

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

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# The use of eCG diluted in D-cloprostenol reduces the number of injections required for TAI protocols in postpartum *Bos indicus* beef cows with same pregnancy efficiency as the convention treatment

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## Resumo

Using eCG in TAI protocols is an essential strategy to improve P/AI in females with low body condition score (BCS), short postpartum period, and in anestrus. We assessed if the dilution of eCG in PGF<sub>2a</sub> would enable administration of both drugs in a single injection, reducing the number of shots to accomplish TAI protocols with same reproduction efficiency. The study was done in 2020 and assigned 642 *Bos indicus* (Nelore) cows (346 primiparous, 296 multiparous) with  $2.60 \pm 0.03$  BCS and ranging 30-60 days postpartum from 3 farms in MS State, Brazil. Cows were kept in pasture. At the onset of the protocol (D0) they received an intravaginal device with 0.5 g P4 (Repro one, Globalgen) and 2 mg estradiol benzoate (Bioestrogen, Biogénesis Bagó) IM. On D8, device was removed and 1 mg estradiol cypionate (Croni-Cip, Biogénesis Bagó) was given IM. At that time, cows were randomly allocated in 3 groups. Cows in Control Group (CG) received only 150 µg D-cloprostenol (PGF; Croniben, Biogénesis Bagó) IM. Cows in Traditional Group (Trad) were treated with 150 µg PGF and 300 IU eCG (Ecegon, Biogénesis Bagó) IM, given in two injections apart. Cows in Group Combined eCG+PGF received eCG diluted in PGF and given in a single injection. The eCG+PGF was set by diluting 3 vials of lyophilized eCG (total 15,000 IU) in 100 mL PGF and the dose used was 2 mL/cow (equivalent to 300 IU eCG + 150 µg PGF). TAI was done 48 h after device removal. Pregnancy was checked 30 days after TAI. The occurrence of estrus was evaluated, and the diameter of the dominant follicle (DDF) was measured on D8 and D10 in the ovaries of all cows to access follicular growth rate (FGR). Data was analyzed with SAS. The DDF on D8 was similar for groups (CG:  $10.3 \pm 0.13$ ; Trad:  $10.2 \pm 0.13$ ; eCG+PGF:  $10.5 \pm 0.15$  mm;  $P = 0.73$ ), yet, it was greater on D10 in cows receiving eCG (CG:  $11.7 \pm 0.16$ b; Trad:  $12.4 \pm 0.12$ a; eCG+PGF:  $12.5 \pm 0.15$ a mm;  $P < 0.0001$ ) with greater FGR in those cows (CG:  $0.7 \pm 0.04$ b; Trad:  $1.1 \pm 0.05$ a; eCG+PGF:  $1.0 \pm 0.04$ a mm/d;  $P = 0.005$ ). The eCG treated cows also had greater estrus rate (CG: 56.2%b; Trad: 66.7%a; eCG+PGF: 66.7%a;  $P = 0.05$ ) and greater P/AI (CG: 50.5%b; Trad: 66.7%a; eCG+PGF: 66.2%a;  $P = 0.0005$ ) than control cows, regardless of form of eCG administration (apart or combined with PGF). Thus, the use of eCG diluted in PGF was as efficient as eCG given apart from PGF in TAI protocols of postpartum *B. indicus* cows, keeping pregnancy outcomes greater than for the control cows (without eCG). The combination of eCG+PGF also has the advantage of reducing the number of injections in TAI protocols, minimizing errors, enabling faster managements and improving cows' welfare.

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# Assisted calving: association with uterine diseases and reproductive efficiency in crossbred dairy cows

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## Resumo

Occasional assistance during calving is important to ensure cow and calf survival. Early intervention has the potential to prevent stillbirths, while unnecessary or premature intervention can also cause injuries in the birth canal due to the lack of proper soft tissue dilation. Although the prevalence of dystocia may appear to be low (between 4.1 and 13.7%), calving assistance rates are high, varying between 10 and more than 50%. Thus, the aim was to evaluate in crossbred lactating dairy cows that had single living calve, the effect of type of calving (normal or assisted) on uterine disease occurrence, and on number of artificial inseminations (AI) per conception and pregnancy rate at 150 days postpartum (DPP). Cows were monitored during calving and the type of calving was classified as normal or assisted. Normal calving needed no interference by humans while assisted calving needed human intervention, such as a calf puller. A total of 825 calvings were recorded, of which 7 were stillbirths (0.85%) and 17 were twins (2.06%), and 801 (97.09%) calvings of a single and live calf were analyzed. The uterine diseases evaluated were retention of the fetal membranes (RFM), metritis, and clinical endometritis (CE). RFM was considered when the cow did not eliminate the fetal membranes within the first 12 hours after calving. Metritis was characterized by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent uterine discharge, which often has a fetid odor. CE was defined by the presence of purulent vaginal discharge containing more than 50% pus, as analyzed by Metrichick®, an involuted uterus at transrectal palpation and no clinical systemic signs diagnosed between 21 and 35 DPP. A total of 801 calving of a single and live calf were analyzed during the study period, of which 766 (95.63%) were normal and 35 (4.36%) were assisted. Most of the cows with normal calving had a healthy postpartum period (73.89%), while most of the cows with assisted calving had uterine diseases (74.29%). The number of AI per conception was similar in cows that had normal or assisted calving ( $2.39 \pm 0.08$  and  $3.00 \pm 0.43$ ,  $P = 0.16$ ). There was no evidence of a negative influence of type of calving on the pregnancy rate at 150 DPP of crossbred lactating dairy cows ( $P = 0.44$ ). Healthy cows had a higher pregnancy rate at 150 DPP than cows affected by uterine diseases in the postpartum period (51.65 vs. 42.92%). In conclusion, the majority of crossbred lactating dairy cows with normal calving had a healthy postpartum period while most of the cows that had assisted calving had uterine diseases, and healthy cows had a higher pregnancy rate at 150 DPP. Crossbred lactating dairy cows that had normal or assisted calving had similar number of AI per conception, and the negative influence of type of calving on the pregnancy rate at 150 DPP was not detected.

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**COMPARISON OF THE VAGINAL MICROBIOTA OF DAIRY COWS PREGNANT OR NOT, TO ESTABLISH POSSIBLE ASSOCIATIONS BETWEEN BACTERIAL COMMUNITIES AND GESTATIONAL STATUS**

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**Resumo**

This study aimed to compare the vaginal microbiota of high-production dairy cows that became pregnant or not after artificial insemination to establish associations between bacterial communities and reproductive performance. Holstein multiparous, high production cows (n = 13) were used. On the day of estrus, the cows were inseminated and after 30 days the diagnosis of pregnancy was made. Cows were classified as pregnant (P) or non-pregnant (NP). The vaginal swab of each female was obtained before artificial insemination. The samples were submitted to DNA extraction and sequencing performed by the PacBio platform. To select and filter the samples within the range of 1,500 bp, the DADA2 software was used, while the SBA analyzer 2.4 software was used to assign taxonomic identification to all readings. Alpha and beta diversities were calculated and PCoA was used to visualize the similarities between the samples. The analysis of variance comparing the relative abundances and alpha diversity indexes was performed by the Minitab 18 program. The comparison of alpha diversity among pregnant and non-pregnant cows was performed by the Kruskal-Wallis test with a 95% confidence interval and using a subsample of 4300 readings per sample. The ANOVA analysis was used to verify if there was a difference between groups (P versus NP). The differential abundance test was performed with LefSe. Of the 13 cows selected for the experiment, seven were pregnant after artificial insemination, and six remained non-pregnant. For the characterization of the vaginal microbiota of these females, 366.509 bacterial readings were used by the PacBio SNG, being grouped into 28 phylum and 652 genera. The relative abundance among the phylum demonstrated the predominance of Firmicutes (58%) and Bacteroidetes (32%) in all vaginal samples evaluated, regardless of the group no significant difference was observed between the P and NP cows ( $p \leq 0.05$ ). In alpha diversity, there was no difference between the groups in the number of coverages ( $p = 0.210$ ) and Chao indexes ( $p = 0.221$ ), and Shannon ( $p = 0.201$ ). In beta diversity, the graphs agree with each other and when submitted to ANOVA showed no difference ( $p = 0.213$ ;  $p = 0.526$ , respectively) comparing the P and NP groups. In addition, the number of bacteria in each sample, according to the Jaccard and Brays Curtis indices, which evaluate the types of bacteria present and the amount of the same community, respectively (Schloss and Handelsman, Applied and Environmental Microbiology, 72:10, 6773-6779, 2006), the non-pregnant cows showed a bacterial community more similar between them, to those present in pregnant cows.

**Keywords:** Bacteria, Beta Diversity, Cattle HPB, SNG, PacBio.

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**INJECTABLE PROGESTERONE (75mg) IS NOT AN ALTERNATIVE TO INTRAVAGINAL PROGESTERONE DEVICES IN *Bos indicus* COWS SUBMITTED TO THE OVULATION SYNCHRONIZATION PROTOCOL**

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**Resumo**

The objective was to evaluate injectable progesterone (P4i) as an alternative exogenous source of P4 in ovulation synchronization protocols. In experiment 1, Nelore cows (n=55) were randomly assigned to five experimental groups to receive different doses of P4i [(P4i60mg, P4i105mg, P4i150mg, P4i195mg and P4i240mg groups; (Sincrogest Injetável®, Ourofino, Brazil)]. Daily blood samples [Day 0 (D0) to D12] were collected to assess the plasma concentration of progesterone (RIA). In experiment 2, Nelore cows (n=48) were divided into three experimental groups to receive an intravaginal device of P4 [(Control group; Sincrogest®, Ourofino, Brazil)] or two different doses of P4i [105 mg of P4i (P4i105 group) 150 mg of P4i (P4i150 group)]. In experiment 3, Nelore cows (n=26) were assigned to two experimental groups to receive an intravaginal device of P4 (Control group) or 75 mg of P4i (P4i75 group) on D0. In experiment 4, Nelore cows (n=28) were divided into two experimental groups to receive received an intravaginal device of P4 (Control group) or 60mg of P4i (P4i60 group). In experiments 2, 3 and 4, concurrent with the treatments cows received 2mg of EB (Sincrodiol®, Ourofino, Brazil) on D0. Eight days later (D8), cows received 1mg of EC (SincroCP®, Ourofino, Brazil), 300IU of eCG (SincroeCG®, Ourofino, Brazil), 500µg of Cloprostenol (Sincrocio®, Ourofino, Brazil) and P4 intravaginal devices were removed from cows in the Control group. In addition, ultrasound examinations were performed every 24 hours for 96 hours or until ovulation. In experiment 5, Nelore cows (n=132) were submitted to the TAI protocol using P4i (P4i75 group) or intravaginal device of P4 (Control group) and administration of 2mg of EB on D0. On D8, the intravaginal device was removed from the cows in the Control group and 1mg of EC, 500µg of Cloprostenol and 300IU of eCG were administered to all cows. After treatments, all cows were submitted to artificial insemination 48 hours after administration of the ovulation inducer. Pregnancy diagnosis and cyclicity rate were evaluated 30 days after TAI (D40). Statistical analysis was performed by SAS. In the P4i release curve, it was observed that the peak occurred one day after the treatments (D1) for the different doses of P4i administered and, from D3 onwards, all groups remained with concentrations below 1ng/mL until the end of the study (D12). Regarding ovarian follicular dynamics, the dose of 75mg showed an ovulation rate at the end of the ovulation synchronization protocol similar to the control group (P=0.24), a result not observed in studies with other doses of P4i (ovulation rate lower). However, the pregnancy rate (experiment 5) was higher in cows, which received the intravaginal P4 device (P=0.0001). It can be concluded that the use of different doses of long-acting P4i at the beginning of the TAI protocol is not an alternative to replace the intravaginal device of P4 in ovulation synchronization protocols in *Bos indicus* cows.

