 0.25) for the different ovulation inducers: 57.9% for the GnRH and 52.3% for EB. It was found an association between animal weight and pregnancy rates (P = 0.04): 49.9% for animals less than 300 kg, 58.3% for animals weighing between 301 and 350 kg and 64.7% for animals over 350 kg. It was concluded that both treatments were equally effective, with satisfactory rates of pregnancy for FTAI protocols in zebu heifers.



A078 FTAI, FTET and AI

### **Evaluation of progesterone release during the second use of an intravaginal device in ewes (preliminary data)**

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**Keywords:** hormone, ovine, reproduction.

In order to evaluate the effect of the reutilization of a new progesterone device for small ruminants, six ovariectomized ewes ( $67.7 \pm 16.3$  kg) were randomly assigned to one of two treatments: New Group (NG, n=3) and Reused Group (RG, n=3). On day 0, ewes from the NG received a new intravaginal progesterone device (product in the process of registration) and ewes from the RG received a used intravaginal progesterone device (previously used for 9 days). Nine days after device insertion (D9), progesterone devices were removed in both experimental groups. Blood samples were collected from the jugular vein on days: D-1, D0 (before device insertion); D0 + 1h (1 hour after device insertion), D0 + 2h; D0 + 4h; D1; D3; D5; D7; D9 (before device removal); D9 + 1h (one hour after device removal); D9 + 2h; D9 + 4h, D10 and D12. Data were analyzed using the MIXED SAS procedure. Prior to the analysis, normality of residues and homogeneity of variances were verified. Before device insertion, mean progesterone concentration in both groups was below 1 ng/mL (NG:  $0.36 \pm 0.068$  and RG:  $0.61 \pm 0.18$ ;  $P=0.68$ ). One hour after the treatments, progesterone concentration increased to  $4.96 \pm 1.23$  in NG and  $3.06 \pm 0.40$  in RG ( $P=0.31$ ). This concentration (over 1 ng/mL) was kept during device maintenance (D0 to D9;  $5.68 \pm 0.48$  in the NG and  $2.25 \pm 0.11$  in the RG). However, progesterone concentration during this period was greater for the ewes that received the new device ( $P=0.05$ ). After 4 hours of device removal, progesterone concentrations in both groups reached values lower than 1 ng/mL ( $0.97 \pm 0.32$  in NG and  $0.43 \pm 0.04$  in RG). Therefore, progesterone concentration was lower in the ewes using the reused device, but the levels were above 2 ng/mL during treatment, which is enough concentration to promote synchronization of ovulation in ewes.

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A079 FTAI, FTET and AI

### **The type of estradiol ester used as ovulatory stimulus in the Tai synchronization protocol does not affect the occurrence of estrus nor the pregnancy rate in beef cattle**

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**Keywords:** estradiol ester, TAI, ovulation.

The aim of this study was to evaluate two different esters of estradiol used as ovulatory stimulus in protocol for synchronization of ovulation for timed artificial insemination (TAI) on the occurrence of estrus and pregnancy per TAI (P/AI) in beef cattle. A total of 538 Nelore females (417 multiparous cows, 62 nonlactating cows and 59 heifers), were divided into one of two treatments. All females received at the beginning of synchronization of ovulation an intravaginal progesterone device (P4, Cronipress Monodose®, Biogenesis-Bagó) plus 2 mg of estradiol benzoate i.m. (EB; Bioestrogen®, Biogênese). Eight days after, device was removed and 0.075 mg of D-Cloprostenol (Croniben®, Biogênese) plus 300 IU eCG i.m. (Novormon®, MSD Animal Health) were administered. At implant removal cows had their tailhead marked with chalk (Raidl-Maxi, Raidex, Germany) and were randomly assigned to receive: 1 mg estradiol cypionate (EC; ECP®, Pfizer Animal Health) at device withdrawal or 1 mg of EB 24 hours afterward. Estrus was deemed to have occurred in cattle without a tailhead chalk mark at TAI. The data were analyzed using the Glimmix procedure of SAS. No differences were found according to the type of estradiol used as ovulatory stimulus (EB vs EC) in the expression of estrus [90.2% (239/265) vs. 89.4% (244/273); P = 0.73] and on pregnancy rate [60.4% (160/265) vs. 52.8% (144/273); P = 0.07]. However, there was an effect of the body condition score (BCS) at first day of synchronization protocol in the occurrence of estrus [ $\leq 2.5 = 82.3\%$  (79/96)<sup>b</sup>; 2.75-3 = 95.1% (197/207)<sup>a</sup>; 3.25-3.5 = 94.0% (125/133)<sup>a</sup>;  $\geq 3.75 = 80.4\%$  (82/102)<sup>ab</sup>], and the P/AI [ $\leq 2.5 = 32.3\%$  (31/96)<sup>b</sup>; 2.75-3 = 62.3% (129/207)<sup>a</sup>; 3.25-3.5 = 62.4% (83/133)<sup>a</sup>;  $\geq 3.75 = 59.8\%$  (61/102)<sup>a</sup>]. In addition, females that displayed estrus had higher P/AI than those females that did not display estrus [Estrus = 58.2% (281/483) vs No Estrus = 41.8% (23/55); P=0.01]. In conclusion, there was no difference between the type of ester of estradiol (EB vs. EC) used as ovulatory stimulus on occurrence of estrus and P/AI following TAI. In addition, cattle presenting higher BCS at beginning of the synchronization have a higher amount of females displaying estrus and higher P/AI. Finally, females that display estrus following the synchronization protocol have a higher P/AI after TAI.



A080 FTAI, FTET and AI

## **Use of sex-sorted sperm with 75% of purity in TAI programs of suckled Nelore cows**

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**Keywords:** Nelore, sex-sorted sperm, TAI.

Sex-sorted sperm efficiency can be changed depending on the desired purity degree (percentage of sex desired). Usually, the commercially available doses present 85-90% of purity. The use of sex-sorted sperm with 75% of purity could be one possibility to increase the birth rate of sex desired and increase yield and production rate in the sex sorted sperm production. Thus, the aim of the present study was to compare the conception rate after the use of sex-sorted sperm with two different degrees of purity (85 vs. 75%) in protocols of timed artificial insemination (TAI) in suckled Nelore cows. A total of 376 cows between 30 and 80 days postpartum, from a single farm were used. At TAI cows were randomly assigned into one of three treatments: CONV (conventional semen, n = 133); SEX85 (sex-sorted sperm 85% of purity, n = 120) or SEX75 (sex-sorted sperm 75% of purity, n = 123). At beginning of the TAI protocol, cows received a norgestomet ear implant (Crestar®, MSD Animal Health) plus 2 mg im estradiol benzoate (EB, Gonadiol®, MSD Animal Health). Eight days later, implants were removed and were administered 0.3975 mg cloprostenol sodium (Ciosin®, MSD Animal Health) plus 300 IU of equine chorionic gonadotropin (eCG; Novormon®, MSD Animal Health). At Day 9, 1 mg of EB was administered (24 hours after removal of the ear implant). The TAI was performed 60 hours after removal of the ear implant. All semen used was produced from a single Nelore bull with a previous history of good fertility in TAI programs. Pregnancy diagnosis was performed 30 days after TAI. Data were analyzed by the GLIMMIX procedure of SAS. No difference was observed (P = 0.56) in conception rates between the groups CONV (45.1%), SEX85 (40.8%) and SEX75 (40.3%). It was concluded that the use of sex-sorted sperm with 75% of purity promote similar conception rate than the use of sex-sorted sperm to 85% of purity during TAI programs in suckled Nelore cows.

**Acknowledgments:** CRV Lagoa and MSD Animal Health.



A081 FTAI, FTET and AI

### **Timing of prostaglandin administration and the number of uses of the progesterone devices in the efficiency of TAI protocol in Girolando heifers**

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**Keywords:** artificial insemination, conception, protocol.

The aim of this study was to evaluate the effect of two different moments of treatment with prostaglandin (PGF<sub>2</sub>α), as well as the number of uses of an intravaginal P4 device on pregnancy per artificial insemination (P/AI) of Girolando heifers (1/2 HO=159, 3/4 HO=120) and 7/8 HO=172). A total of 451 cyclic heifers (CL present at first day of TAI protocol), were divided into one of four treatments (2x2 factorial): 1) treatment with ½ dose of PGF<sub>2</sub>α (12.5 mg im of Dinoprost; Lutalyse®, Pfizer) on D0 and D9 (PGFD0/D9), 2) treatment with full dose of PGF<sub>2</sub>α on D7 (PGFD7), 3) new P4 device, and 4) used P4 device (previously used for 9 d). On D0, all heifers were treated with 2 mg estradiol benzoate i.m. (Sincrodiol®, Ourofino) and a P4 device (CIDR® used or new, Pfizer) was inserted. On D9, the P4 devices were removed and heifers were treated with 1 mg estradiol cypionate im (ECP®, Pfizer). The TAI was performed 48 h after P4 device removal (D11). Pregnancy diagnosis was performed 30 d after TAI. Ultrasound examination was performed on D0 (presence of CL) of all heifers. A subgroup of heifers (n = 76) was also evaluated on D7, D9, D11, and D18 to assess the diameter (Ø) of the dominant follicle (DF) and ovulation. Statistical analysis was performed using the GLIMMIX procedure of SAS. There was no interaction between the time of treatment with PGF<sub>2</sub>α and the type of P4 device on the DF diameter or ovulation rate. The DF Ø on D7 (8.7±2.0 vs 9.4±1.4 mm, P=0.18), D9 (10.9±2.4 vs. 11.4±2.1 mm, P=0.46), and D11 (12.7±2.8 vs. 12.9±2.1 mm, P=0.64), and ovulation rate within 48 h of P4 removal (i.e. earlier ovulations = 28.6% vs 30.6%, P=0.89) and overall ovulation rate (91.4% vs. 88.9%, P=0.68) were similar among treatments and PGFD0/D9 PGFD7, respectively. Likewise, the number of uses of the device P4 (New vs. Used) did not alter the DF Ø on D7 (8.8 ± 1.7 vs. 9.2 ± 2.2 mm, P=0.45), on D9 (10.8 ± 2.1 vs. 11.6 ± 2.4 mm, P=0.18) or on D11 (12.7 ± 2.0 vs. 13.0 ± 3.0 mm, P=0.62). Also no difference was found on the rates of earlier ovulation (24.3% vs. 35.3%, P=0.32) or on the overall ovulation (86.5% vs. 94.1%, P=0.29). No interactions were found (P>0.05), and the P/IA were similar (P>0.05) among treatments [(PGFD0/D9=40.3%, 92/228 vs. PGFD7=42.1%, 94/223) and (New=40.7%, 92/226 vs. Used=41.8%, 94/225); P>0.05]. The degree of HO blood did not affect (P>0.05) the P/AI (1/2 HO=42.8%, 68/159; 3/4 HO=43.3%, 52/120; or 7/8 HO=38.4%, 66/172). In conclusion, the time of prostaglandin administration and the number of uses of the P4 devices did not affect the ovarian response and P/AI of Girolando heifers receiving a TAI.