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# Fertility programs for lactating dairy cows: a novel Presynch + Timed-AI program (ESALQ-Synch) produces similar reproductive outcomes as Double-Ovsynch

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## Resumo

Fertility programs were implemented for the 1st postpartum timed-AI (TAI) in 798 (primiparous and multiparous) lactating dairy cows, evaluating 2 presynchronization (presynch) strategies and 2 TAI protocols, in a 2×2 factorial design. Weekly, cows were enrolled into 1 of 4 groups (Ovs+Ovs, Ovs+OvsP4/E2, PreP4/E2+Ovs and PreP4/E2+OvsP4/E2). On d-17 (34 ± 3 days in milk), the Ovs presynch initiated with 10 µg busserelin acetate (GnRH), and cows received 0.5 mg cloprostenol (PGF) on d-10, and 10 µg GnRH on d-7. The PreP4/E2 presynch initiated on d-17 with a used 2 g P4 implant. On d-10, implant was removed and 0.5 mg PGF and 1 mg E2 cypionate (EC) were given. For TAI protocols, Ovs was Ovsynch: d0: 20 µg GnRH (double-dose), d7: PGF, d8: PGF, d9.5: 10 µg GnRH, and d10: TAI (16 h after GnRH). Cows submitted to OvsP4/E2 received on d0: 20 µg GnRH (double-dose) and a 2 g P4 implant, d7: PGF, d8: P4 implant removal, PGF and EC, and d10: TAI. For all cows, expression of estrus until TAI was evaluated and ultrasound was performed on d-17, d0, d7 and d17. The GLIMMIX procedure of SAS 9.4 was used for analyses (P≤0.05). The presence of CL on d-17 (average = 68.8% [549/798]) was similar among treatments and parity. Presence of CL on d0 of TAI protocols was high, and Ovs as a presynch slightly increased the proportion of cows with CL (95.5 [381/399] vs. 90.7% [362/399]). However, at the first PGF of breeding protocols, there was no effect of presynch, and 98.5% (786/798) of the cows had at least 1 CL. Ovulation after d0 was greater in cows submitted to PreP4/E2 than Ovs (71.9 [287/399] vs. 64.1% [256/399]), and those ovulating had greater P/AI (51.0 [277/543] vs. 41.6% [106/255]). Overall, multiple ovulation after TAI was low and similar between TAI protocols and presynch strategies (7.2% [54/751]). Expression of estrus in OvsP4/E2 protocols was greater than Ovs (69.3 [273/394] vs. 41.6% [168/404]), and an interaction was detected, in which cows not expressing estrus ovulated more after TAI in Ovs compared to OvsP4/E2 protocol (93.2 [220/236] vs. 77.7% [94/121]). Cows expressing estrus had greater P/AI in both Ovs (58.3 [98/168] vs. 42.0% [99/236]) and OvsP4/E2 (57.5 [157/273] vs. 24.0% [29/121]), but there was an interaction, and cows not showing estrus had greater P/AI on Ovs compared to OvsP4/E2 (42.0 vs. 24.0%). There was no interaction between presynch and TAI protocol on P/AI on d32 (48.2, 49.7, 53.3, and 52.8% for Ovs+Ovs [Double-Ovsynch], Ovs+OvsP4/E2, PreP4/E2+Ovs and PreP4/E2+OvsP4/E2 [ESALQ-Synch], respectively), and no differences on pregnancy loss between days 32 and 60 (average = 17.8% [68/383]). In summary, the two presynchronization strategies and both TAI protocols, despite differences in pharmacological bases, induced similar and well-controlled ovarian dynamics, high synchronization, and excellent fertility outcomes, providing 4 outstanding options of high fertility TAI programs.

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# Pre-synchronization with injectable P4 for GnRH/P4-based TAI protocol in *Bos indicus* beef cows

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## Resumo

This study evaluated the effect of pre-synchronization with injectable P4 on the ovarian follicular dynamics of cows treated with a GnRH/P4-based TAI protocol. Nelore females (n=50), multiparous, between 5 and 8 years of age, with 30 to 60 days postpartum were used in this study. Ten days before (D-10) starting the TAI protocol antral follicle count (AFC; follicles  $\geq 3$  mm), ovarian condition (diameter of the largest follicle and CL presence), and body condition score (BCS: 1-5) were evaluated by a single technician to establish two groups: Control (n=23) and Pre-sync (n=27; injectable P4). On the same day, the Pre-sync group received i.m. 150mg injectable P4 (Sincrogest® Injetável, Ourofino, Cravinhos, Brazil). On D0, the ovarian evaluation was repeated, and the TAI protocol was started with the insertion of a P4 intravaginal device (0.5g, Cronipres®, Biogénesis Bagó, Curitiba, Brazil) and administration of 10.5µg buserelin acetate/GnRH (Gonaxal®, Biogénesis Bagó) in all animals. On D7, the P4 device was removed and 300IU eCG (Ecegon®, Biogénesis Bagó), 150µg D-cloprostenol (Croniben®, Biogénesis Bagó) and 1mg estradiol cypionate (Croni-Cip®, Biogénesis Bagó) were i.m. applied. On the same day, the CL presence was evaluated, the largest follicle was measured, and the tail was painted to evaluate the estrus expression. On D9, the measurement of the largest follicle was repeated, and TAI was performed in all cows using semen from a single bull. Those animals with no estrus or low expression received 10.5µg GnRH. Numerical variables did not present normal distribution, then the data were analyzed by the Mann-Whitney test. Binary data were analyzed by Fisher's exact test (5%). The Control and Pre-sync groups did not differ regarding the BCS at the beginning of pre-synchronization (D-10;  $2.4 \pm 0.1$  and  $2.5 \pm 0.1$ ;  $P=0.59$ ) or of the TAI protocol (D0;  $2.5 \pm 0.1$  and  $2.6 \pm 0.1$ ;  $P=0.43$ ), respectively. The AFC was similar between Control ( $40.5 \pm 5.7$  follicles) and the Pre-sync group ( $49.7 \pm 6.1$  follicles;  $P=0.16$ ). The diameter of the largest follicle (mm) was also similar between the Control and Pre-sync groups on D-10 ( $9.2 \pm 0.7$  and  $10.1 \pm 0.6$ ;  $P=0.33$ ), on D0 ( $9.9 \pm 0.6$  and  $10.7 \pm 0.6$ ;  $P=0.33$ ), on D7 ( $11.0 \pm 0.8$  and  $10.1 \pm 0.8$ ;  $P=0.21$ ) and D9 ( $11.3 \pm 0.7$  and  $12.0 \pm 0.7$ ;  $P=0.20$ ), respectively. The Control and Pre-sync groups were also similar regarding the CL presence on D7 of the protocol [56.5% (13/23) and 77.8% (21/27);  $P=0.11$ ], the estrus expression rate [91.3% (21/23) and 96.3% (26/27);  $P=0.44$ ] and conception rate [39.1% (9/23) and 44.4% (12/27);  $P=0.45$ ], respectively. Ovarian follicular dynamics of cows treated with injectable P4 10 days before the start of the TAI protocol did not differ from the control group.

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## Effects of pre-TAI protocol with different injectable progesterone (P4i) on pregnancy rate in beef cows

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### Resumo

The aim of the study was to evaluate the efficiency of two different injectable progesterone (P4i) on pre-TAI protocol in beef cows. A total of 490 Nelore multiparous cows with  $38.3 \pm 1.2$  DDP and  $2.76 \pm 0.02$  BCS (1-5 scale) from a commercial farm in Rondonópolis, MT state (Brazil) were used. Ten days before the beginning of the TAI protocol (D-10), cows were randomized according to DDP and BCS into 2 experimental groups: 1) Sincrogest: cows received 150mg i.m of Sincrogest injetável® (Ourofino Saúde Animal, Brazil; n=243) and 2) Progecio: cows received 140mg i.m of Progecio® (Agener União Saúde Animal, Brazil; n=247). On D0 of the TAI protocol, all animals received an intravaginal P4 device (Sincrogest®, Ourofino) and 2 mg of estradiol benzoate (EB; Sincrodiol®, Ourofino). On D8, heifers received 1 mg of estradiol cypionate (EC; SincroCP®, Ourofino), 0.53mg of sodic cloprostenol (PGF; Sincrocio®, Ourofino) and 300 IU of eCG (SincroeCG® Ourofino). At the same time, heifers were painted with chalk on their tailheads, and removal of chalk on D10 was used as an indication of estrus. TAI was performed 48h later (D10) in both groups. Pregnancy diagnosis was evaluated by US (Mindray® DP-2200Vet) after 30 days of insemination. Statistical analyses were performed using Glimmix of SAS 9.4. No difference was observed between groups for P/AI [Sincrogest = 52.7% (128/243); Progecio = 46.2% (114/247); P=0.15]. The P/AI was affected by estrus expression, where cows that demonstrated estrus had greater pregnancy rate [Estrus = 56.7% (140/274); No estrus = 42.0% (102/243); P=0.001]. Additionally, an interaction between treatment and estrus expression was found, in which cows without estrus expression from Progecio group had lower pregnancy rate when compared to others groups [Sincrogest\_Estrus = 54.3% (69/127)A; Sincrogest\_No estrus = 50.9% (59/116)AB; Progecio\_Estrus = 59.2% (71/120)A; Progecio\_No estrus = 33.9% (43/127)C; P=0.02]. In conclusion, the administration of Sincrogest injetável® on pre-synchronization protocol showed satisfactory P/AI, regardless of estrus expression.

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## Effects of different doses of estradiol benzoate (1 vs. 2mg) on D0 and estradiol cypionate (0.5 vs. 1mg) on D7 (P4 device removal) on TAI protocol efficiency in Nelore heifers

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### Resumo

The experiment evaluated different doses of estradiol benzoate (EB) at the beginning of TAI protocol (D0), and different doses of estradiol cypionate (EC) at the P4 device removal (D7) in Nelore heifers (*Bos indicus*). A total of 794 heifers [349 aging 14 months (BW=254.3±2.8 Kg and BCS=2.88±0.01) and 445 aging 24 months (BW=322±3.2 Kg and BCS=2.86±0.02)] from a commercial farm were used. On D0, heifers were randomized into 4 groups using a 2x2 factorial design. Heifers received a reused intravaginal P4 device (Sincrogest®, Ourofino) and 0.53mg sodic cloprostenol (Sincrocio®, Ourofino). Experimental treatments were: administration 1 (EB1) or 2 (EB2) mg EB (Sincrodiol®, Ourofino) on D0 and, 0.5 (EC0.5) or 1 (EC1) mg EC (SincroCp®, Ourofino) on D7, resulting in 4 treatments: EB1-EC0.5 (n=209); EB2-EC0.5 (n=196); EB1-EC1 (n=182) and EB2-EC1 (n=207). On D7, heifers received 0.53mg of PGF and 200 IU of eCG (SincroeCG® Ourofino), concomitant with P4 device removal. At the same time, heifers were painted with chalk on their tailheads, and removal of chalk on D9 was used as an indication of estrus. TAI was performed 48h later (D9) in all groups. A subset of heifers (n=184) was evaluated by the US in order to measure the diameter of the dominant follicle (DF) on D7 and D9. Statistical analyses were performed by GLIMMIX of SAS 9.4, using BE and CE doses as fixed effects and heifer category interactions were verified as secondary effects. Since there was no interaction between EB and EC (P> 0.10), data is shown by main effects. No differences were found between EB or EC treatments for diameter of DF on D7 and D9, as well as daily follicular growth. However, the early ovulation rate (between D7 and D9) was higher in EB1 than EB2 group [11.1% (10/90) vs. 5.3% (5/94); P=0.05], but no effect of EC was observed (P=0.35). Furthermore, 24m heifers presented an increased early ovulation rate than 14m heifers [24m=10.8% (14/130) vs. 14m=1.9% (1/54); P=0.03]. No effect of EB was observed (P=0.40) for expression of estrus, but the expression of estrus was higher in CE1 when compared to CE0.5 [88.9% (363/407) vs. 94.6% (368/389); P=0.01]. An interaction between EB and heifer category for pregnancy rate was found (P=0.04), which 14m heifers presented a decreased pregnancy rate in EB2 than EB1, but 24m heifers presented a similar pregnancy rate [14mEB1=37.0% (64/173)B vs. 14mEB2=26,1% (46/176)C vs. 24mEB1=53,2% (116/218)A vs. 24mEB2=54,6%(124/227)A]. Additionally, a tendency for decreasing pregnancy rate was observed (P=0.06) for EC groups (EC0.5=46.4% (188/405) vs. EC1=41.7% (162/389)]. In conclusion, the early ovulation rate was higher in heifers that received 1mg of EB on D0. Heifers that received 1mg of EC on D7 presented greater expression of estrus. The administration of 2mg BE decreased the pregnancy rate only in 14m heifers. Regardless of heifer category, a lower pregnancy rate was observed when 1mg EC on D7 was administered.



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## Evaluation of hCG as a gonadotropic support to timed embryo transfer protocol in beef cattle

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### Resumo

This study compared the reproductive performance of embryo recipients treated with a TET protocol employing hCG or eCG. Nulliparous/heifers (n=194, 18 Nelore and 176 crossbred Nelore x A. Angus) and multiparous cows (n=147, 75 Nelore and 72 crossbred Nelore x A. Angus) with BCS 3.0 to 4.0 were used in this study. On a random day of the estrous cycle (D-10) females receiving a TET protocol with EB (2mg, i.m., Gonadiol®, Zoetis, Sao Paulo, Brazil) and an intravaginal P4 device (CIDR®, Zoetis) that remained until D-8.5. On D-8.5, the animals receiving i.m. 150µg D-cloprostenol (Croniben®, Biogénesis Bagó, Curitiba, Brazil) and 1mg EC (ECP®, Zoetis) and then were randomly divided into two groups: eCG-group (n=179; i.m. treatment with 300IU eCG; Novormon®, Zoetis) and hCG-group (n=162; i.m. treatment with 150IU hCG; Vetecor®, Ceva, Paulinia, Brazil). Estrus intensity (sacrococcygeal painting) and diameter of the dominant follicle (DF) were monitored on D0 and CL Doppler evaluation (Pugliesi et al., 2019) was assessed on D7 to in ovulation of the fresh embryo and in vitro produced. Then, pregnancy was assessed 23 days later. Data were analyzed by ANOVA using a mixed-effect model (gonadotropic treatment, category, breed, and estrus expression intensity) and the Tukey test. Rates were analyzed by logistic regression using the same variables as the previous model (P<0.1). There was no isolated breed effect or interaction (P>0.1). The diameter of the DF (mm) was influenced (P=0.01) by gonadotropic treatment\*category interaction (eCG-multiparous 10.3±0.3a, eCG-nulliparous 10.4±0.3a, hCG-multiparous 10.7±0.3a and hCG-nulliparous 9.3±0.3b). Treatment with hCG and eCG showed a high estrus rate, but the proportion of females with high-intensity estrus was higher in the hCG group (79.8% vs 68.6%, respectively; P=0.03). The utilization rate was influenced (P=0.06) by gonadotropic treatment\*category interaction (eCG-multiparous 88.9%a, eCG-nulliparous 81.3%ab, hCG-multiparous 82.7%ab and hCG-nulliparous 72.4%b). The hCG-group resulted in CL with a better Doppler evaluation score (1.83±0.08 vs 1.62±0.07; P=0.04), central blood flow (1.90±0.08 vs 1.65±0.07; P=0.03) and peripheral blood flow (2.16±0.08 vs 1.97±0.07; P=0.08) in relation to eCG-group. There was an interaction effect of gonadotropic treatment\*category that revealed a lower conception rate (eCG-multiparous 40.3%ab, eCG-nulliparous 29.8%bc, hCG-multiparous 42.3%a and hCG-nulliparous 18.0%c; P=0.01) and pregnancy rate (eCG-multiparous 37.5%a, eCG-nulliparous 24.3%b, hCG-multiparous 37.3%a and hCG-nulliparous 13.8%c; P=0.001) in nulliparous treated with hCG. Treatment with hCG during the TET protocol resulted in greater estrus expression intensity and CL with a higher Doppler score, which determined rates of utilization, conception, and pregnancy similar to conventional protocols using eCG. However, the nulliparous category treated with hCG exhibited lower overall reproductive rates in the TET program.

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# Removal of estradiol treatments from synchronization protocols decrease TAI outcomes in *Bos indicus* heifers and COWS

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## Resumo

This experiment evaluated the outcomes of removing estradiol (E2) administration for synchronization of follicular wave (SYN) and ovulation induction (IND) on the reproductive performance of Nelore heifers (HE) and cows (CO) submitted to TAI. A total of 1,111 HE and 1,290 suckled CO from commercial farms (TO, PA, MT, MS) were used. On day 0 (D0), animals received an intravaginal P4 device (P4d; Primer®, Agener União) and were randomized according to cyclicity (HE=45%; CO=27%) and BCS (HE=2,8±0,2; CO=2,8±0,3) to either one of the treatments (2x2 factorial): 1)EB/EC: 2mg of benzoate estradiol (EB; RIC BE®, Agener União) on D0 and 1mg of estradiol cypionate (EC; Cipiotech®, Agener União) on D8 (HE=279; CO=328); 2)EB/GnRH: 2mg of EB on D0 and 25µg of leirelin (GnRH; Tec-Relin®, Agener União) on D10 (HE=277; CO=314); 3)GnRH/EC: 50µg of GnRH on D0 and 1mg of EC on D8 (HE=281; CO=327) and 4)GnRH/GnRH: 50µg of GnRH on D0 and 25µg of GnRH on D10 (HE=274; CO=321), i.m. The experimental protocol was adjusted for heifers [0.265mg of PGF (Estron®, Agener União) on D0 and 0.5mg of EC on D7 (7days of P4d treatment) instead of 1mg on D8 in CO]. All animals received 0.530mg of PGF and eCG (HE=200IU; CO=300IU; Novormon®, Zoetis) i.m, concomitant with P4d removal. At the same time, animals were painted with chalk on their tailheads, and removal of chalk on the day of TAI was used as an indication of estrus. The TAI was performed 48h (HE=D9; CO=D10) after P4d removal. A subset of animals (HE=477; CO=772) was evaluated by US (Mindray®, DP-2200) on day of P4d withdrawal and at TAI. The following variables were evaluated: dominant follicle diameter (DFD; mm±SEM), CL at P4d removal (CLP), early ovulation rate (EOV; ovulation between P4 device removal and TAI), estrus detection rate (EDR) and pregnancy rate (P/AI). Statistical analyses were performed using PROC GLIMMIX of SAS 9.4. No interaction (P>0,05) between SYN\*IND was observed for any variable and data was presented by main effects (EB at the beginning or EC at ending of protocol). In SYN, CO treated with EB on D0 had greater P/AI compared to those that received GnRH [EB=44.0% (280/642) vs. GnRH=33.0% (216/648); P<0.0001]. Similar effect was observed for HE [BE=43.1% (201/466) vs. GnRH=37.2% (174/468); P=0,003]. CO and HE treated with GnRH on D0 showed a larger DFD at P4d removal than animals treated with EB (HE=9.8±0.1 vs. 8.0±0.1mm; P<.0001; CO=10.8±0.1 vs. 9.6±0.1mm; P<.0001). Furthermore, CO treated with GnRH on D0 presented higher CLP [EB=10.1% (39/347) vs. GnRH=20.0% (77/309); P=0.0002]. Also, it was verified that CO treated with GnRH at D0 had a higher EOV [6.51% (22/316) vs. 1.8% (6/331); P=0.02] than EB treatment. In the IND group, the EDR was greater in HE [88.8% (53/520) vs. 83.7% (75/385); P=0.01] and CO [78.6% (121/445) vs. 68.1% (178/380); P=0.02] that received EC instead of GnRH. In conclusion, removal of E2 treatments on TAI protocol had a negative impact on reproductive efficiency in HE and CO.

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# Injectable progesterone, follicular dynamics and estrous synchronization in cyclic ewes

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## Resumo

The aim of the present study was to evaluate the pharmacokinetics of two different doses of injectable long-acting commercial progesterone (P4i) in cyclic ewes, and thus to evaluate its effects on sexual behavior and follicular dynamics. For this, the estrous cycle of 30 adult multiparous Santa Ines was synchronized with the administration of two doses of prostaglandin (120 µg cloprostenol i.m.; Estron, Agener União, São Paulo, Brazil), separated 7 days. After the second dose of prostaglandin, ewes were immediately allocated to the experimental groups of 10 ewes each. One group received 20 mg (G20) of P4i (progesterone i.m.; Progecio Agener, São Paulo, Brazil), 40 mg (G40) or remained as a control group (GCon), with no administration of P4i. In order to determine the pharmacokinetics of P4i, blood samples were collected every 12h for four days for posterior dosage of circulating progesterone by radioimmunoassay. After the administration of P4i, the females were kept with rams for estrous detection and the ovaries were daily scanned through ultrasound. The progesterone concentration was analyzed with a mixed model which treatment, time, and their interaction as main factors, and including time as a repeated measurement. The other data were compared with Mann-Whitney and Kruskal Wallis test, or the Fisher exact probability test. A significance of  $P < 0.05$  was used. There was a significant interaction between time and treatment in progesterone concentrations ( $P < 0.0001$ ), which reached the maximum concentration 12 h after its' administration, being 1.94 ng/mL and 1.62 ng/mL for G40 and G20 ewes, while remained in 0.0 ng/mL in GCon ewes (pooled SEM=0.2;  $P < 0.0001$  in both comparisons, without differences between G40 and G20). Progesterone concentrations remained above 1 ng/mL in both, G40 and G20 until 24 h after the administration (1.4 ng/mL and 1.0 ng/mL respectively vs 0.0 ng/mL in GCon;  $P < 0.0001$ , without differences between G40 and G20). Thirty-six hours after treatment, progesterone concentrations did not differ between groups, with only a tendency to have greater concentrations in G40 than GCon (0.5 vs 0.02, ng/mL  $P = 0.067$ ). The administration of 20 mg of progesterone delayed the estrous onset ( $104.0 \pm 25.6$  h vs  $94.0 \pm 15.9$  h and  $16.7 \pm 9.4$  h in G40, G20 and GCon,  $P < 0.05$ ; respectively). More GCon than G40 ewes ovulated (9/10 vs 2/10;  $P = 0.003$ , and G20 than G40 tended to ovulate - 6/10 vs 2/10;  $P = 0.075$ ). The size of the ovulatory follicle in G20 and GCon ewes did not differ ( $5.6 \pm 0.4$  mm vs  $5.4 \pm 0.6$  mm, ns). In conclusion, circulating progesterone concentrations remained at luteal levels ( $>1$  ng/mL) only for 24 h, with similar profiles despite administering 20 or 40 mg. The doses of P4i used in this experiment seems no to be useful to control the estrus in ewes.