A082 FTAI, FTET and AI

### **Effect of proestrus length on fertility in dairy cows submitted to ovulation synchronization protocols based on E2/P4**

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**Keywords:** fertility, proestrus, progesterone.

The aim was to evaluate whether the time between PGF and TAI (proestrus) alter fertility in an E2/P4 ovulation synchronization protocol. Binomial variables were analyzed using PROC GLIMMIX and continuous using MIXED. Significance was considered when  $P < 0.05$ . In Exp.1, cows ( $n=893$ ) were randomly assigned to receive PGF im (5.0 mL, Lutalyse, Pfizer Animal Health, Sao Paulo, Brazil) on either one d before (3d proestrus) or d of (2d proestrus) intravaginal P4 device (CIDR) removal in the following protocol: d0–2mg im EB (2.0mL, Estrogin, Farmavet, SP, Brazil) +CIDR (1.9g of P4, Pfizer Animal Health, SP, Brazil); d8–CIDR removal+1mgECP (0.5mL, Pfizer Animal Health, SP, Brazil); d10-TAI or d17-TET. As expected, 3d of proestrus decreased P4 at CIDR removal compared to 2d. Fertility (P/AI or P/ET) was affected by breeding technique (AIxET) and proestrus length (3dx2d) at the 30d pregnancy diagnosis (PD) at AI (3d-36%[238] x 2d-20%[168]) and ET (3d-47%[243] x 2d-41%[244]) and 60d PD at AI (3d-32%[238] x 2d-18%[168]) and ET (3d-37%[243] x 2d-33%[244]). The P4 at TAI altered fertility with higher P/AI in cows with P4  $< 0.1$ ng/mL, compared to cows with P4 from 0.1 to 0.29 and  $> 0.3$ ng/mL. Longer proestrus length (3d) increased percentage of cows with P4  $< 0.1$  ng/mL on d10. The P4 on d17 was higher ( $2.7 \pm 0.3$ ng/mL) for 3d of proestrus than 2d ( $2.3 \pm 0.3$ ng/mL). In Exp.2, a longer period of proestrus was tested (3dx4d). All cows ( $n=914$ ) were treated with the E2/P4 protocol with d7 PGF as described above, however cows were randomly assigned to have the CIDR removed on d8 with TAI on d10 (3d) or CIDR removed on d9 with TAI on d11 (4d). Cows were evaluated for presence/absence of a CL on d0. Cows with a CL on d0 had similar P/AI at the 30d PD (3d-43%[385] x 4d-43%[377]). However, cows without a CL with 3d of proestrus had lower P/AI (31%[70]) than all other groups (4d no CL-44%[82]). At 60d PD, no difference was detected between treatments in cows with CL (3d-37%[385] x 4d-39%[377]) or without CL (3d-26%[70] x 4d-36%[82]) on d0. Furthermore, cows with a CL on d0 had higher pregnancy loss with 3d of proestrus (14%[175]) compared to 4d (7%[166]). The P4 7d after TAI was lower for 3d of proestrus ( $3d-2.8 \pm 0.2$ ng/mL x  $4d-3.1 \pm 0.2$ ng/mL) compared to 4d, independent of CL presence. Fewer cows in 3d proestrus showed estrus (65%[455]) compared to 4d (72%[459]). Cows detected in estrus had greater P/AI at 30d PD (48%[609] x 30%[305]) and 60d PD (43%[609] x 24%[305]) and lower pregnancy loss (9%[301] x 17%[99]) compared to cows not detected in estrus, independent of treatment. Longer proestrus have a positive impact on fertility of dairy cows during ovulation synchronization protocols, and this is more important in TAI than in TET.



A083 FTAI, FTET and AI

### **Effect of bull on conception rate of zebu cows in a fixed-time artificial insemination program**

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**Keywords:** FTAI, Nelore, semen.

The use of protocols for fixed-time artificial insemination (FTAI) in order to control the growth of follicular waves, regulate the function of the corpus luteum and ovulation, is responsible for the expansion of artificial insemination in the country. However, many are the factors that may influence the results, among them stands out the individuality of the bull, since the FTAI insemination is performed before ovulation, requiring semen from bulls with characteristics to favor high viability in the female genital tract for a long time. This experiment aimed to evaluate the individual effect of three different bulls in the conception rate of *Bos taurus indicus* cows in a FTAI program. For this study, we used 307 females Nelore in different categories (calved cows, single cows and heifers), with mean body condition score of  $2.94 \pm 0.7$  (range 1-5) and submitted to the following hormonal protocol: in a random stage of the estrous cycle (D0) all cows received an intravaginal progesterone device (Sincrogest®, Ourofino, São Paulo, Brazil) and an application of 2.0mg of estradiol benzoate (Sincrodiol®, Ourofino) intramuscularly (im). On D8, the P4 device was removed and cows received 500µg Sodium cloprostenol (Sincrocio®, Ourofino) im, 1.0mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) im and 300 IU of Equine chorionic gonadotropin (Folligon®, MSD Saúde Animal, São Paulo, Brazil) im. On D10 all females were distributed according to category and body condition score into three groups: T1 (insemination with semen from the bull number 1, n = 103), T2 (insemination with semen from the bull number 2, n = 83) and T3 (insemination with semen from the bull number 3, n = 121) and were inseminated. The doses of semen from each bull belonged to the same batch and had a concentration above  $26 \times 10^6$  spermatozoa, with a minimum of 60% of progressive motility and 80% of spermatozoa with normal morphology. The pregnancy diagnosis was performed 30 days after insemination by transrectal ultrasonography (Mindray, DP2200vet, São Paulo, Brazil) and the results compared by chi-square test using SPSS software ( $P < 0.05$ ). The total conception rate found was 51.5% (158/307). There was no difference in conception rates among animals inseminated with the bull number 1 and 2 (T1: 39.8% vs. T2: 47.0%  $P = 0.325$ ), however, there were differences between the conception rates of cows inseminated with semen from bulls number 2 and 3 (T2: 47.0% vs. T3: 64.5%,  $P = 0.013$ ), as well as between the bulls number 1 and 3 (T1: 39.8% vs. T3: 64.5%,  $P = 0.0001$ ). The data presented show that there is a significant bull effect on sperm viability in the female genital tract. Bull certification should be conducted by insemination centers, so that bulls have satisfactory results when used in fixed-time artificial insemination.



A084 FTAI, FTET and AI

### **Pregnancy rate in embryos recipients is influenced positively by manifestation of estrous behavior and by plasma progesterone concentration at timed-embryo transfer**

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**Keywords:** cattle, conception rate, progesterone.

In timed-embryo transfer (TET) the possibility of selecting recipients with higher odds of becoming pregnant would significantly improve the success of this biotechnology. In cattle, plasma progesterone (P4) concentrations during the first week post-estrus are positively associated with pregnancy rates. Hypothesis was that P4 concentrations on days 5 (D5) and 7 (D7) post-estrus and the occurrence of estrous behavior are positively associated with pregnancy rates. Therefore, our aims were (1) to assess the effects of P4 concentrations on D5 and D7 on pregnancy rates, (2) to compare P4 concentrations on D5 and D7 between heifers that became pregnant or not after embryo transfer, and (3) to assess the effect of showing estrous behavior on pregnancy rates. Crossbred heifers (*Bos taurus taurus* x *Bos taurus indicus*; n=236) received a previously used intravaginal P4 device (Cronipress® - Biogênese) and an intramuscular (IM) injection of 1 mg of estradiol benzoate (Bioestrogen®; Biogênese). Devices were removed 8 days later, and heifers received 0.075 mg of D-Cloprostenol (Croniben®; Biogênese), 300 IU of eCG (Novormon® 5000 IU; Intervet Schering) and 0.6 mg of estradiol cypionate (ECP®; Pfizer), all by IM injections. The day of expected estrus (D0) was considered the second day after the removal of device. Estrous behavior was monitored twice daily during 60 hours after P4 device removal. Blood was collected on D5 and D7, and P4 concentrations measured by RIA. On D7 all heifers received an *in vitro*-produced embryo by TET. Pregnancy diagnosis was performed on D30 by ultrasonography. Data were analyzed by proc GLIMMIX from SAS. Heifers were divided in the ones that showed and the ones that did not show estrous behavior, according to pregnancy status and in quartiles (Q) of P4 concentrations on D7. Overall pregnancy rate was 58.05% (137/236). Heifers showing estrous behavior had higher pregnancy rates [62.4% (106/170) vs. 47.0% (31/66); P=0.01]. Pregnancy rates and P4 concentrations on D7 for Q1, Q2, Q3 and Q4 were 45.8% (27/59)<sup>c</sup> - 0.64±0.16 ng/mL, 52.25% (31/59)<sup>bc</sup> - 1.70±0.04 ng/mL, 66.1% (39/59)<sup>ab</sup> - 2.90±0.07 ng/mL and 67.8% (40/59)<sup>a</sup> - 5.52±0.27 ng/mL (different superscript letter indicates statistical difference; P=0.01). Heifers that became pregnant had higher P4 concentrations on D7 (2.87 ± 0.16 ng/mL vs. 2.45 ± 0.24 ng/mL; P<0.01), but not on D5. In conclusion, the pregnancy rate following TET of an *in vitro*-produced embryo is positively influenced by the manifestation of estrous behavior and P4 concentration at the moment of embryo transfer.