**Keywords:** estrus synchronization, ultrasound, sexual behavior

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## Reduction in the pregnancy rate caused by the excitable temperament of Nelore cows can be circumvented by the administration of GnRH or long-acting P4.

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### Resumo

This research aimed to determine the effect of temperament on reproductive parameters including cortisol and progesterone (P4) in Nelore cows. Additionally, two methods for increasing plasma progesterone (P4) levels in excitable animals to enhance pregnancy rate (P/AI) and reduce pregnancy loss were investigated. In total, 939 cows were subjected to timed artificial insemination (TAI) and divided into three groups: (P4LA; n = 305) 150 mg of injectable long-acting progestogen 7 days after TAI; (GnRH; n = 306), 10 µg of buserelin acetate on day 7 after TAI; control group (CG; n = 328) without hormonal treatment. Ultrasound evaluations of the preovulatory follicle (FL) (Mode B) were performed on the day of insemination and of the corpus luteum (CL) (Color Doppler) 7 and 16 days after TAI. Blood samples were obtained on the day of insemination and after 7 and 16 days to measure cortisol and progesterone, respectively. At the time of insemination, subjective temperament evaluations were performed with the animals being classified as excitable (EXC) or adequate (ADQ). The SAS GLIMMIX procedure was used to compare the pregnancy rate (P/AI) and gestational loss within each temperament for the three experimental groups. Continuous variables were analyzed utilizing SAS PROC MIXED procedure. Cortisol concentration was higher in excitable females ( $P < 0.01$ ) and FL ( $P = 0.03$ ) and CL ( $P = 0.02$ ) volumes, at the time of insemination and 7 days after AI, respectively, were lower in EXC than in ADQ animals. No significant difference was observed between the number of pixels, CL intensity, and plasma concentration of P4, 7 days after TAI. However, 16 days post-insemination, among the animals classified as EXC, higher concentrations of P4 were observed in the GnRH and P4LA groups than in the control. P4 concentration was lower in animals classified as EXC than in ADQ within the control group ( $P = 0.06$ ), while rate of blood flow from the CL was lower in EXC animals than in ADQ animals ( $P = 0.04$ ). Among the ADQ animals, the GnRH and P4LA groups showed a lower flow rate than that observed in the control ( $P = 0.04$ ). Among EXC animals, a higher pregnancy rate was observed in the GnRH and P4LA groups than in the control group ( $P = 0.01$ ). In the control group, the pregnancy rate (P/AI) of the ADQ animals was higher than that of the EXC animals ( $P = 0.05$ ). No statistically significant differences were observed between gestational losses when the treatments or temperaments were compared. In conclusion, the use of GnRH or P4LA, 7 days after insemination, improves pregnancy rates in excitable animals and is a viable alternative to minimize the negative impact of stress and improve reproductive efficiency in beef cattle.

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# The influence of the antral follicle count and estrus expression on the conception rate in Nelore cows submitted to timed artificial insemination

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## Resumo

Antral follicle count (AFC) has been related to bovine fertility and response to reproductive biotechnologies, but its relationship to estrus expression during timed artificial insemination (TAI) is unknown. The study aimed to evaluate the relationship between the AFC and estrus expression on the conception rate in beef cows that received an ovulation synchronization protocol for TAI. For this, multiparous Nelore cows with up to 40 days postpartum were evaluated by an ultrasound to determine the AFC and were classified into low ( $\leq 15$  follicles;  $n=150$ ), intermediate ( $\geq 19$  and  $\leq 26$  follicles;  $n=150$ ), and high count ( $\geq 30$  follicles;  $n=150$ ). AFC evaluation was performed on Day 0 of the protocol for synchronization of the ovulation, on a random day of the estrous cycle. The animals received a conventional TAI protocol based on progesterone and estradiol benzoate. On day 8, the animals received cloprostenol, equine chorionic gonadotropin, estradiol cypionate, and a painting in the sacrococcygeal region. On day 10, TAI was performed, and estrus expression was evaluated. The estrus expression was classified as present or absent, present when the paint was totally removed, and absent when partially removed or full paint. Pregnancy diagnosis was performed by ultrasound 30 days after TAI. The data were analyzed using a generalized linear model and a binary logistic regression model ( $P<0.05$ ). At the end of the TAI protocol, regardless of AFC, a high proportion of cows showed estrus expression (low – 61.3%; intermediate – 67.3%, and high – 58.7%), and no significant difference in estrus expression was observed between the AFC groups. The conception rate was influenced by AFC (low: 69.3% vs. high: 56.7%;  $P=0.04$ ) and estrus expression (estrus present: 67.3% vs. estrus absent: 45.4%;  $P=0.003$ ). In addition, an interaction ( $P=0.008$ ) between AFC and estrus expression showed that low AFC cows and estrus present expression had the highest conception rate to TAI. In conclusion, AFC and estrus expression influenced the conception rate of Nelore cows in the TAI program. Furthermore, the interaction of AFC with estrus expression revealed that the best conception rate was observed in females with low AFC that express estrus.

**Keywords:** Bos indicus, ultrasound, synchronization, TAI, fertility.

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**IMPACT OF PROGESTERONE SUPPLEMENTATION ON MORPHOFUNCTIONAL ASPECTS OF THE CORPUS LUTEUM, PREGNANCY AND GESTATIONAL LOSS IN NELORE COWS**

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**Resumo**

The aim of the present study was to evaluate the impact of different strategies to increase plasma progesterone (P4) on luteal morphology and function in bovine females. Additionally, the effect of increasing P4 on pregnancy rate and gestational loss in Nelore cows submitted to Timed Artificial Insemination (TAI) was evaluated. A total of 939 cows were divided into three groups: P4LA (n=305) - 150mg of injectable long-acting progestin, seven days after the TAI; GnRH (n=306) - 10µg of buserelin acetate, seven days after TAI; Control Group (n=328) no hormone application. The treatments (CONTR, GnRH and P4LA), cyclicity, body condition score, farm, inseminator, bull and interactions were included in the model. For the final model, the variables were removed, based on the criterion of Wald's statistics when  $P > 0.20$ . The variables included in the final model for the analysis of pregnancy rate were treatments. Doppler ultrasound assessments and progesterone measurements were performed on the seventh and sixteenth day after TAI. Pregnancy rate and pregnancy loss as a function of treatments were evaluated with the aid of transrectal ultrasound at 30 and 60 days after insemination, respectively, and compared using the SAS GLIMMIX procedure. The number of pixels and intensity of each corpus luteum (CL) were evaluated using the Image J® program (version 1.45s, National Institutes of Health, Bethesda, MD, USA). CL vascular perfusion and volume and plasma P4 concentrations were analyzed using the SAS PROC MIXED procedure. No significant difference was observed between treatments in the variables volume, number of pixels and CL intensity, as well as in plasma progesterone concentration, 7 days after ovulation. The CL blood flow, 16 days after ovulation, was lower in the P4LA and GnRH groups ( $P < 0.01$ ). Plasma concentrations of P4, 16 days after ovulation, were higher in the P4LA and GnRH groups compared to the control group ( $P = 0.04$ ). There was a significant difference in pregnancy rate (CONTR 47,8%, GnRH 55,5%, and P4LA 57,4%;  $P = 0.003$ ) and a trend in gestational loss ( $P = 0.07$ ) as a function of the treatments. Under the conditions of the present study, P4 supplementation using injectable, long-acting P4 or GnRH on the seventh day after TAI affected CL perfusion and increased the plasma concentration of progesterone sixteen days after ovulation, increasing the pregnancy rate compared to the control group.

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## Relationship between health problems and fertility in lactating dairy cows submitted to timed-artificial insemination fertility programs.

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### Resumo

The study evaluated the relationship between health problems (HP) and fertility in lactating Holstein cows submitted to fertility programs for the first postpartum timed-artificial insemination (TAI). All cows (387 multiparous and 411 primiparous), from a commercial dairy herd, were submitted to fertility programs (presynchronization + TAI protocol initiating with GnRH), differing in their pharmacological bases (Ovsynch-type or estradiol [E2]/progesterone [P4]-based programs), and received the first TAI postpartum with  $60 \pm 3$  days in milk. Using the IDEAGRI software, data related to the following diseases were retrieved: retained placenta (RP), metritis, endometritis, and mastitis. For analyses, cows were classified as healthy (HLT) or with health problems (HP), and as having one (1HP) or more HP ( $\geq 2$ HP). Pregnancy diagnoses were performed 32 and 60 d after TAI, and pregnancy loss (PL) was evaluated. Statistical analyses were performed using the GLIMMIX procedure of SAS 9.4 ( $P \leq 0.05$ ). There was no interaction between HP and neither presynchronization strategy nor TAI protocol on pregnancy per AI (P/AI) and PL. A total of 35.5% (283/798) of cows were classified as with HP (RP: 5.5%; metritis: 17.2%; endometritis: 6.5%; and mastitis: 17.0%). Multiparous cows had a greater proportion of HP than primiparous (39.5 [153/387] vs. 31.6% [130/411]), and there was no interaction between parity and HP on fertility. The P/AI on d 32 was greater in HLT than HP cows (52.4 [270/515] vs. 39.9% [113/283]), as well as for the 60-d pregnancy diagnosis (43.5 [224/515] vs. 32.2% [91/283]). The P/AI on d 32 differed among classes of cows, with HLT cows having the highest P/AI, followed by an intermediate fertility on cows with 1HP, and the lowest fertility in cows with  $\geq 2$ HP (52.4a [270/515], 43.7b [94/215], and 27.9%c [19/68], respectively). The same pattern was detected for P/AI on d60 (43.5a [270/515], 35.4b [94/215], and 22.1%c [19/68], for HLT, 1HP and  $\geq 2$ HP, respectively). Curiously, there was no difference on PL among HLT, 1HP and  $\geq 2$ HP cows (17.0, 19.2 and 21.1%, respectively). This study is novel in terms of associating HP with fertility after TAI with a large number of cows in a Brazilian commercial dairy herd. In summary, HP had a clear negative effect on fertility of lactating dairy cows, which in this study was evidenced by the substantial decrease on P/AI, although no significant effect on PL was detected. Modern dairy herds must have a holistic vision, aiming to understand and control aspects associated with HP increasing overall performance and profitability.