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A085 FTAI, FTET and AI

### **Influence of estrus on follicle dynamics in synchronization of ovulation protocol for FTAI in anestrus Nelore cows**

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**Keywords:** follicle, reproduction, ovary

The objective of this study was to evaluate the influence of estrus expression on follicle dynamic and ovulation in a synchronization of ovulation protocol with eCG in anestrus Nelore cows. In this sense, it was hypothesized that the cows showing estrus after the synchronization protocols have a greater dominant follicle, presenting anticipated ovulation. Adult, Nelore cows (n=24) were selected by ultrasonography for the absence of corpus luteum (CL). In the beginning of the experiment, the animals had a body condition score of  $2.82 \pm 0.23$  (1-thin to 5-obese, point scale) and were maintained under pasture conditions. The protocol for synchronization of ovulation used was D0 – intravaginal P4 device (Sincrogest®, Ourofino, Cravinhos-SP) and 2 mg of estradiol benzoate i.m. (BE, Sincrodiol®, Ourofino); D8 – 1 mg of estradiol cypionate i.m. (CE, E.C.P.®, Pfizer, São Paulo-SP), 0.4 mg of sodic cloprostenol i.m. (Sincrocio®, Ourofino), 300 IU of eCG i.m. (Novormon®, Intervet) and P4 device removal; D10 – FTAI. The ultrasonographic evaluations were done at 24, 48, 60, 72, 80 and 92 h following the P4 device removal. The estrus occurrence was assessed by the tail-head marked with chalk (Raidl-Maxi, RAIDEX GmbH, Dettingen/Erms, Germany). Estrus was deemed to have occurred in cattle with a tail-head chalk mark at TAI. The data were analyzed by Glimmix procedure of the SAS. After analysis, it was observed that 54.2% (n=13) of the cows showed estrus. All the follicle dynamic variables were similar ( $P > 0.05$ ) considering the presence or absence of estrus. The dominant follicle diameter of cows with estrus was  $7.3 \pm 2.2$  mm on the D8 and  $10.0 \pm 2.2$  mm at FTAI; and for the cows without estrus was  $6.7 \pm 3.1$  mm on D8 and  $8.5 \pm 3.0$  at the FTAI. Cows with presence of estrus have similar ( $P > 0.05$ ) ovulation rate (76.9%) compared with those cows with no estrus (45.4%), identical for the mean of the follicle growth (estrus 1.2mm/day vs. no estrus 0.9mm/day). The ovulation time was equal ( $P > 0.05$ ) in estrus cows ( $72.0 \pm 12.0$  h after the P4 device removal) and in no estrus cows ( $80.0 \pm 9.9$  h). Briefly, there was no effect of estrus occurrence on the ovarian response of anestrus Nelore cows submitted to a synchronization of ovulation protocol.



A086 FTAI, FTET and AI

### **Use of GnRH analogs to induce and synchronize ovulation in cattle**

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**Keywords:** estrous cycle, lecorelin acetate, TAI.

The GnRH agonist has an important application in treatments of the reproductive disorders and, more recently, it has been widely used in protocols for timed artificial insemination (TAI). The aim of this study was to evaluate the dose of a GnRH analogous molecule, 0.050mg of the lecorelin acetate (Dalmarelin®, Vallée, São Paulo, Brazil), to induce and synchronize ovulations in a hormone protocol for bovine. Twenty animals were first selected by the presence of the corpus luteum and assembled in a hormone protocol: D0 – progesterone (P4) implant and 2mg of Estradiol Benzoate (Sincrodiol® - Ourofino, Brazil); D8 – P4 implant withdraw and 0.5mg of cloprostenol (Sincrosin® - Vallée, Brazil); D9: the animals were randomized in one of the two groups – G1 (n=12) Lecirelin acetate and G2 (n=8) control. Transrectal examinations of the ovaries (ultrasound with linear array of 7.5 MHz - Mindray, DP2200) were performed on days D8, D9, D10 (morning) and D10 (afternoon) and, the diameter of the dominant follicle was measured and the ovulations were detected within this period. For each examination, the Student t test was used to compare the follicle diameter between groups. Differences in ovulation rate were accessed by Fisher exact test. The diameter of the dominant follicle did not change ( $P<0.05$ ) between groups on day 8 ( $9.3\pm 2.3$  vs  $9.5\pm 3.2$  mm) and 9 ( $10.0\pm 2.6$  vs  $10.4\pm 2.5$  mm for G1 and G2, respectively) of the hormone protocol. All ovulations were detected on the afternoon of D10 and were observed in 100% (12/12) of the animals of the G1. The ovulation rate was lower ( $P<0.05$ ) in control group (12.5% - 1/8), even though the follicle was larger ( $P<0.05$ ) than Lecirelin treated group ( $12.1\pm 2.2$  vs  $10.8\pm 2.5$  mm for G2 and G1, respectively) at previous ultrasound exam - D10/morning, approximately 20 hours after treatment. The GnRH analog – Lecirelin acetate - used in this hormone protocol was efficient to induce and synchronize ovulations in these experimental conditions.

**Support:** FAPEMIG, CNPq and Biotran.



A087 FTAI, FTET and AI

## **TAI improves the reproductive efficiency of primiparous nelore cows during the breeding season**

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**Keywords:** primiparous, reproductive efficiency, resynch.

The aim of this study was to compare the reproductive performance of primiparous Nelore cows submitted to different reproductive programs during the 84-d of breeding season (BS). Three programs are used: 1) Natural service (NS)+Suckling Restriction (T1; n=159) - cows were submitted to NS throughout the BS and suckling restriction period between 20 and 50 days of the BS, when the calves were separated and suckling only a once daily; 2) Timed artificial insemination (T2; n=154) - cows were submitted to a TAI at the first day of BS, followed by NS until the end of BS; or 3) T3 (n=160) - cows were submitted to a TAI and after 40 days, non-pregnant cows at first TAI were submitted to a second TAI (resynchronization). Cows of the groups T2 and T3 were synchronized using a TAI protocol 10 days before the onset of BS. Both synchronized and resynchronized received a P4 device (DIB®, MDS Animal Health) plus 2mg of estradiol benzoate (Gonadiol®, MSD Animal Health). Eight days after, the devices were removed and were associated with i.m. injections of 0.3975 mg of sodium cloprostenol (Ciosin®, MSD Animal Health) and 300 IU of equine chorionic gonadotropin (eCG; Novormon®, MSD Animal Health) plus 1 mg of estradiol cypionate (ECP®, Pfizer Animal Health). All synchronized and resynchronized cows received TAI 48 h after P4 device removal, with the same inseminator and semen batch. In T2 and T3, the bulls were introduced 15 days after the last TAI. Pregnancy diagnosis was performed by ultrasound 30 days after the first TAI and every 30 days after NS. Conception rates at 42 (42d) and at 84 (84d; end of NS) days of BS were analyzed by logistic regression using Glimmix of SAS. The conception rate of first TAI was similar ( $P=0.12$ ) between T2 (39.0%, 60/154) and T3 (30.0%, 48/160). The conception rate of resynchronization in T3 group was 33.9% (38/112). Cows that received TAI (T2 and T3) had higher ( $P<0.001$ ) proportion of cows pregnant at 42 days of BS [43.5% (67/154) and 56.8% (86/160)] than cows exposed only to the natural service and suckling restriction [T1; 17.6% (28/159)]. However, the proportion of cows pregnant at the end of BS did not differ ( $P = 0.21$ ) among the treatments [T1 = 47.8% (76/159); T2 = 48.7% (75/154); T3 = 55.0% (88/160)]. The interval in days between the onset of BS and the occurrence of pregnancy was different between groups (T1 =  $47.6\pm 1.7^c$  days; T2 =  $11.9\pm 2.9^a$  days; T3 =  $20.5\pm 2.4^b$  days;  $P < 0.0001$ ), when the T2 group showed the shorter interval. It was concluded that the use of TAI at the beginning of BS increases the proportion of pregnant primiparous cows by artificial insemination and induced cows to become pregnant faster than those subjected only to natural service plus suckling restriction.

**Acknowledgments:** MSD Animal Health; HoRa Agronegócio.



A088 FTAI, FTET and AI

### **Ovulation synchronization with EB or GnRH in buffalo TAI during the non breeding season**

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**Keywords:** buffaloes, ovulation inducers, TAI

The aim of this study was to evaluate the efficiency of different ovulation inducers - Estradiol Benzoate (EB) and Buserelin acetate (GnRH) - at different times - 24 (BE), 36 (BE) and 48 (GnRH) hours after PGF2 $\alpha$  injection - and two categories of buffaloes - nulliparous and multiparous - submitted to TAI protocol during non breeding season. In the study, 354 buffaloes were divided according to category, age, weight, body condition score and ovarian activity in one of three groups (GBE24, n = 112; GBE36, n = 125; GGn48, n = 117). At random stage of the estrous cycle (D0, pm), all buffaloes received an intravaginal progesterone device (P4; Sincrogest®, Ourofino Agronegócio, Brazil) and 2.0 mg im of estradiol benzoate (EB; Sincrodiol®, Ourofino Agronegócio). On D9 (pm), females received im 0.53 mg PGF2 $\alpha$  (Cloprostenol, Sincrocio®, Ourofino Agronegócio) and 400 IU of eCG (Novormon®, MSD Saúde Animal, Brazil), followed by P4 removal. After 24h (D10, pm) the GBE24 ovulation was induced by 1.0 mg im of EB injection (Sincrodiol®, Ourofino Agronegócio). The GBE36 and GGn48 received im 1.0 mg of EB and 10  $\mu$ g of GnRH (Sincroforte®, Ourofino Agronegócio) on D11 (am and pm), respectively. One portion of animals (GBE24, n=47; GBE36, n=50; GGn48, n=44) was randomly selected for ultrasound evaluation (DP2200Vet Mindray, China) performed on D0 to check ovarian activity, on D9 to verify the follicular diameter and from D11 to D14 (12/12h for 60h) to establish the time of ovulation. All animals were TAI 64h after PGF2 $\alpha$  injection and 30 days later submitted to the pregnancy diagnosis (D42; Mindray DP2200Vet). The variables were analyzed using the GLIMMIX procedure of SAS®. There was no interaction between the ovulation inducers and animal categories (P>0.05). In addition, there was no difference between the experimental groups (GBE24, and GBE36 GGn48) to ovulatory follicle diameter ( $\emptyset$ ; 13.1 $\pm$ 0.3mm; 13.7 $\pm$ 0.3mm; 13.7 $\pm$ 0.3mm) and to ovulation [78.7% (37/47); 82.0% (41/50); 84.1% (37/44)] and pregnancy rates [51.3% (60/117); 45.5% (51/112); 46.4% (58/125)], respectively (P>0.05). However, the multiparous were superior to nulliparous on dominant follicle  $\emptyset$  on D9 (13.3 $\pm$ 0.3mm and 8.6 $\pm$ 0.2mm), on ovulatory follicle  $\emptyset$  (14.1 $\pm$ 0.3mm and 13.1 $\pm$ 0.2mm), on ovulation rate [91.1% (51/56) and 75.3 (64/85)] and on the interval between PGF2 $\alpha$  injection and ovulation (70.3 $\pm$ 1.3h and 66.5 $\pm$ 1.4h), respectively (P<0.05). This range was also higher for GBE36 (72.4 $\pm$ 1.4h) compared to GBE24 (64.0 $\pm$ 1.5h) and to GGn48 (67.6 $\pm$ 1.9h). In conclusion, ovulation induction with EB or GnRH results in satisfactory follicular response, ovulation and pregnancy rates in buffaloes synchronized for TAI during the non breeding season.



A089 FTAI, FTET and AI

### **Effects of eCG on follicular, luteal and embryonic development of Nelore cows submitted to a synchronization of ovulation protocol for timed AI**

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**Keywords:** corpora lutea, follicle, reproduction.

The aim of this study was to evaluate the effects of equine chorionic gonadotrofin (eCG) on follicular dynamics, corpora lutea (CL) development and conceptus size on day 16 after timed AI (TAI) in Nelore cows. At random day of the estrous cycle (D0), a total of 124 cows received 2 mg of estradiol benzoate i.m. (EB; Gonadiol®-MSD Animal Health) and a progesterone intravaginal device (P4; CIDR®, Pfizer Animal Health). At the P4 device removal (D8), cows received 0.15 mg of D-cloprostenol i.m. (Prostaglandina Tortuga® - Tortuga Companhia Zootécnica Agrária) and 0.5 mg of estradiol cypionate i.m. (E.C.P.® - Pfizer Saúde Animal) and were randomly assigned in two groups: eCG (n=60), where cows received 300 IU of eCG i.m. (Novormon® - MSD Saúde Animal) or control (n=64). The diameter of the dominant follicle (DF) on D8 and D10 and CL on D15 were determined by ultrasound exam. Cows were timed inseminated 48 h after P4 device removal and were slaughtered 16 days after (D26). Blood samples were collected on D15 and D26 to determine serum P4 concentration. The genital tracts were recovered immediately after slaughter and the embryos were collected by uterine flushing using phosphate-buffered saline. The recovered conceptuses were photographed and their sizes were determined using the software AutoCAD 2007®. The CLs were dissected, weighed and the diameter was measured using a caliper rule. Data were analyzed by the GLIMMIX procedures of SAS. The diameter of the DF at the moment of eCG administration (D8) was similar between groups (P=0.70). The follicular growth rate from D8 to D10 (3.5±0.3 mm vs 2.6±0.2 mm; P=0.03), the diameter of CL on D15 (16.3±0.5 mm vs 14.7±0.5 mm; P=0.03) and on D26 (19.6±0.3 mm vs 17.9±0.4 mm; P=0.003) and CL weight on day 26 (2.8±0.1 g vs 2.4±0.1 g; P=0.04) were greater in eCG group. There was no difference between groups for embryo recovery [30% (18/60) and 29.7% (19/64); P=0.97], conceptus size (118.5±21.8 mm vs 98.1±17.2 mm; P=0.23), P4 concentration on D15 (1.4±0.2 ng/ml vs 1.5±0.5 ng/ml) and on D26 (5.5±0.8 ng/ml vs 5.5±0.8 ng/ml) respectively for eCG and control groups. In conclusion, despite the fact that eCG promoted follicular growth and increased CL diameter, the conceptus length and progesterone serum concentration did not change.

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A090 FTAI, FTET and AI

### **Fixed time artificial insemination in dairy goats with cooled semen stored for 24 or 48 hours**

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**Keywords:** artificial insemination, cooled semen, goat

The fertilizing capacity of goat semen chilled at 5°C for 24 (T24) or 48 (T48) hours was evaluated. Three Canarian's Bucks were used. The insemination dose was 150 million mobile spermatozoa. The extender was tris – egg yolk at 2.5%, for cooling and keeping the semen at 5°C a Botutainer® (Biotech Botucatu, Animal Reproduction, Botucatu - SP) was adapted and used. The estrus was synchronized by using an intravaginal sponge containing 60 mg of medroxyprogesterone acetate during 6 days; also 37.5 µg of D-cloprostenol and 200 IU of eCG, both injected 24 hours before removing the sponges. Transcervical artificial insemination at fixed time was performed 37 hours after sponge removal in 133 goats (native of Cape Verde) that were divided, randomly, into the two treatments T24 and T48. The means of sperm motility and calving rates were evaluated by Kruskal-Wallis test. Data regarding the range of sponge withdrawal to artificial insemination (SWAI), calving rate (CARA) and depth of semen deposition (DSD) were subjected to variance analysis (ANOVA). There was no difference ( $P > 0.05$ ) between the seminal patterns for the two different periods of cooling (T24 - 58.8%±11.1 for motility and 2.9±0.5 for strength; T48 - 51.3%±2.5 for motility and 2.8±0.3 for strength), which allowed to obtain similar pregnancy rates in both treatments (T24 – 26.5% and T48 – 21.5%). The efficiency of the tested protocols allowed the dissemination of goat's genetic material in the Republic of Cape Verde. There was a correlation ( $r = 0.27$ ,  $P < 0.05$ ) between SWAI and DSD. There was also a correlation ( $r = 0.29$ ,  $P < 0.05$ ) between SWAI and CARA, indicating that late inseminations favored the results. It was concluded that goat semen, cooled for 48 hours at 5°C, has the same fertility capacity that semen cooled for 24 hours at 5°C and that the artificial inseminations were done too early for the suggested protocol.



A091 FTAI, FTET and AI

## **Timed artificial insemination improves the reproductive performance of suckled beef cows during a breeding season**

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**Keywords:** artificial insemination, breeding season, synchronization.

The objective of the present study was to compare the performance of different reproductive programs that used natural service (NS) and timed artificial insemination (TAI) on a 90 day (90d) Breeding Season (BS). Also, the present study aimed to evaluate the effect of body condition score at onset of BS, cows breed (Nelore vs. Crossbred) and parity (primiparous vs. multiparous) on the reproductive efficiency. A total of 507 suckled beef cows (Nelore, n=302 and crossbred, n=205) were enrolled in this study. Cows were randomly assigned into one of two treatments: TAI+NS (n=252); cows received TAI on Day 11 of the BS followed by NS until the end of the BS (90 days). The NS group (n=255) only received NS during entire BS. Cows in the TAI+NS group were synchronized with an estradiol plus progesterone-based TAI protocol. Cows were examined for pregnancy by transrectal ultrasonography 30 d after the TAI or every 30 d after exposure to bulls to evaluate gestational age. Pregnancy rates at 45 and 90 d of the BS was evaluated using the GLIMMIX procedure. The hazard of pregnancy was analyzed by the Cox proportional hazard model using the PHREG procedure of SAS. The pregnancy rate following TAI (P/TAI) was 52.4% (132/252). The TAI+NS group had greater (P=0.001) proportion of cows pregnant at 45 d of the BS (63.5%; 160/252) than the group of cows exposed only to NS (46.3%; 118/255). However, proportion of cows pregnant at end of the BS did not differ (P=0.31) between treatments [(TAI+NS=77.0% (194/252) vs. NS=71.0% (181/255)]. Regardless of the breeding program, multiparous cows had greater (P<0.01) proportion of pregnant cows at 45 d of BS [(Multiparous=72.4% (186/257) vs. Primiparous=36.8% (92/250)] and at the end of the BS [(Multiparous=87.6% (225/257) vs. Primiparous=58.0% (145/250)] than primiparous cows. Cows with BCS  $\geq$  3.0 had greater (P<0.01) proportion of pregnant cows at 45 d [ $\geq$ 3.0=69.6% (183/263) vs. <3.0=38.9% (95/244)] and in the end of BS [ $\geq$ 3.0=86.3% (227/263) vs. <3.0=58.6% (143/244)] than cows with BCS < 3.0. Crossbred cows also had greater (P<0.01) proportion of pregnant cows at 45 d [Crossbred=67.3% (138/205) vs. Nelore=46.4% (140/302)] and at end of the BS [Crossbred=84.4% (173/205) vs. Nelore=62.5% (197/302)] than Nelore cows. In conclusion, the use of TAI improves the proportion of pregnant cows in the onset of BS when compared to the exclusive use of NS. In addition, multiparous cows, having greater BCS, and crossbred cows had greater pregnancy rates at 45d and at the end of BS. Data are indicative that the establishment of reproductive programs that use TAI in the onset of the BS is an important tool to improve reproductive efficiency of suckling beef cows.

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A092 FTAI, FTET and AI

### **The effect uterine massage on conception rate of nelore cows submitted to FTAI protocol**

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**Keywords:** conception rate, FTAI, uterine massage.

The aim of this trial was to evaluate the uterine massage effect on conception rates of Nelore cows submitted to fixed time artificial insemination (FTAI). The work was made in a farm located in Santa Amélia/PR. Two hundred and seven Nelore lactating cows were studied. Their body condition score were from 2.5 to 3.5 and they were divided into three groups with similar body score and postpartum period. The animals from the first group (control group, n=87) received 1.9g implant of intravaginal progesterone (CIDR, Pfizer, Brazil) and 2 mg estradiol benzoate IM (Estrogin, Farmavet, Brazil), in random stages from estrous cycle. Nine days later the implants were removed and the animals received an administration IM of 12.5 mg Dinoprost (Lutalyse, Pfizer, Brazil), 400 IU of eCG (Novormon, MSD, Brasil) and 1mg of estradiol cypionate (ECP, Pfizer, Brasil). All animals were inseminated 50 to 54 hours after implants removal, with semen from a bull. The second (massage 30 days prior group, n=61) and third groups (massage group, n=59) received the same hormone treatment as control group, preceded by uterine massage for one minute, 30 days prior to the beginning or on the day of the FTAI protocol, respectively. The pregnancy diagnosis was made by transrectal ultrasonography after 30 days from artificial insemination. The results were analyzed by Anova ( $P < 0.05$ ). Pregnancy rates were similar ( $P > 0.05$ ) among the experimental groups resulting in 55.1% (48/87), 55.7% (34/61), and 54.2% (32/59), respectively, for the control group, massage 30 days prior group and massage group. From the results it was concluded that the uterine massage had no effect on the conception rate of Nelore cows submitted to the FTAI protocol.