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

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# The use of a recombinant equine chorionic gonadotropin (reCG) in fixed-time AI programs in beef cattle

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An experiment was designed to evaluate the effect of the addition of a recombinant eCG (reCG, Zoovet, Argentina) on pregnancy rates to AI (P/AI) in suckled beef cows submitted to an estradiol (E2)/progesterone (P4)-based FTAI protocol. In Experiment 1, Angus and Angus x Hereford beef cows (n=1244), with 45 to 60 d post-partum and a BCS of 2.6±0.4 (1 to 5 scale) were used. On Day 0, all cows were scanned by ultrasonography (289, 23% had a CL, 684, 55% had a follicle >8 mm in diameter, and 271, 34% had follicles < 8 mm diameter) and were randomly assigned to 1 of 3 treatment groups, to receive two dosages of reCG [105 IU (1.5 mL) or 140 IU (2 mL)] at the time of P4 device removal or no reCG treatment (control). On Day 0, all cows received 2 mg of estradiol benzoate (EB, Zoovet) i.m. and a device containing 0.75 g of P4 (Proclinar, Zoovet). On Day 7, P4 devices were removed, and all cows received 150 µg of D+cloprostenol (PGF2α, Zoovet), 1 mg of ECP (Zoovet) i.m., had their tail painted for estrus detection and received reCG or no further treatment. All cows were AI 48 to 54 h after P4 device removal and those with >50% of their tail paint intact also received 10 µg buserelin (GnRH, Zoovet) at the same time. In Experiment 2, cross-bred (Bos taurus x Bos indicus) beef cows (n=905), with 40 to 90 d post-partum and a mean BCS of 2.6±0.4, were used. Cows were examined by ultrasonography on Day 0 (182, 20% had a CL, 509, 56% had a follicle >8 mm in diameter, and 215, 24% had follicles < 8 mm diameter) and were randomly assigned to 1 of 4 treatment groups, to receive three dosages of reCG [84 UI (1.2 mL), 105 UI (1.5 mL) or 126 UI (1.8 mL)] at the time of P4 device removal or no reCG treatment. All cows were synchronized and AI as those in Experiment 1. Pregnancy was diagnosed by ultrasonography 30 days after AI and P/AI was analyzed by GLMM for binary data with a logit link. In Experiment 1, P/AI was greater (P<0.05) in cows treated with reCG than in those in the control group (105 UI reCG: 52.3%, 216/413; 140 IU reCG: 53.5%, 224/419 and control: 44.4%, 183/412). There was a significant (P<0.01) effect of ovarian structure on Day 0 (CL: 60.2%, 174/289; follicles >8 mm: 49.7%, 340/684 and follicles <8 mm: 40.2%, 109/271); but no interaction. In Experiment 2, although differences among groups only tended to differ (84 IU reCG: 38.6%, 78/202; 105 IU reCG: 38.5%, 100/260; 126 IU reCG: 36.8%, 84/228 and control: 27.9%, 60/215; P=0.1), there was a significant effect of giving or not reCG, regardless of dose, on P/AI (reCG: 38%, 262/690 vs control: 27.9%, 60/215; P<0.01). There was a significant (P<0.01) effect of ovarian structure on Day 0 (CL: 41.8%, 76/182; follicles >8 mm: 36.9%, 188/509 and follicles <8 mm: 27.1%, 58/214); but no interaction. In conclusion, the results confirm those previously reported (Villarraza et al., Theriogenology, 172: 223-229, 20221) in which treatment with reCG increased P/AI in suckled cows submitted to E2/P4-based FTAI protocols.



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## Prostaglandin F2a administration at the beginning of FTAI protocol in primiparous beef cows: preliminary results

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### Resumo

The aim of the study was to evaluate the dominant follicle size and rates of estrus expression and pregnancy in primiparous beef cows submitted to 7 days or 8 days FTAI protocol. Primiparous [*Bos taurus* (Aberdeen Angus) and *Bos taurus* x *Bos indicus* (Braford and Brangus)] suckling with a mean body condition score (BCS) of  $2.82 \pm 0.15$  (1= thin and 5=obese) and 7,7% of cyclicity (CL on D0) were distributed in experiment used a 2 x 2 factorial arrangement of four treatments. The two factors were: 1) time of exposure to progesterone (P4) (7 and 8 days); 2) prostaglandin treatment: one PGF (PGF at removal P4) and two PGF (PGF at D0 and moment of P4 removal). Thus, the treatments were PGFd0d7 (n=70), PGFd7 (n=70), PGFd0d8 (n=72) and PGFd8 (n=72). The hormones used were all from the same company (Zoetis) - estradiol benzoate (2mg) i.m. (Gonadiol®), P4 intravaginal device (DIB® 0.5mg), estradiol cypionate (0.6mg) i.m. (ECP®), equine chorionic gonadotropin (300 IU) i.m. (Novormon®) and PGF (25 mg Dinoprost Tromethamine) i.m. (Lutalyse®). The basis of the tail was painted at P4 device removal for observation of estrus at the FTAI. The follicular diameter was measured at P4 device removal and FTAI. FTAI was performed 48h after P4 device removal using semen from a single sire. The pregnancy diagnosis was performed 30 d after AI using a transrectal B-mode ultrasound. Analyses of binomial outcome variables (expression of estrus and P/AI) and continuous outcomes (follicle diameter on P4 device removal and FTAI) were performed using the Fit Mixed Effects Model procedure. Values are presented as percentage (%; binomial variables). The results of continuous outcome variables are expressed as means  $\pm$  standard error of the mean. The follicular diameter (mean  $\pm$  SE mm) at the time of device removal was: PGFd0d7 ( $11.4 \pm 0.25$  mm) and PGFd7 ( $9.5 \pm 0.21$  mm); PGFd0d8 ( $11.8 \pm 0.21$  mm) and PGFd8 ( $11.6 \pm 0.2$  mm) (Protocol length:  $P < 0.0001$ ; PGF  $P = 0.0001$ ). The follicular diameter (mean  $\pm$  SE mm), at AI was: PGFd0d7 ( $13.5 \pm 0.26$  mm) and PGFd7 ( $12.1 \pm 0.19$  mm); PGFd0d8 ( $14.8 \pm 0.27$  mm) and PGFd8 ( $14.85 \pm 0.19$  mm) (Protocol length:  $P < 0.0001$ ; PGF  $P = 0.001$ ). No difference was observed in estrus rate: PGFd0d7 (69.3%), PGFd7 (60.0%), PGFd0d8 (72.8%) and PGFd8 (65.72%) (Protocol length  $P = 0.15$ ; PGF  $P = 0.12$ ). The pregnancy rate was similar between treatments PGFd0d7 (41.4%), PGFd7 (28.8%), PGFd0d8 (45.8%) and PGFd8 (37.3%) (Protocol length  $P = 0.19$ ; PGF  $P = 0.08$ ). The preliminary results indicate that prostaglandin analogue administration at the beginning of the 7d-protocol is associated with an increased dominant follicle size in *Bos taurus* primiparous cows. There was no difference in estrus and conception rate between the protocol of seven and eight days of exposure to P4 device (0.5g), but further studies with a large number of animals are indicated.

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# Timed-artificial insemination protocols without estradiol esters for *Bos indicus* (Nelore) cattle

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## Resumo

The study evaluated strategies of timed-artificial insemination (TAI) protocols without estradiol (E2) esters for postpartum *Bos indicus* (Nelore) cows, and the inclusion of a long-acting injectable progesterone (injP4) before the beginning of the protocols. Primiparous (n=293) and multiparous (n=798) cows (46.7±0.4 d postpartum and body condition score [BCS]=3.1±0.02) were submitted to synchronization protocols, following a 2×2 factorial arrangement, resulting in 4 treatments: injP4+E (n=279), E (n=266), injP4+G (n=267) and G (n=279). On D-10, cows were randomly assigned to receive or not 150 mg injP4. On D0, all cows received an intravaginal P4 device (1 g), concomitant with the experimental treatments, that were: 2 mg E2 benzoate (EB; E2-based groups) or 16.8 µg buserelin acetate (GnRH; G-based groups). On D6, cows from G-based groups (injP4+G and G) received 0.5 mg cloprostenol sodium (PGF) and 300 IU eCG. On D7, all cows received 0.5 mg PGF concomitant with P4 device withdrawal, and cows from E2-based groups (injP4+E and E), received 300 IU eCG and 1 mg E2 cypionate (EC). TAI was performed on D9, and all cows received 8.4 µg GnRH. Ultrasound examinations were performed on D0, 7, 9, 39, and 69 to evaluate dominant follicle (DF) diameter, presence of CL, ovulatory response after D0 and pregnancy per AI (P/AI). Statistical analyses were performed by PROC GLIMMIX of SAS 9.4 (P≤0.05). Treatment with injP4 was associated with less cows with CL on D0 (19.2 [104/542] vs. 30.2% [163/540]), and larger DF on D0 (13.3±0.2 vs. 12.1±0.1 mm) and on D7 (11.8±0.1 vs. 11.3±0.2 mm), with no effect on P/AI (with: 57.9 [316/546] vs. without: 58.5% [319/545]). Moreover, cows from G-based groups had larger DF on D7 (12.3±0.01 vs. 10.8±0.1 mm) and D9 (13.9±0.1 vs. 10.8±0.1 mm) than cows from E2-based groups. Ovulation after D0 was higher in G-based groups (73.1 [350/479] vs. 12.9% [62/482]), without effect or interaction with injP4. More cows from E2-based groups were detected in estrus (83.1 [453/545] vs. 74.0% [404/546]) and these groups had greater P/AI than cows from G-based groups (62.4 [340/545] vs. 54.0% [295/546]). Expression of estrus affected P/AI only in E2-based groups, in which cows that expressed estrus had greater fertility (65.3 [296/453] vs. 47.8% [44/92]). Pregnancy loss (PL) between D39 and 69 was similar among groups, but primiparous had higher PL than multiparous cows (12.9 [13/101] vs. 5.2% [21/407]). In conclusion, although P/AI was greater in cows treated with the E2-based protocol, this study presents data related to GnRH-based protocols for TAI in which Nelore cows had high ovulatory response after D0, adequate ovulatory follicle size and a 54% P/AI. More studies are required to determine if, with some adjustments, GnRH-based protocols result in similar fertility compared to E2-based protocols in *Bos indicus* cows. Finally, injP4 had no effect on P/AI.

## Acknowledgements

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**Effect of postpartum period on pregnancy rate of beef cattle submitted to TAI.**Alicia Pires <sup>1</sup>, Beatriz Alves Paulino <sup>1</sup>, Thiago Barros Almeida <sup>1</sup>, Samuel Rodrigues Bonamichi do Couto <sup>1</sup>, Marco Roberto Bourg de Mello <sup>1</sup><sup>1</sup> UFRRJ - Universidade Federal Rural do Rio de Janeiro (Km 07,Zona Rural, BR-465, Seropédica - RJ, 23890-000)**Resumo**

The fixed-time artificial insemination (TAI) is now a reality in the Brazilian beef cattle production systems. Its use can directly impact the reproductive performance of the herd, improving productivity. However, there are some known factors of failure in the implementation of TAI programs that should be studied, such as uterine condition in the postpartum period (PP). The hypothesis of this study is that cows presenting PP less than 40 days have a lower pregnancy rate than cows with PP higher. Thus, the objective of the present study was to evaluate the effect of PP on the pregnancy rate (P/AI) of Nelore cows submitted to TAI. The experiment was carried out in a commercial farm in the interior of the State of São Paulo, in the 2020/2021 breeding season, where 865 multiparous cows, presenting different PPP, were kept in *Urochloa* spp (*U. decumbens*) pastures, receiving mineral supplementation and water ad libitum. The animals were submitted to synchronization of ovulation, initiated on a random day of the estrous cycle (D0), with the introduction of a new intravaginal device containing 0.5 g of progesterone (Primer®, Tecnopec, São Paulo, Brazil) and application of 2.0 mg of estradiol benzoate (Sincrodiol®, Ouro Fino Saúde Animal), by intramuscular route. After 8 days (D8), the P4 device was removed and 500 µg of cloprostenol (Sincrocio®, Ouro Fino Saúde Animal, São Paulo, Brazil) + 400 IU of eCG (Folligon®, MSD Saúde Animal, São Paulo, Brazil) + 1.0 mg of estradiol cypionate (SincroCP®, Ouro Fino Saúde Animal, São Paulo, Brazil) were applied, all by intramuscular route. Two days after the removal of the device (D10), the TAI was performed. The pregnancy diagnosis was performed 30 days after TAI, with ultrasound (Mindray, model Z5VET). To evaluate the influence of PP on pregnancy rate, cows were divided into three experimental groups: early PP, consisting of cows calved at less than 40 days (GE; n = 226), intermediate PP with cows between 40 and 70 days postpartum (GI; n = 396) and late PPP, consisting of cows calved more than 70 days (GL; n = 243). Pregnancy rates were compared by the nonparametric binomial test using Bioestat 5.0 software. Significant differences were considered for  $P < 0.05$ . The overall pregnancy rate (P/AI) of the experiment was 56.6% (490/865) and the GE, GI and GL groups had pregnancy rates of 41.2a% (93/226), 59.3b% (235/396) and 66.7c% (162/243), respectively. Significant difference ( $P < 0.001$ ) was observed between the groups, confirming the hypothesis of the study. Therefore, we conclude that the postpartum period interferes in the pregnancy rate of beef cattle submitted to TAI and that, aiming to increase the efficiency of this technique, this criterion should be taken into consideration at the beginning of the program (D0), not including animals with less than 40 postpartum days.