A093 FTAI, FTET and AI

### **Comparison of recovery rates and pregnancy rates of embryos from quarter horse mares of different ages: preliminary results**

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**Keywords:** donor mares, pregnancy rate, transfer of embryos.

The objective of this study was to evaluate the rate of embryo recovery, embryo quality, and pregnancy rate in mares of different ages. The study was made in two farms located in Limeira/SP, in breeding season 2011/2012. Were analyzed 200 uterine flushings of Quarter Horse mares (n=113). The procedures were divided into three groups according to age: mares from 2 to 7 years old (G1, n = 112), 8-14 years old (G2, n = 50) and over 15 years old (G3, n = 38). Donors with follicles of 35 mm and significant uterine edema were treated with 1 mg of deslorelin or 1250 IU of hCG. The mares were inseminated with cooled semen 24h after ovulation induction and examined 24 h later to verify the occurrence of ovulation. Semen of 70 stallions at a dose of 500 million to 2 billion sperm with progressive motility were used. Uterine flushing were performed 7 to 9 days after ovulation and embryo transfer was done in healthy mares, between the fifth and ninth days of the estrous cycle, with a closed cervix, uterus with homogeneous echogenic, and content without edema on ultrasound evaluation. Pregnancy diagnosis was performed at 15 days of gestation by transrectal ultrasonography. Results were compared by analysis of variance ( $P < 0.05$ ). The results were grouped because there was no effect of location on the variables analyzed. The recovery rate of embryos was similar ( $P > 0.05$ ) between groups (G1: 74.1% (83/112), G2: 64% (33/50) G3: 65.7% (25/38). The stage of embryonic development was similar between groups resulting in 86.7% (72/83), 78.1 (25/32) and 87.1% (21/25) of expanded blastocysts, 1.2% (1/83), 12.5% (4/32) and zero blastocysts and 12.3% (10/83), 12.5% (4/32) and 8% (8/25) of morula, respectively for G1, G2 and G3. There was a predominance of embryos of excellent quality among the groups (G1: 89.1%, G2: 96.8% and G3: 76%). Pregnancy rates were similar between groups G1: 79.5% (80/83), G2: 81.1% (27/33), and G3: 80% (20/25). From the preliminary results we observed no effect of age on the efficiency of the programs of embryo transfer in mares.



A094 FTAI, FTET and AI

### **TAI synchronization protocols using intravaginal device Vallée® containing 0.6 or 1.2 g of progesterone present satisfactory results on ovarian follicle and pregnancy responses in bovine females**

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**Keywords:** bovine, progesterone device, TAI.

The present study evaluated the ovarian follicular dynamics and conception rate following synchronization of ovulation using intravaginal devices containing 1.2 (DMax; Dispolcel Max®; Vallée S.A.) or 0.6g (DMonouso; Dispolcel Monouso®; Vallée S.A.) of progesterone (P4). The number of use of each devices (1X=used for 8 days or 2X=used for 16 days) was tested. Four experiments were conducted to evaluate the efficacy of the protocol [Exp. 1 = Follicular dynamics and Exp. 2 (A, B and C) = Pregnancy rate]. In Exp. 1, 81 Holstein females (40 cows and 41 heifers) were divided into one of six treatments: New P4C (DIB®; MSD Animal Health; n=13); New DMax (n=14); DMax 1X (n=13); DMax 2X (n=13); New DMonouso (n=14) and DMonouso 1X (n=14). The synchronization protocol used consisted in the administration of 2mg of EB (P4C=Gonadiol®, MSD Animal Health or Vallée Groups=Estradiol Benzoate Vallée®) plus insertion of the device according to the treatment in the beginning of the protocol (D0). At D8, the devices were removed and was administered sodium cloprostenol (P4C=530µg Sincrocio®, Ourofino Animal Health and Vallée Groups=500µg, Sincrosin®, Vallée®), 1mg of estradiol cypionate (P4C=ECP®, Pfizer Animal Health and Vallée Groups=Estradiol cypionate Vallée®) plus 300IU of eCG (Folligon®, MSD Animal Health). Ultrasonographic examinations were performed (Chison 8200Vet, 7.5MHz) from removal up to 96 hours to determine the moment of ovulation. All animals received TAI 48 hours after the devices removal. In Exp. 2, 1599 Nelore postpartum cows were treated with the same protocol mentioned above, however, divided in experiments 2A (New P4C vs. New DMax vs. New DMonouso), 2B (P4C 1X vs. DMax 1X vs. DMonouso 1X vs. DMax Novo) and 2C (P4C 2X vs. DMax 1X vs. DMax 2X vs. New DMax). The pregnancy diagnosis was performed 30 days after TAI. Data were analyzed using the PROC Glimmix of SAS. In Exp. 1, no differences were found between treatments for the moment (P=0.38) and ovulation rate (P=0.99) (New P4C=69.2±14.0h and 100% vs. New DMax=60.9±12.9h and 100% vs. DMax 1X=68.3±12.4h and 76.9% vs. DMax 2X=64.8±12.9h 85.7% vs. New DMonouso =70.0±10.0h and 85.7% vs. DMonouso 1X=62.0±16.8h and 85.7%). In Exp. 2, no differences were verified on the pregnancy rates in either experiments: 2A [New P4C (62.3%; 91/146), New DMax (61.5%; 91/148) and New DMonouso (66.0%; 95/144); P=0.35]; 2B [P4C 1X (54.0%; 81/150); DMax 1X (48.0%; 71/148); DMonouso 1X (54.9%; 79/144) and New DMax (56.4%; 84/149); P=0.16] and 2C [P4C 2X (51.0%; 74/145); DMax 1X (48.6%; 70/144); DMax 2X (51.8%; 72/139) e New DMax (52.8%; 75/142); P=0.81]. In conclusion, it is possible to use Dispolcel Max® (New, 1X or 2X) or Dispolcel Monouso® (New or 1X) devices for the synchronization of ovulation and TAI in bovine females with satisfactory results.

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A095 FTAI, FTET and AI

## **CL presence and PGF2 $\alpha$ treatment effect at the beginning of the resynchronization protocol on lactating Nelore cows**

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**Keywords:** prostaglandin, synchronization, TAI.

The effect of the CL presence and prostaglandin administration at beginning of the resynchronization protocol were evaluated on pregnancy rate and estrus expression in lactating Nelore cows. In the pregnancy diagnosis (30 days) from the first TAI, 635 Nelore cows (492 primiparous and 143 multiparous) were selected among the ones with negative diagnosis for pregnancy. At this time, females were distributed according to their ovarian status (presence or absence of CL) in one of three treatments (without CL Group, CL Group and CL+PGF Group). Also, in the same day (D0), all cows were synchronized with 2mg estradiol benzoate IM (RIC-BE, Agener Union, Brazil) and an intravaginal progesterone device previously used for 16 days (Primer, Agener Union, Brazil). Moreover, on CL+PGF group administrated 250 $\mu$ g cloprostenol sodium (Sincrocio, Ouro Fino, Brazil). On D8, the progesterone device was removed and 1 mg of estradiol cypionate (ECP®, Pfizer, Brazil), 300 IU of eCG (Novormon®, MSD, Brazil) and cloprostenol sodium (500 $\mu$ g im without CL Group and CL Group and 250 $\mu$ g in CL+PGF Group) were administered. A subgroup (n = 421, 279 multiparous and 142 primiparous) was used to detect estrus after of synchronization protocol. A marking stick stamped on the tail set at the moment of progesterone device removal was used. Was considered in estrus, females who had stick stamped removed at the TAI. Statistical analysis was performed using SAS software by PROC GLIMMIX. A statistical difference was observed between multiparous and primiparous cows in CL presence at the beginning of the resynchronization protocol [60.0% (295/492) vs. 14.7% (21/143), P=0.001], expression of estrus [62.0% (173 / 279) vs. 43.0% (61/142), P=0.001] and pregnancy rate [45.9% (226/492) vs. 28.7% (41/143), P=0.001], respectively. On the evaluation of the treatments, only multiparous cows were used due to the high incidence of primiparous cows without CL that promoted imbalance between experimental groups. The cows from the CL+PGF Group [81.1% (60/74)<sup>a</sup>] had higher estrus expression rate (P = 0.004) than the other experimental Groups [48.5% (65/134)<sup>c</sup> for Without CL Group and 67.6% (48/71)<sup>b</sup> for CL Group]. Furthermore, the pregnancy rate was greater in cows that had CL at the beginning of the protocol [58.3% (88/151)<sup>a</sup> for PGF+CL Group, 50.0% (72/144)<sup>a</sup> for CL Group and 33.5% (66/197)<sup>b</sup> to Without CL Group]. In conclusion, multiparous Nelore cows have higher CL presence at the beginning of resynchronization, higher expression of estrous (multiparous and primiparous) and higher pregnancy (multiparous). Moreover, cows with CL at the beginning of the resynchronization protocol had higher estrous and pregnancy rates. Additionally, the administration of prostaglandin at the beginning of resynchronization protocol increases the expression of estrus, but does not alter the pregnancy rate of cows undergoing resynchronization protocol.



A096 FTAI, FTET and AI

## Evaluation of the efficacy of ECEGON® on ovarian response and pregnancy in cattle subjected to FTAI

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**Keywords:** eCG, fertility, reproduction efficiency.

The objective of this study was to evaluate the effect of im administration of Ecegon® (eCG commercialized by Biogénesis-Bagó, Argentina) on follicular growth, diameter of the dominant follicle (ØDF), ovulation rate, volume of the CL and pregnancy rate of Nelore (*Bos indicus*) and crossbred (*B. indicus* x *B. taurus*) cows in postpartum anoestrus (25-45d) cows synchronized for FTAI. The study was conducted in Vera Cruz Farm, Nova Xavantina – MT. At random days of the estrous cycle (D0), cows (n=254) received a new progesterone-releasing intravaginal device (Cronipres Mono Dose®, Biogénesis-Bagó, Argentina) and 2 mg estradiol benzoate (Bioestrogen®, Biogénesis-Bagó, Brazil) IM. On D8, device was removed and 150µg D-Cloprostenol was administered (Croniben®, Biogénesis-Bagó, Brazil) IM. At this same time cows were homogenously allocated (according to body weight, BCS and ØDF) to receive or not 400IU of Ecegon®. On D9, 1mg estradiol benzoate was given IM. FTAI was performed 54h after device withdrawal. Ultrasonografic evaluations were performed on D0 and D8 to verify presence of CL, on D8 and D10 to evaluate the ØDF, on D17 to verify presence and volume of the CL, and on D40 to diagnose pregnancy. Only cows without CL on D0 and D8 were used herein. Daily follicular growth was calculated dividing by two the difference among ØDF on D8 and D10. Ovulation was considered by the presence of CL on D17 in the same ovary where the DF was observed on D10. A blood sample was collected on D17 to evaluate serum progesterone concentration (RIA method; Coat-A-Count®, Genese, USA). Data were analyzed using PROC GLIMMIX of SAS. No interaction was found among treatment and breed. The ØDF on D8 was similar among control and Ecegon®-treated cows (10.9 ± 0.2 vs. 11.0 ± 0.2 mm; P = 0.96), demonstrating the homogeneity before treatment. Cows receiving Ecegon® had greater ØDF on D10 (13.6 ± 0.2 vs. 12.2 ± 0.2 mm; P < 0.00001), greater daily follicular growth rate (1.40 ± 0.06 vs. 0.70 ± 0.05 mm/day; P < 0.00001), greater ovulation rate (95.9 vs. 70.3%; P < 0.00001), greater volume of the CL (3,927.4 ± 141.1 vs. 3,127.0 ± 145.8 mm<sup>3</sup>; P < 0.00001) and greater progesterone concentration on D17 (3.99 ± 0.16 vs. 2.14 ± 0.13 ng/mL; P < 0.00001). Also, pregnancy rate tended to increase when cows were treated with Ecegon® (50.0 vs. 39.4%; P = 0.09). Thus, Ecegon® was efficient to increase follicular and luteal dimensions, leading to an increase in pregnancy rates in anestrous postpartum cows.

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A097 FTAI, FTET and AI

### Effect of progesterone, duration of proestrus and follicular diameter on the conception rate of timed-inseminated pubertal Nelore heifers

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**Keywords:** heifers, proestrus, TAI.

The aim of this study was to evaluate the effect of progesterone, follicular diameter and length of proestrus on conception rate of pubertal heifers submitted to TAI. In this study, 1083 cycling heifers with a CL in at least two consecutive ultrasound evaluations (Aloka - SSD 500) were synchronized with the protocol: D0: estradiol benzoate (EB, 2.0 mg, Estrogin®) + CIDR®; D7: dinoprost tromethamine (PGF2 $\alpha$ , 12.5 mg, Lutalyse®) and D9: removal of the device. In D0, the animals were randomly distributed to receive a new (CIDR1) or a previously used CIDR for 18 days (CIDR3). The heifers were inseminated 48 h (0.5 mg, im, ECP® on day 9), 54 or 72 h (100  $\mu$ g, im., Fertagyl®) at the time of TAI) after removal of the devices performing 6 experimental groups (2 x 3 factorial). The largest diameter of the follicle ( $\emptyset$ DF) was assessed in a subgroup of heifers on day 7 (n = 255), D9 (n = 532) and in all animals at TAI. Blood samples for P4 were collected from the animals assessed on D7, D9 and D19 in all animals (7 d after TAI). Pregnancy diagnosis was performed 40 days after TAI. Continuous variables were analyzed using PROC MIXED and PROC GLIMMIX for binomial. Heifers with P4 concentration  $\geq$  1.0 ng/mL on D19 were considered as ovulated, and used for analysis of conception rate (CR = pregnant heifers/ovulated). In the analysis of  $\emptyset$ DF at TAI heifers that ovulated early ( $\emptyset$ DF  $\leq$  6.2 mm and concentration of P4D19  $\geq$  1.0 ng/ml, n = 283) were removed from the analysis. Significance was set when  $P \leq 0.05$  and tendency when  $P \leq 0.10$ . In the analyses all interactions were tested and variables with  $P > 0.2$  were removed from the final model. Heifers treated with CIDR3 had lower P4 concentration (D7:  $4.77 \pm 0.27$  and D9:  $2.86 \pm 0.11$  ng/mL) than CIDR1 (D7:  $6.61 \pm 0.27$  and D9:  $4.31 \pm 0.11$  ng/mL) and larger  $\emptyset$ DF (D7:  $7.60 \pm 0.13$  and D9:  $9.62 \pm 0.40$  mm) compared to CIDR1 (D7:  $6.84 \pm 0.13$  and D9:  $8.53 \pm 0.40$  mm). The P4 concentration negatively affected  $\emptyset$ DF on D7 ( $r = 0.21$ ) and  $\emptyset$ DF on D9 ( $r = 0.18$  and  $r = 0.15$ ). There was an effect of number of CIDR uses on  $\emptyset$ DF-TAI (CIDR1:  $11.20 \pm 0.10$  and CIDR3:  $11.65 \pm 0.11$ ) and [ ] P4D19 (CIDR1:  $3.62 \pm 0.09$  and CIDR3:  $3.97 \pm 0.09$  ng/mL). The [ ] P4D19 positively influenced the CR. There was a positive effect of  $\emptyset$ DF-TAI on conception, ovulation and [ ] P4D19 ( $r = 0.26$ ). Treatments did not have effect on CR (43.63%, 404/926) and pregnancy (PR = 37.31%, 404/1083), but heifers with  $\emptyset$ DF-TAI between 11.0 and 15.5 mm had a greater CR (53.35, 263/493) compared to animals with  $\emptyset$ DF-TAI  $< 11.0$  mm (30.77%, 88/286) which did not differ from animals with  $\emptyset$ DF-TAI  $> 15.5$  mm (38.10%; 8/21). At the time of TAI 36% of the heifers had follicles  $< 11.0$  mm constituting an aggravating factor for this animal category. It is concluded that the proestrus was not the limiting factor in the conventional protocol of 9 days and the main factor that interferes with the TC is the follicular diameter, however the use of a device previously used (CIDR3) did not enhance the CR and PR, therefore others factors may affect follicular development and fertility in heifers.



A098 FTAI, FTET and AI

## **Reduction of prostaglandin (Ciosin®) dose during timed artificial insemination protocols in cyclic nelore heifers and suckled Nelore cows**

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**Keywords:** *Bos indicus*, luteolysis, TAI.

Two experiments were designed to evaluate effect of reduction of sodium cloprostenol dose (Ciosin®) on the pregnancy rates in cyclic Nelore heifers and suckled Nelore cows subjected to synchronization of ovulation protocol for timed artificial insemination (TAI). In Experiment 1, heifers (n = 457) received one of three different doses of sodium cloprostenol (500µg vs. 375µg vs. 250µg). In Experiment 2, lactating Nelore cows (n = 626; 30 to 60 days postpartum) were randomly assigned to received one of two doses of cloprostenol (375µg vs. 250µg). On Day 0, all animals received 2.0 mg im estradiol benzoate (EB, Gonadiol®, MSD Animal Health) and norgestoment ear implant (heifers, P4, Crestar®, MSD Animal Health) or an intravaginal device containing progesterone (cows, P4, DIB®, MSD Animal Health). On day 8, exogenous P4 were removed and 300 IU eCG i.m. (Novormon®, MSD Animal Health) plus estradiol cypionate im (EC, heifers = 0.5 mg and cows = 1.0 mg, ECP®, Pfizer Animal Health) were administrated. Animals were randomly assigned in experimental groups: Experiment 1 – heifers [500µg (2.0 mL) or 375µg (1.5 mL) or 250µg (1.0 mL) im cloprostenol (PGF, Ciosin®, MSD Animal Health)] and Experiment 2 – cows [375µg (1.5 mL) or 250µg (1.0 mL) im of cloprostenol]. All animals were submitted to TAI 48h after P4 device removal. The data were analyzed by Glimmix procedure of the SAS. No differences were found between treatments on the pregnancy rate in suckled cows [375µg = 55.1% (173/314); 250µg = 59.3% (185/312), P = 0.88] or in cyclic heifers [500µg = 60.8% (93/153); 375µg = 50.2% (76/151); 250µg = 59.5% (91/153), P = 0.14]. In conclusion, it is possible to reduce the dose of prostaglandin (250µg of cloprostenol) on synchronization of ovulation for TAI in cyclic Nelore heifers and suckled Nelore cows, with no detrimental effects on pregnancy outcomes.

**Acknowledgment:** MSD Animal Health; Hora Agronegócio.



A099 FTAI, FTET and AI

### **Progestogen or GnRH-hCG supplementation after artificial insemination in postpartum beef cows**

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**Keywords:** cattle, estrus resynchronization, GnRH, hCG, norgestomet.

This study determined pregnancy rate (PR) of cows after fixed time artificial insemination (FTAI=Dzero) and supplementation with progestogen or a GnRH-hCG association to optimize ovarian function of these cows. Crossbred beef suckled cows (N=274; ½ Nelore and ½ either Simental or Aberdeen Angus) from Embrapa Pecuária Sudeste (BCS=5.6; body weight=563.5kg and more than 60 days postpartum) were randomly assigned to groups: SYNC (n=99) – received 3 mg norgestomet/5 mg estradiol valerate IM and an auricular 3 mg norgestomet implant, which was removed nine days later and 0.5 mg of estradiol cypionate was given IM. FTAI was performed 54 hours after that; RE-SYNC (n=103) – treated as SYNC and re-implanted with one once-used implant on D12 after FTAI. This implant was withdrawn on D21 and a second FTAI was carried out 48h later only in females in heat within this two-day interval; GNRH-HCG (n=72) - treated as SYNC and injected (IM) with 200mcg of gonadotropin releasing hormone (GnRH) on D5 and 2500 IU of hCG on D12. All cows were thereafter monitored for estrus displaying and AI was done according to the am:pm/pm:am rule. Ultrasound diagnosis took place 30 days post-FTAI and parturition rate was recorded. Data were analyzed by chi-square test. PR to the FTAI did not differ ( $P>0.05$ ;  $\chi^2_{GL=2}=2.03$ ) between SYNC (67.7%) and RE-SYNC (64.1%) or GNRH-HCG (70.8%) and PR to the following AIs did not vary either. Overall parturition rate was 87.9% after the breeding period (BP). The number of pregnant cows within the first 23 days of that BP in RE-SYNC was not greater ( $0.20>P>0.10$ ;  $\chi^2_{GL=2}=4.19$ ) than the other groups, but those cows conceived earlier. Indeed, the proportion of parturitions due to the 1st and 2nd FTAI of RE-SYNC compared ( $P>0.05$ ;  $\chi^2_{GL=2}=0.27$ ) to overall values in SYNC and GNRH-HCG after finished the entire BP. This trial did not show differences among treatments, but the resynchronization protocol allowed for high PR within the first 23 days of the breeding period. The use of male teasers was shortened to only two days within that entire period.