**Keywords:** Uterine involution, reproductive efficiency, fertility.

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# Effect of the dose of estradiol cypionate and GnRH treatment at the time of artificial insemination on fertility of crossbred beef heifers

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## Resumo

The study evaluated the effect of the dose of estradiol cypionate (EC; 0.5 vs. 1.0 mg) at the time of intravaginal progesterone (P4) device removal and GnRH treatment (with vs. without) at timed-artificial insemination (TAI) on expression of estrus and pregnancy per AI (P/AI) in *Bos taurus* (Angus) x *Bos indicus* (Nelore) heifers. A total of 317 heifers (BCS = 2.84 ± 0.01 and BW = 294.1 ± 1.1 kg) were enrolled in a 2 × 2 factorial arrangement. On D0, heifers were submitted to ultrasound examination and synchronized with 2 mg estradiol benzoate im, an intravaginal P4 device (0.5 g), and 0.53 mg cloprostenol sodium (PGF) im. On D7, heifers received 0.53 mg PGF and 200 IU eCG im, concomitant with P4 device removal, and were randomized to receive 0.5 mg (EC0.5) or 1.0 mg EC (EC1.0) im. Moreover, heifers were painted with chalk on their tailheads for posterior evaluation of expression of estrus. Two days later (D9), the removal of chalk was evaluated to determine the expression of estrus. In addition, on D9, TAI was performed, and heifers were randomized to receive (G1) or not (G0) 8.4 µg buserelin acetate (GnRH) im. Thus, 4 experimental groups were established: EC0.5-G0 (n = 78), EC0.5-G1 (n = 80), EC1.0-G0 (n = 78) and EC1.0-G1 (n = 81). Pregnancy diagnosis was performed by ultrasound 30 days after TAI. Hormones were from GlobalGen vet science (Jaboticabal, Brazil) and semen was from Solução Genética (Santa Maria, Brazil). Statistical analyses were performed by the GLIMMIX procedure of SAS 9.4 ( $P \leq 0.05$ ). On D0, 10.1% (32/317) of heifers had a corpus luteum. Expression of estrus was greater in EC1.0 than EC0.5 group (74.8% [119/159] vs. 62.0% [98/158]), and heifers that expressed estrus had greater P/AI (43.3% [94/217] vs. 24.0% [24/100]). Despite numerical differences, no main effect of EC dose was detected on P/AI (EC0.5 = 41.1%, [65/158] vs. EC1.0 = 33.3%, [53/159];  $P = 0.14$ ). The GnRH treatment at TAI increased P/AI (42.9% [69/161] vs. 31.4% [49/156]), but there was no interaction between EC dose and GnRH treatment (EC0.5-G0 = 33.3% [26/78], EC0.5-G1 = 48.8% [39/80], EC1.0-G0 = 29.5% [23/78] and EC1.0-G1 = 37.0% [30/81]). Moreover, there was no interaction between GnRH treatment and expression of estrus on P/AI (without estrus: G0 = 24.5% [13/53] vs. G1 = 23.4% [11/47]; with estrus: G0 = 35.0% [36/103] vs. G1 = 50.9% [58/114]). In conclusion, the power of this study was not enough to detect effect of EC dose on fertility, but it was able to demonstrate a positive effect of GnRH treatment at TAI on P/AI in crossbred beef heifers, regardless of the EC dose.

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# Reproductive outcomes of high producing Holstein cows initiating timed artificial insemination protocols with estradiol benzoate or double dose of GnRH, and receiving or not a double dose of GnRH 2 days later

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## Resumo

The study evaluated strategies to initiate progesterone (P4)-based timed artificial insemination (TAI) protocols in high producing Holstein cows with estradiol benzoate (EB) or a double dose of buserelin acetate (GnRH), associated or not with a double dose GnRH 2 d later. Primiparous (n=291) and multiparous (n=622) cows with milk production of 41.2±0.40 kg/d were randomized into four groups in a 2×2 factorial arrangement (Groups: EBd0, EBd0-Gd2, Gd0, and Gd0-Gd2). On d0, all cows received a 2 g P4 implant, and groups EBd0 and EBd0-Gd2 received 2 mg EB, whereas Gd0 and Gd0-Gd2 groups received 20 µg GnRH. Additionally, on d2, cows from EBd0-Gd2 and Gd0-Gd2 groups received an additional 20 µg GnRH treatment. The remaining treatments were similar for all cows: 0.53 mg cloprostenol sodium (PGF) on d7, followed by a second PGF on d8, concomitant with P4 implant removal and 1 mg estradiol cypionate. Cows were inseminated on d10 in all groups. Statistical analyses were performed using GLIMMIX of SAS 9.4 (P≤0.05). The proportion (% [n/n]) of cows with corpus luteum (CL) on d0 of the protocol was similar among groups (77.8% [710/913]). Ovulation after d0 was greater in cows treated with GnRH on d0, and GnRH on d2 increased ovulation in cows receiving EB on d0 (EBd0 = 16.7 [38/227]; EBd0-Gd2 = 26.8 [61/228]; Gd0 = 59.2 [138/233]; Gd0-Gd2 = 63.6% [143/225]). The EB treatment was associated with greater CL regression from d0 to d7 compared to GnRH (45.1 [160/355] vs. 29.8% [106/356]). These effects resulted in a greater number of cows with CL at PGF treatment in groups initiating the protocol with GnRH compared to EB. Moreover, treatment with GnRH on d2 increased CL presence on d7 of cows receiving EB on d0 (EBd0 = 60.8 [138/227]; EBd0-Gd2 = 68.0 [155/228]; Gd0 = 88.8 [207/233]; Gd0Gd2 = 90.2% [203/225]). Ovulation after d0 increased pregnancy per AI (P/AI) of cows without CL at the beginning of the TAI protocol compared with cows that did not ovulate (42.6 [52/122] vs. 25.9% [21/81]). Similarly, cows without CL on d0 were benefited from GnRH on d0 compared to EB (44.6 [46/103] vs. 27.0% [27/100]). Moreover, cows with CL at PGF treatment had greater P/AI than cows without CL (44.2 [311/703] vs. 31.9% [67/210]). Regarding P/AI among treatments, there was a main effect of treatment on d0 (P=0.05) and on d2 (P=0.04), but no interaction (P=0.90). When the four TAI protocols were compared, only EB on d0 promoted the lowest fertility (EBd0 = 35.7 [81/227]b; EBd0-Gd2 = 43.0 [98/228]a; Gd0 = 41.3 [97/233]a; Gd0-Gd2 = 45.3% [102/225]a). In conclusion, initiating TAI protocols in high producing dairy cows with a double dose of GnRH or including a GnRH at the beginning of the protocol in cows receiving EB promoted improved ovarian dynamics and fertility, reinforcing that only EB to initiate TAI protocols in dairy cows does not optimize fertility.

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# Can the estradiol valerate be an effective alternative to use for timed-AI programs in suckled *Bos indicus* beef cows?

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## Resumo

The use of protocols based on estradiol benzoate (EB) and P4-device associating is widespread in South America. But, the prolonged half-life of estradiol valerate (EV) makes this ester an alternative to induce luteolysis without the need to apply the ovulatory and luteolytic agents during the timed-AI (TAI) protocols. We aimed to evaluate pregnancy rate (P/AI) in TAI protocols using EV in suckled beef cows. In Exp. 1, 568 primiparous Nelore cows with  $30 \pm 0.4$  days postpartum and BCS of  $2.99 \pm 0.01$  (scale from 1 to 5) were used. On D-11 (D0=day of TAI), cows received 2 mg EB (Fertilcare Sincronização®, MSD) associated to a NEW [n=96] or USED [n=94] P4-device (Fertilcare1200®, MSD), or received 5 mg EV (Valerol®, MSD Animal Health, Brazil) associated to a NEW [n=184] or USED [n=194] P4-device. On D-2, devices were removed and cows of EB group received 0.265 mg PGF2 $\alpha$  analogue (Ciosin®, MSD), 1 mg estradiol cypionate (Fertilcare Ovulação®, MSD) and 300 IU eCG (Folligon®), whereas cows in EV group received only 300 IU eCG. Cows in the EB group were submitted to TAI 48 hours after withdrawal and, in cows in the EV group TAI was performed 48 [n=201] or 54 [n=177] hours after withdrawal. In Exp. 2, 396 multiparous Nelore cows with  $29 \pm 0.4$  days postpartum and BCS of  $3.2 \pm 0.02$  were used. On D-11, cows received 2.5 mg of EV (Valerol®) associated to a MULTIDOSE (Fertilcare 1200®, MSD) or SINGLE-DOSE (Fertilcare 600®, MSD) P4-device [n=198 cows/group]. On D-2, devices were removed, cows received 300 IU eCG (Folligon®) and, TAI was performed 54 hours later. Pregnancy diagnosis was performed using ultrasound 30-40 days after TAI. Statistical analyses were performed using PROC MIXED and GLIMMIX of SAS 9.2. In Exp.1, pregnancy data were also separated by orthogonal contrasts (EB vs EV and EV-48h vs EV-54h) to assess the effect of drug given on D0 and the timing of insemination in the group that received EV. In Exp. 1, P/AI did not differ ( $P>0.1$ ) between EB [42% 80/190] and EV [38% 145/378] groups. Regarding to contrast 2, we observed a tendency ( $P\leq 0.1$ ) of interaction between the type of P4-device and time of TAI. Using a NEW P4-device, P/AI tended to be greater when TAI was performed 54h [44%, 37/84] after withdrawal, compared to 48h [31%, 31/100]; whereas, using a USED P4-device, P/AI tended to be greater when TAI was performed 48h [43% 43/101] after withdrawal, compared to 54h [37% 34/93]. In Exp.2, P/AI did not differ ( $P>0.1$ ) between MULTI [46% 90/198] and SINGLE [44% 85/198] groups. We conclude that in primiparous cows, the use of the EV-based protocol proved to be effective compared to the EB-based protocol. When using a new device, the insemination is indicated to be performed 54h after device removal; whereas with an used device, insemination is preferred 48h after device removal. In multiparous cows, the use of the EV-based protocol can be done in association with multi or single dose devices.