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A100 FTAI, FTET and AI

## **Pregnancy rates obtained through transfer of bovine embryos at different stages of development**

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**Keywords:** cattle, embryo transfer, embryonic stages.

The embryo transfer (E.T.), is a technique which aims to increase the number of offspring of a female during her reproductive lifetime. The embryonic stages are divided into morula, compact morula, early blastocyst, blastocyst, expanded blastocyst and hatched blastocyst. In bovine, the best pregnancy rates (PR) can be obtained through the use of ET in morula and blastocyst stage. Thus, the aim of this study was to evaluate the PR in different embryonic stages through ET performed on farms in western Pará state. In the methodology, the protocol used was accomplished with the application progesterone (P4), two days before the beginning of hormone application in the Nelore donors as well as in the crossbred Nelore receptors and this time was designated as (D-2). The P4 (Sincrogest, Ourofino, Brazil) was used along with 2 ml of estradiol benzoate (E.B.) (EB-RIC, Tecnopec, Brazil). Two days later (D0) the protocol of the donor started and it was used PRID with P4 and 2.5 ml of EB was applied in the donor females. On day 4 (D4) at 08:00 it was applied 40 IU of follicle-stimulating hormone (F.S.H.; Pluset, Hertape Calier), and at 18:00 it was applied 40 IU of FSH which corresponded to 40% of the whole dose. Further, on day 5 (D5) twice at 08:00 and 18:00 it was applied 34 IU FSH which comprised to 30% of the corresponding dosage, respectively. On day 6 (D6) at 08:00 it was applied 28.4 IU FSH corresponding to 20% of the whole dose, plus 2 ml of Prostaglandin (PGF2 $\alpha$ ) (Prolise, RARS, Argentina). At 12:00 it was done the withdrawal the PRIDs from the recipients female implanted and applied 2 mL of PGF2 $\alpha$ , 1 mL of EB plus 300 IU of equine chorionic gonadotropin (eCG) (Novormom, Intervet, Holland). Furthermore at 18:00 it was applied in the donors 28.4 IU FSH plus, 1 mL of PGF2 $\alpha$ . On day 7 (D7) at 08:00 and 18:00 it was applied 22.7 IU FSH and took out the PRID of the donors. On day 8 (D8) at 08:00 it was applied 2 mL of gonadotropin-releasing hormone (GnRH) (Sincroforte Ouro Fino, Brazil) and 18:00 it was performed the first artificial insemination (A.I.) as well as on D9 at 08:00 a second AI was done. Likewise, the estrus cycle of the recipients was synchronized through the D8. On day 15 (D15) or 7 days after the first AI at 08:00, it was carried out the flushing for embryo collection, which were selected and transferred fresh to the recipients. An overall of 160 embryos were transferred to the recipients, in two different embryonic stages, morula and blastocyst being the pregnancy diagnosis performed on average of 23-30 days after the FTET and data were analyzed by SAS Proc GLIMMIX. The PR obtained were 28.7% (19/66) vs. 50.0% (47/94) respectively, which were significantly different (P=0.0070). The results here obtained showed that embryo transfer at the blastocyst stage enables higher PR when confronted to morula stage.



A101 FTAI, FTET and AI

**Effect of lecorelin and LH to induce ovulation in the pregnancy rate of nelore heifers pre-synchronized with progesterone and estradiol benzoate in a protocol of fixed-timed artificial insemination (FTAI)**

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**Keywords:** heifers, FTAI, LH.

The aim of this experiment was to compare the efficacy of two FTAI protocols using two different inducers of ovulation: lecorelin (Gestran Plus, Tecnopec/União Química, ARSA, Argentina), a synthetic analogue of GnRH, or LHp (Lutropin V Tecnopec /União Química, Bioniche, Canada). For this purpose, we used 448 Nelore heifers, with estimated mean body weigh of 300 kg and mean body condition score of 3 (range 1-5). Initially, the heifers were evaluated by ultrasound and classified according to the ovarian condition in cyclic (with corpus luteum) and prepubertal (without corpus luteum). The animals were divided into 3 homogeneous groups. Animals from Groups LH and GnRH were submitted to protocols of pre-synchronization with progesterone reused devices (Primer, Tecnopec/União Química, Embu-Guaçu, Brazil), and application of 2 mg of estradiol benzoate (RIC-BE, Tecnopec/União Química, Embu-Guaçu, Brazil) at the time of insertion of devices (Day 0) and 1 mg of estradiol benzoate at its removal (Day 8). After 12 days, heifers were subjected to the following FTAI protocols: D0 – insertion of progesterone devices and application of 2 mg estradiol benzoate; D9 - removal of devices in both groups and application of 0.5 mg of estradiol benzoate, 5 mg FSHp (Folltropin V, Tecnopec/União Química, Bioniche, Canada) and 125 µg of D-cloprostenol (Prolise, Tecnopec/União Química, Embu-Guaçu, Brazil). After 46 hours, the Group GnRH received 25 µg of lecorelin and Group LH received 3.15 mg LHp. Animals from both groups received the induction of ovulation treatment immediately after FTAI. The Control Group remained with the standard FTAI protocol of the farm (without pre-synchronization and BE to induce ovulation). The protocol used in Control Group was: D0 – progesterone device (Primer) associated to 2 mg estradiol benzoate; D9 - removal of the device and application of 0.5 mg of estradiol benzoate, 5 mg FSHp and 125 µg D-cloprostenol, and FTAI 46 hours after the devices were removed. Data of pregnancy between groups were compared by Chi-square test. The rate of cyclicity of animals before puberty induction protocol was 75.1%. Pregnancy rates were similar in the Group GnRH (45.6%, 63/138) and LH (51.7%, 76/147) (P = 0.31). These groups showed better results than the Control Group, which showed pregnancy rate of 27.6% (45/163) (P <0.001). The results of this study indicated that breeding programs for heifers that combine pre-treatment with progesterone and BE and a FTAI protocol with GnRH or LH appear to be greater than the conventional protocol of FTAI without pretreatment and the use of BE to induce ovulation. New experiments should be performed to isolate each of the factors possible responsible for the difference in pregnancy rates observed.



A102 FTAI, FTET and AI

### **Effect of recombinat bovine somatotropin associated to superovulation protocol on embryo fertility**

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**Keywords:** embryo, fertility, recombinat bovine somatotropin.

Recombinat bovine somatotropin (bST) stimulates insulin growth factor I (IGF-I) production and increases milk production. Some studies in lactating dairy cows detected higher pregnancy rates when bST was administrated at the beginning of the TAI protocol or at AI (Moreira, et al., 2001. J.Dairy Sci. 84:1646-1659). The aim of this study was to evaluate if administration of bST associated to superovulation protocols affect embryo fertility. Dry Holstein dairy cows (n=5) and heifers (n=86) were used as embryo donors, and synchronized with an intravaginal progesterone device (CIDR, 1.9g of P4, Pfizer Animal Health, SP, Brazil) and 2mg of EB (2.0mL of Estrogen, Farmavet, SP, Brazil) on Day -8. The superovulatory stimulus began on Day -4, with decreasing doses of FSH (Folltropin-V, im; Bioniche Animal Health, Belleville, ON, Canada) twice daily for 4 days (400mg). The PGF (5.0mL, im Lutalyse, Pfizer Animal Health, SP, Brazil) was given on Day -2 and Day -1 at the time of CIDR removal. On Day 0 the donors received 200µg of gonadorelin (GnRH; 2.0mL im of Fertagyl, Intervet/Schering-Plough, SP, Brazil), and AI was performed 12 and 24 hours after GnRH. Embryos were collected on Day 7 by non surgical method, and transferred to the recipient synchronized previously. Only grade 1 and 2 embryos were transferred. On the beginning of the superovulation protocol, cows were randomly assigned to receive: saline solution (Days 0 and 9; Control Group), two (Days 0 and 9) or one dose (Day 9) of bST (0.8ml of bST, 333mg of sometribove zinco, sc; Lactotropin, Elanco Animal Health). Binominal variables were analyzed by PROC GLIMMIX of SAS. Significance were considered when P<0.05 and tendency when P<0.10. Embryo from donors that received two doses of bST had greater pregnancy rate at 30 and 60 days (52.7% and 42.4%; 203) compared to one dose (38% and 34.2%; 266) or Control (38.9% and 31.7%; 180). Some studies report that IGF-I have a beneficial effect in oocytes and in early embryo development (Izadyar et al., 1997. Mol. Reprod. Dev. 45:175-180), as well increases the number of cells and reduce apoptotic blastomeres in bovine embryos (Jousan and Hansen, 2004. Biol. Reprod. 71:1665-70). The bST use associated to superovulation protocol improved embryo fertility and this effect could be on the follicle or in early embryo development.



A103 FTAI, FTET and AI

## Induction of puberty 45 or 90 days before the breeding season increases the cyclicity and pregnancy rates of nelore heifers

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**Keywords:** heifer, induction, puberty.