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Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

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## ASSOCIATION OF DIFFERENT STEROID HORMONES AND GONADOTROPINS IN PROTOCOLS OF ESTRUS SYNCHRONIZATION AND OVULATION INDUCTION FOR FTAI IN NELORE BOVINE FEMALES

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### Resumo

The aim of this study was to evaluate the reproductive performance of Nelore bovine females submitted to Fixed Time Artificial Insemination (FTAI) and treated with different estradiol esters (BE and 17 $\beta$ E2) and gonadotropins (eCG and hCG), which act in a similar way when used to synchronize the emergence of a new follicular wave and to stimulate follicular growth, respectively. A total of 587 females in reproductive activity were submitted to FTAI using an 8.5-day hormonal treatment protocol based on P4 and sodium Cloprostenol, so that estradiol esters were administered on day zero (D0) and gonadotropins on day 8.5 (D8.5), with artificial insemination (AI) on day 10 (D10). Treatments were distributed in a completely randomized design with a 2 x 2 factorial arrangement, being: Group BEE (BE + eCG), group BEH (BE + hCG), group 17EE (17 $\beta$ E2 + eCG) and group 17EH (17 $\beta$ E2 + hCG). The follicular diameter of the dominant follicle (DDF) was measured by B-mode transrectal ultrasound on D8.5 and on D10. Pregnancy diagnosis was performed 40 days after AI. Data were analyzed by logistic regression (proc logistic; SAS 9.4) considering pregnancy per AI (P/AI) as a binary dependent variable. The nominal variables considered in the statistical model were gonadotropins (eCG or hCG), estrogens (BE or 17 $\beta$ E2), breeding season (2019-2020 or 2020-2021), ECC ( $\leq 3$  or  $> 3$ ), parity (heifer or cow), ovarian condition on D0 (presence or absence of CL), bull used for AI (1, 2 or 3) and presence of ovulation on the day of AI (ovulated or not ovulated). DDF data on D8.5 and D10 and follicular growth rate from D8.5 to D10 were analyzed by multivariate regression (proc reg; SAS 9.4) as dependent variables. The nominal variables considered in this statistical model were the same as used for logistic regression, excluding the variables bull and presence of ovulation on the day of AI. Significance level  $P < 0.05$  was used for all tests. P/AI, DFD on D8.5 and D10 and follicular growth rate between D8.5 and D10 did not differ when compared between the four treatments ( $P > 0.05$ ). Considering both breeding seasons, P/AI was 46.3% (272/587). For BEE, BEH, 17EE and 17EH, respectively, the results obtained were: P/AI of 50.0% (79/158), 42.5% (62/146), 46.1% (65/141), and 46.5% (66/142). DDF (mean $\pm$ SME) at D8.5 of 11.35 $\pm$ 0.17, 11.37 $\pm$ 0.14, 11.08 $\pm$ 0.18, and 11.06 $\pm$ 0.17. DDF (mean $\pm$ SME) on D10 of 12.55  $\pm$  0.17, 12.51  $\pm$  0.18, 12.14  $\pm$  0.17 and 12.35  $\pm$  0.19. Follicular growth rates of 0.49  $\pm$  0.05, 0.42  $\pm$  0.05, 0.40  $\pm$  0.05, and 0.48  $\pm$  0.05. Therefore, it is concluded that the different gonadotropins and estrogens used in this study can be combined and act similarly in estrus synchronization and ovulation induction protocols in Nelore bovine females submitted to FTAI.

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## Use of the $\beta$ -hCG rapid immunochromatographic test as qualitative analysis to identify the plasma concentration of hCG administered by different routes in dairy goats

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### Resumo

The hCG has been used to improve goats reproductive efficiency. The intravaginal (i.vag.) route has been advocated of having a "first uterine/ovary pass effect". The immunochromatographic analysis is a qualitative method to detect hCG in serum and plasma blood. Thus, this study aimed to evaluate if hCG administered by the intramuscular (i.m.) or intravaginal (i.vag.) route is detected by a rapid  $\beta$ -hCG test in the blood plasma, to propose a quantitative detection method for scores-based hCG and in which group and moment the tape marking is more intense. To synchronize the estrus, 22 alpine goats received two injections i.m. of 30  $\mu$ g d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) at intervals of 7.5 days. One day after onset of estrus, the goats were equally allocated to three groups of animals that received: (1) Control (n=7): 0.3 mL i.vag. of saline solution; (2) hCG-im (n=7): 300 IU i.m. of hCG (Vetecor®; Hertape-Calier, São Paulo, Brazil); and (3) hCG-ivag (n=8) 300 IU i.vag. of hCG. Blood samples collection to rapid  $\beta$ -hCG test (ECO Diagnóstica, Corinto, Brazil) in the blood plasma were performed one hour before (-1h), 3, 6, 9 and 24h after, and on Days 3, 7, 10, 13, 17 and 21 after treatment. The tapes tested in these concentrations were evaluated using a scale ranging scores from 1 to 4 – briefly, 1: unidentified; 2: very weak (similar or less intense than 10mIU/mL); 3: weak (similar 25 and 50mIU/mL and less intense than 75mIU/mL); 4: very strong (similar or more intense than 75mIU/mL). Data were analyzed by the Mann-Whitney test, Friedman test and Fisher's exact test at 5% minimal level of significance. All animals tested negative at -1h and all animals in the control group also test negative at all times of evaluation. During the analyses, only 50% goats from the hCG-ivag tested positive. All animals in the hCG-im tested positive from 3h until D3. From animals that tested positive at some point on both treated groups, 100% of the animals remained positive until D3; on D7 the rate decreased to 83.3% and 75.0% for hCG-im and hCG-ivag, respectively; on D10 the rate was 33.3% in hCG-im and 25% in hCG-ivag; and on D17 and D21 there were no positive in hCG-im and only one goat (25%) in hCG-ivag. There was difference between groups hCG-im and hCG-ivag only from 3h until 24h (P<0.05). In the quantitative scores-based hCG evaluation, the hCG-im animals presented higher score than hCG-ivag (P<0.05) in 3, 6, 9 and 24h (median 3, 4, 3 and 2 Vs 1, 1, 1 and 1, respectively). In hCG-im, there was higher intensity of hCG marking (P<0.05) in 3, 6 and 9h, while in hCG-ivag, all positive animals presented the score 2 regardless of the time of evaluation (P>0.05). In conclusion, the hCG administered by the different routes could be identified with the rapid  $\beta$ -hCG test and classified into scores, while the intensity of tape marking is higher in hCG-im and in the first 9 hours.

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## Use of GnRH prior TAI improves conception rates in *Bos taurus* beef cows

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### Resumo

Estradiol cypionate (EC) is used as ovulation inducer in time AI (TAI) protocols, despite the dispersive time of ovulation (42-96 h after P4 removal). The present study tested the hypothesis that GnRH 34h after intravaginal device (IVD) removal increases the conception rate in *Bos taurus* beef cows. A secondary objective was to determine the effect of GnRH on the morphology and function of the corpus luteum (CL). Procedures were approved by UFRGS Ethics Committee. Data were analyzed by ANOVA and Chi-square. In Experiment 1, lactating multiparous cows (n=157; BCS±2.9) kept on ryegrass pasture received an IVD (0.96g P4; Boehringer), 5.5mg 17b estradiol and 50mg P4 (Boehringer; im) on Day 0 (D0). Cows were treated with cloprostenol (PGF; 500ug, Boehringer; im), eCG (300UI im; Ourofino; im) and estradiol cypionate (EC; 0.5mg, Zoetis; im), simultaneously to IVD removal on D8. On D9, cows were allocated to Control (no treatment; n=79) or GnRH (25µg leirelin; Agener; im; n=78) groups 34h after IVD removal. Control cows that did not show estrus received GnRH at TAI (48h after IVD removal). Cows in the GnRH group had higher pregnancy per AI (P/AI) compared to Control (67.9% and 51.9%; p<0.05). In Experiment 2, we tested whether these results would be repeated without im P4 on D0 and GnRH at TAI in cows not detected in estrus. Multiparous cows (n=342; BCS±2.9) kept in native pasture had the follicular wave synchronized as previously described, except for estradiol benzoate (EB 2mg; Ourofino; im) and IVD (1g P4, Ourofino) on D0; and EC (1mg Ourofino; im) on D8. On D9, cows were allocated to Control (no treatment; n=144) or GnRH (n=198) groups. No difference (p>0.1) in the P/AI was detected between groups (52% and 50%, for GnRH and Control groups, respectively). In Experiment 3, primiparous (n=14) and multiparous (n=16) lactating Brangus cows had the follicular wave synchronized with a protocol of 8 and 9 days, respectively. The animals received EB (2mg im; Ourofino) and IVD (1g P4, Agener). At IVD removal, cows received PGF (0.5mg; Ourofino), eCG (300UI; Ourofino) and EC (0.5mg; Agener) im. The follicles were scanned by ultrasound at IVD removal and at GnRH treatment. Cows were distributed according to the follicular diameter: primiparous (Control 11.3±0.6mm, n=7; GnRH 11.9±0.6mm, n=8; p>0.5) and multiparous (Control 12.4±0.8mm, n=5; GnRH 12.3±0.5mm, n=7; p>0.5). Ovulation was confirmed 72h after IVD removal. Serum P4 concentration and CL diameter did not differ between treatments (p>0.05) in both categories 7 and 12 days after TAI. In conclusion, GnRH treatment prior to TAI promoted a significant increase in P/AI in cows kept on winter pasture, whereas no significant effect was observed in cows grazing on native pasture. The positive effect of GnRH in Experiment 1 is more likely related to ovulation synchrony because no significant effect was observed on CL morphology and function.

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## Effect of long-acting injectable progesterone during the seasonal anestrus in ewes

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### Resumo

The effects of long-acting injectable progesterone (iP4) on an ovulation induction protocol during the seasonal anestrus was evaluated. In experiment 1, 39 ewes were allocated (D0) into three groups: control (n=14), non-treated; iP4 37.5, which received 37.5 mg of iP4 (Sincrogest injetável, Ourofino, Cravinhos, Brazil); and iP4 75, which received 75 mg of iP4. Ten days after iP4 (D10), all animals received an intravaginal device (IVD; 60 mg Medroxyprogesterone Acetate, Fagron, Anápolis, Brazil), which was removed on D21, concomitantly to im administration of 400 IU eCG (Novormon, Zoetis, São Paulo, Brazil) and exposition of ewes to chest-painted rams (marking was monitored every 12 h). Blood samples were collected on D0, 2, 4, 7, 10 and 15 of the hormonal protocol and on day 6 (D6C) and 12 (D12C) of estrous cycle. In experiment 2, 36 ewes were allocated into two groups: control (n=10), which remained with an IVD for 9 days and received 300IU of eCG at IVD removal; and iP4 75 (n=26), which received 75 mg of iP4 im. After 9 days, the iP4 75 ewes were subdivided equally into other two groups: eCG D9 or eCG D10, differing on the day of eCG (300IU) administration. Afterwards, the ewes remained with chest-painted rams to assess estrus (every 12 h), conception and pregnancy rates. P4 concentrations were determined on D11C. Student's T, chi-square or ANOVA tests were used (p<0.05 considered as significant). In experiment 1, iP4 75 had greater P4 concentration on D4 (2.1±0.2 vs. 1.3±0.2 ng/mL) and on D7 (1.0±0.1 vs. 0.75±0.1ng/mL) compared to iP4 37.5. After the conventional IVD+eCG protocol, estrus and pregnancy rates did not differ among groups; however, the iP4 75 had lower conception rate (90.9% for control and 88.9% for iP4 37.5 vs 44.4% for iP4 75). P4 concentrations on DC6 and DC12 were lower in the iP4 75 group (6.5±1.4 and 11.5±1.4 ng/mL) compared to control (10.5 ±1 and 16.9±1 ng/mL) and iP4 37.5 (9.0±1.3 and 14.8±1.3 ng/mL). In experiment 2, estrus, pregnancy and conception rates did not differ between iP4 subgroups, which were regrouped. Estrus and conception rates were lower in iP4 75 (90% and 80% for control and 26.9% and 11.5% for iP4 75, respectively). Eleven days after estrus, P4 concentrations observed in ewes from control, eCG D9 and eCG D10 did not differ (7.0±0.8; 8.5±1.2 and 6.2±0.2 ng/mL, respectively). Regardless of the group, 14 of the 15 ewes had P4 concentration above 1ng/mL, indicating that 93.3% of the ewes ovulated. Therefore, iP4 treatments allowed plasma P4 concentrations greater than 1ng/ml, for at least 4 (35.7mg) and 7 days (75mg). The use of iP4 prior to the conventional IVD+eCG ovulation induction protocol did not increase the response of the animals, while the higher dose impaired reproductive performance. The use of iP4+eCG was not effective for estrus synchronization, even though the vast majority of the ewes ovulated.