Protocols for induction of puberty have presented satisfactory efficiency in Nelore heifers. However, the fertility may be influenced by the moment that the protocol is initiated in relation to the onset of the breeding season (BS). The objective of this study was to evaluate two moments to initiate the induction protocol before the onset of BS in Nelore heifers. A total of 670 heifers were selected from two farms (Colina-SP and Araguaiana-MT) by presenting no corpus luteum (CL) during ultrasonography evaluation. Females were randomly assigned into one of three treatments: control (n=218; no treatment); P4-90d (n=227) and P4-45d (n=225). The heifers of P4-90d and P4-45d groups received an induction protocol at 90 or 45 days before the onset of the BS, respectively. The animals received an intravaginal progesterone device (P4; CIDR® Pfizer Animal Health), previously used for 27 days. The device was removed 10 days after insertion and 0.5 mg of estradiol cypionate (ECP, Pfizer Animal Health) was administered at that time. Only in Araguaiana farm, heifers with CL were submitted to a synchronization of ovulation protocol to TAI at onset of BS. The protocol consisted in the insertion of a previously used for 16 days P4 device (CIDR) plus 2 mg of estradiol benzoate (Gonadiol, MSD Animal Health) at onset of the treatment. Eight days later, the implants were removed and were administered 375µg of PGF2α (Ciosin, MSD Animal Health), 300 IU of eCG (Folligon®, MSD Animal Health) plus 0.5 mg of ECP. The heifers were classified according to their body condition (BCS) and the presence of CL was evaluated by transrectal ultrasonography on days -90, -45 and 0 (onset of BS). Data were analyzed by the GLIMMIX procedure of SAS. On day -45, it was found higher (P<0.01) presence of CL in Heifers from P4-90d group [52.0% (118/227)] than heifers from control group [19.3% (42/218)] and P4-45d group [21.8% (49/225)]. At onset of BS (day 0) it was found higher presence of CL (P<0.01) in both treated groups [P4-45=60.4% (136/225) and P4-90=63.9%, (145/227)] than control group [45.4% (99/218)]. Heifers with BCS≥3.5 had higher presence of CL (P<0.01) 45 days before [43.7% (76/174)] and at onset of BS [69.5% (121/174)] than heifers with BCS=3.0 [d-45=30.3% (104/343) and d0=52.2%, (179/343)] and those with BCS=2.0 [d-45=19.0% (29/153) and d0=52.3%, (80/153)]. There was no difference in conception rates (P=0.72) between the three groups [P4-90d=51.9% (54/104) vs. P4-45d=54.1% (60/111) vs. control=45.3% (34/75)]. However, pregnancy rate was higher (P=0.01) in treated groups [P4-90d=27.3% (54/198) and P4-45d=31.3% (60/192)] than control group [18.1% (34/188)]. Therefore, the induction, either at 45 and 90 days before the onset of BS, increases cyclicity and pregnancy rates of Nelore heifers submitted do TAI. Furthermore, the higher is the BCS, the greater is the number of heifers with CL at the onset of BS.

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### Utilization of DIB 0.5 g® in TAI programs of postpartum Nelore cows

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**Keywords:** insemination, progesterone device, veal.

The objective of this study was to evaluate the efficiency of new and previously used for 8 days intravaginal devices containing 0.5 g of progesterone (P4; DIB® 0.5 g, Syntex, Argentina), in the conception rate of Nelore cows submitted to time-fixed artificial insemination (TAI) programs. For both, 929 females between 30 and 60 days postpartum were randomly divided into four treatments: new intravaginal device containing 1.0 g of P4 (n=244; New DIB, DIB 1g®, MSD Animal Health); intravaginal device with 1.0 g of P4 previously used for 8 days (n=235; DIB 1X; DIB 1 g®, MSD Animal Health); new intravaginal device containing 0.5 g of P4 (n=238; New DIB0.5; DIB 0.5 g®, Syntex SA.); and intravaginal device with 0.5 g of P4 previously used for 8 days (n=212; DIB0.5 1X; DIB 0.5g®, Syntex SA.). All females were submitted to the same TAI protocol, the only cause of variation was the intravaginal device utilized. On day 0, the P4 device was inserted, associated to 2 mg of estradiol benzoate i.m. (Gonadiol®, MSD Animal Health). After 8 days, the P4 devices were removed and 0.375 mg of Sodic Cloprostenol (Ciosin®, MSD Animal Health), 300 IU of equine chorionic gonadotropin (eCG; Novormon®, MSD Animal Health) and 1 mg of estradiol cypionate (Cipiosyn®, Syntex SA.) were administrated i.m. TAI was done 48 hours after device removal, using semen of sires with historical good fertility in TAI programs and with homogeneous distribution of the inseminators and the semen batches with in replicates and treatments. Pregnancy diagnosis was performed 30 days after TAI by transrectal ultrasonography and data were analyzed by GLIMMIX procedure of SAS. The conception rate of New DIB0.5 group (59.24%<sup>a</sup>, 141/238) was similar to those obtained in New DIB group (63.52%<sup>a</sup>, 155/244) and DIB 1X group (59.15%<sup>a</sup>, 139/235). However, the use of DIB0.5 1X (46.23%<sup>b</sup>, 98/212) significantly reduced the TAI conception rate (P=0.001). It was concluded that the utilization of New DIB 0.5 g® promotes satisfactory results in the pregnancy rates at suckled Nelore cows TAI. However, the reuse of the DIB 0.5 g® provides reduced conception rates to TAI.

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A105 FTAI, FTET and AI

### **Conception rate and diameter of the ovulatory follicle from zebu cattle submitted to FTAI protocol with estrus detection**

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**Keywords:** bovine, estrus, FTAI.

It is known that high levels of estrogen around insemination are related to higher fertility in cows. Thus, the expression of estrus can positively influence the reproductive performance of Zebu cattle in a program of fixed-time artificial insemination (FTAI). The aim of this study was to evaluate the expression of estrus between the removal of the progesterone device (P4) and the artificial insemination on the diameter of ovulatory follicle (DFOL) and the conception rate in a FTAI protocol. Were used 74 Zebu females (*Bos taurus indicus*), with average for body condition score of  $2.9 \pm 0.17$  (scale 1-5); weight of  $467.88 \pm 63.4$  kg and age  $6.5 \pm 2.6$  years. Females were submitted to the following synchronization protocol: on a random day of the estrous cycle (Day 0), the animals received an intravaginal P4 device (PRIMER®, Tecnopec, São Paulo, Brazil) combined with 2.0mg estradiol benzoate (RIC-BE®, Tecnopec, São Paulo, Brazil) intramuscular (im.). On the 8th day, the intravaginal P4 device was removed and applied 10mg of FSHp (Folltropin®, Tecnopec, São Paulo, Brazil) im., 150µg of d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) im. and 1.0 mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) im. Then, the cows received the estrus detector adhesive (Estrotec®, IVP Brazil, São Paulo, Brazil) placed between the tail base and the hips of each cow. On the 10th day, immediately before each insemination, the DFOL measurement was preceded by transrectal ultrasonography by using a 5.0 MHz linear transducer (Mindray, DP2200vet, São Paulo, Brazil). At this time, the animals were divided into two groups according to the expression (Group with Estrus, n = 48) or not estrus (Group without Estrus, n = 26). The expression of estrus was determined by the activation of the estrus detector adhesive. The pregnancy diagnosis was performed 45 days after FTAI. For statistical analysis the statistical package SPSS (version 19) was used, considering a significance level of 5%. The difference in the averages for DFOL was analyzed by Student's t test and the conception rate was compared by Chi-square test (c2). The total conception rate was 45.9% (34/74). There were differences ( $P = 0.16$ ) between the conception rate of the cows from the Group with Estrus (56.3%) compared with the cows from the Group without Estrus (26.9%). Furthermore, the DFOL was greater ( $P = 0.001$ ) in the Group with Estrus ( $12.77 \pm 1.61$ mm) compared to the Group without Estrus ( $10.70 \pm 1.36$ mm). Based on these data, it is verified that the expression of estrus in a synchronization protocol for FTAI is an important indicator of high ovarian response and a higher probability of conception in cows.



A106 FTAI, FTET and AI

### **Evaluation of timed artificial insemination protocols in dairy cattle: presynchronization with GnRH, increasing estradiol benzoate, and limitations to success during the protocol**

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**Keywords:** dairy cattle, synchronization, TAI.

With the objective to optimize TAI protocols based on estradiol and progesterone (P4), we performed two experiments using presynchronization with a single GnRH treatment 3d or 7d before initiation of the protocol (Expt 1) or increased estradiol benzoate (EB; 2 vs. 3mg; Expt 2). In Expt 1, 82 lactating cows (33.8±1.0 kg/d [Mean±SE]), DIM 161.3±18.35; BCS 2.7±0.03) were randomly assigned to G-7 (n=40; 10µg busserelin 7d before protocol) or G-3 (n=42; 3d before). At the start of the protocol (D0), cows were treated with P4 intravaginal device of 1g (Sincrogest, Ourofino) and 2mg EB (Sincrodiol, Ourofino, i.m.). On D7, cows were given 25 mg dinoprost (Lutalyse, Pfizer, i.m.) and on D8, 1mg estradiol cypionate (ECP, Pfizer, i.m.) and withdrawal of P4 device. In Expt 2, 44 lactating cows (23.6±1.46 kg/d; DIM 242.8±36.20; BCS 2.8±0.05) were randomly assigned to receive 2mg EB (EB2; n=23) or 3mg EB (EB3; n=21) at D0. The remainder of the protocol was the same as Expt 1. In both Expts, AI was performed on D10 and ultrasound evaluations (US) were performed daily between D0 and D12. In Expt 1, US was also performed on D-7 and D-5 (to determine ovulation to GnRH in G-7), and on D-3 (cows in G-3). Statistical analyses were performed with Glimmix procedure of SAS. In Expt 1, for cows that ovulated to GnRH (54.9%; 45/82), conception rate (30d post-AI) was 44.0% vs 35.0% (P=0.24) for G-3 and G-7. Concentrations of P4 (RIA) on D0 were greater for cows in G-7 than G-3 (P=0.02), although there were no differences between groups in P4 on D7 or D10. Regardless of group, cows with lower P4 at time of AI (D10: <0.1 ng/mL vs D10: 0.1 to 0.3 ng/mL) had increased percentage of ovulation to the protocol (79.8±0.05 vs 60.6±0.08%), and consequently, increased pregnancies per AI (P/AI) at the 30d pregnancy diagnosis (49.0±0.07 vs 27.3±0.07%; P=0.05). In Expt 2, EB3 compared to EB2: there was no increase in time to wave emergence (3.6±0.20d vs 3.4±0.15d, P=0.36), however EB3 reduced time to ovulation (10.5±0.1d vs 11.0±0.1d; P<0.01), tended to increase CL regression between D0 and D7 (68% vs 38%; P=0.17), and reduced optimal synchronization (wave emergence D1 to D6; single ovulation to protocol; 26% vs 69%; P=0.03). A descriptive analysis of data from both Expts showed that only 77.8% (98/126) of cows ovulated at the end of the protocol and only 59.5% (75/126) had synchronized wave emergence between D1 and D6. Nevertheless, fertility was high in synchronized cows (P/AI 61.3%; 46/75) compared to cows not optimally synchronized (15.7%; 8/51). Thus, although current TAI protocols using EB and P4 produce satisfactory results for lactating dairy cows, there is still room for improvement since less than 60% of cows were correctly synchronized. Presynchronization with GnRH so that cows were on a specific day of the follicular wave or increasing the dose of EB to 3mg were not effective in increasing the synchronization rate.

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