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# Follicular wave synchronization with injectable hormones before ovum-pick up in *Bos taurus* cows

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## Resumo

Ovum-pick up (OPU) in *Bos indicus* cows results in greater number of cumulus-oocyte complexes (COCs) by each session compared to *Bos taurus* cows. Therefore, the in vitro embryo production (IVP) systems are more efficient in zebu cattle. Thus, it is necessary to evaluate alternatives to improve IVP in taurine cows. The objective of this study was to evaluate the effect of an injectable hormonal treatment to synchronize the follicular wave of *Bos taurus* donors on progesterone (P4) concentration, number, and quality of COCs recovered by OPU and IVP efficiency. *Bos taurus* cows (Hereford and Aberdeen Angus; n=11) were submitted to four OPU sessions (n=44 procedures) in a crossover design. On day zero (D0) the cows were allocated to control (not synchronized) or treated group, which received 2 mg estradiol benzoate (Agener União, Brazil), 150 µg of D-cloprostenol (PGF; Biogenesis-Bagó, Argentina) and 300 mg of injectable progesterone (iP4; Ourofino, Brazil). OPU sessions were performed on D6. Follicle number at OPU and serum P4 concentration were normally distributed, and the means were compared by t test for unpaired samples (P<0.05). The other dependent variables were not normally distributed, and the medians were compared, between the different factors (treatments or bull), by non-parametric analysis using the Mann-Whitney test (P<0.05). The mean number of aspirated follicles did not differ between groups (P > 0.05; 14.7±1.7 in the control and 14.1±1.6 in the treatment group) nor the percentage of cows with CL on the day of OPU (54% for both groups). There was no significant difference (P > 0.05) on the medians (95% CI) of total structures [12 (6-15) and 10 (9-19), for control and treatment] neither viable structures [5.5 (5-8) and 6 (5-9), for control and treatment]. The cleavage rate/cultured COCs was 58% (50-71%) for control and 65% (50-80%) for treatment, and no significant effect of the bull was observed (P > 0.05). Embryo production rate did not differ neither considering embryo/cultured COCs (28% (21-46%) for control and 33% (22-55%) for treatment) nor embryo/cleaved (50% for both groups; CI 32-75% for control and 36-75% for treated group). Serum progesterone level (ng/mL) was significantly (P < 0.05) lower on D6 in treated (1.1±0.2 ng/mL) than in control cows (2.8±0.8 ng/mL). In conclusion, on the conditions of this study, the hormonal treatment for pre-aspiration synchronization did not improve the evaluated parameters, consequently, its application is not recommended.

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

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# Progesterone treatments before timed artificial insemination in heifers from taurine and synthetic breeds

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## Resumo

Progesterone treatment advance puberty in *Bos indicus* heifers (Lima et al., *Theriogenology*, 154:128-34, 2020). Thus, the aim of the present study was to evaluate the effect of one or two injections of long-acting injectable progesterone (iP4) before TAI in taurine and synthetic heifers. In exp. 1, 462 Aberdeen Angus heifers (14-18 months) maintained on improved native pastures at a farm located in Acéguá-RS were used, with BCS of 2.5 to 3. On D -48 the animals were weighed, the reproductive tract score (RTS) and BCS were evaluated, both on a scale from 1 to 5. The heifers were assigned to one of three groups: 1) control (n=188): no treatment before TAI protocol; 2) 2 iP4 (n=129): two administrations of iP4 (175 mg i.m; Progécio- Agener União, Brazil) on D -48 and D -24 before the beginning of the TAI protocol (D 0); 3) iP4 (n=145): one iP4 administration (175 mg i.m.) on D -24. The TAI protocol consisted of a P4 device (0.5 g; ReptoOne- Biogénesis, Brazil) and estradiol benzoate (2 mg; Bioestrogen- Biogénesis, Brazil) i.m, on D0. On D8, tailheads were painted for estrus detection, the devices were removed and 0.5 mg of estradiol cypionate (CroniCip- Biogénesis, Brazil), 300 IU of eCG (Ecegon- Biogénesis, Brazil), and 150 µg of D-cloprostenol sodium (Croniben-Biogénesis, Brazil) were administered i.m. On D10, TAI was performed, and the heifers that did not show estrus received 10.5 µg of buserelin acetate (Gonaxal- Biogénesis, Brazil) i.m. Data were analyzed by logistic regression considering the effect of group, RTS, BCS and body weight. In exp. 2, 51 Brangus heifers from a farm located in Eldorado do Sul- RS, with BCS between 2.5 and 4, were allocated to: control (n=16), 2 iP4 (n=18) or iP4 (n=17) groups and submitted to a TAI protocol, as described in Exp 1, but without eCG and GnRH. Ovulation rate was evaluated by chi-square test, whereas P4 concentration were compared seven and 14 days after TAI using Student's paired T test. In exp. 1 the mean weight of heifers on D -48 and D 0 was 226±1.9 and 270±2.3 kg, respectively (average daily gain of 0.76±0.02 kg). No difference was observed on pregnancy rates 30 days after TAI [Control=49% (93/188); 2 iP4=46% (59/129); iP4=46% (66/145); P>0.05]. There was no interaction of treatments with RTS, BCS and weight evaluated on D -48 (P>0.05). In exp. 2, the heifers presented 302.1±4.1 kg of live weight on the day of TAI. There was no effect of treatments on P4 concentrations on D7 and D14 [Control= 3.7±0.5 and 9.6±0.9; 2 iP4= 5.5±0.7 and 12.2±1.5; iP4= 4.7±0.8 and 11.3±1.8 ng/mL P>0.05], being observed an effect of day (P≤0.05). Ovulation rate did not differ among groups [Control=56.2% (9/16); 2 iP4=61.1% (11/18); iP4=58.8% (10/17) P>0.05]. It is concluded that iP4 did not affect pregnancy rate, ovulation rate and progesterone synthesis. Future studies will be conducted to evaluate the effect of iP4 under extensive conditions.

## Acknowledgements

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Synchronization protocol for FTAI in *Bos indicus* cows treated with GnRH or estradiol

Guilherme Felipe Ferreira dos Santos<sup>1</sup>, Laís Ângelo de Abreu<sup>1</sup>, Bruna Lima Chechin Catussi<sup>1</sup>, Pietro Sampaio Baruselli<sup>1</sup><sup>1</sup> USP - University of São Paulo (São Paulo, SP, Brazil)**Resumo**

This study aimed evaluate the effects of administration of lecoreline (GnRH) or estradiol benzoate (EB) on D0 for synchronization of follicular wave (SYN) and estradiol cypionate (EC) on D8 or GnRH on D10 for induction of ovulation (IND). Forty-eight suckled multiparous Nelore cows were randomized according to the BCS ( $2.44 \pm 0.12$ ;  $P=0.87$ ), presence of CL on D0 (14.6%;  $P=0.71$ ) and according to the classification of the largest follicle to one of four treatments: 1) EB/EC: 2.0 mg EB (RIC BE®; Agener União, São Paulo, SP-Brazil) on D0 and 1.0 mg EC (Cipiotec®; Agener União) on D8, (n=11); 2) GnRH/EC: 50 µg GnRH (Tec-relin®; Agener União) on D0 and 1.0 mg EC on D8, (n=14); 3) EB/GnRH: 2.0 mg EB on D0 and 25 µg GnRH on D10, (n=11) and 4) GnRH/GnRH- 50 µg GnRH on D0 and 25 µg GnRH on D10, (n=12). All cows received an intravaginal P4 device (PRIMER®; Agener União) on D0, 0,53 mg of PGF (Estron®; Agener União) and 300 IU of eCG (Novormon®; Zoetis, Guarulhos, SP-Brazil) at the time of P4 device removal (D8). Ultrasound was performed from D8 to FTAI (D10; every 24 hours) and from FTAI to ovulation (every 12 hours). The following variables were evaluated: LF diameter at D8 (mm;  $\pm$ SEM), estrus detection rate at D10 (%; n/n), time of estrus (hours;  $\pm$ SEM), presence of CL at D8 (%; n/n), LF diameter at D10 (mm;  $\pm$ SEM), diameter of the ovulatory follicle (mm;  $\pm$ SEM), ovulation rate (%; n/n) and time of ovulation (hours;  $\pm$ SEM). Statistical analyses were performed using GLIMMIX of SAS 9.4. There was no interaction ( $P>0.05$ ) between SYN and IND groups for any of the studied variables. The presence of CL at D8 (18.7%;  $P=0.31$ ) and ovulation rate (76.7%;  $P=0.87$ ) were not significant between the groups. In the SYN group, cows treated with GnRH on D0 showed greater LF diameter on D8 when compared to cows treated with EB ( $10.85 \pm 0.53$  vs.  $8.66 \pm 0.40$ mm;  $P<0.001$ ). Similar effect was observed for ovulatory follicle diameter on D10 (GnRH= $12.82 \pm 0.43$  vs. EB= $11.66 \pm 0.43$ mm;  $P=0.05$ ). In the IND group, the estrus detection rate on D10 was higher ( $P<0.01$ ) in cows that received EC on D8 (68%; 17/25) compared to those that received GnRH on D10 (22%; 5/22). However, cows that received GnRH on D10 expressed estrus later ( $P<0.01$ ) when compared to cows that received EC on D8 (GnRH= $61.2 \pm 2.28$ h vs. EC= $52.7 \pm 1.64$ h). LF diameter on D10 was also greater ( $P=0.01$ ) in cows that received GnRH on D10 ( $12.52 \pm 0.42$ mm) compared to those that received EC on D8 ( $10.88 \pm 0.52$ mm). However, ovulation occurred later ( $P<0.01$ ) in cows that received GnRH on D10 (GnRH= $78.0 \pm 2.46$ h vs. EC= $68.6 \pm 2.07$ h). In conclusion, cows that were synchronized with GnRH presented larger follicle diameter at D8 and larger ovulatory follicle diameter when compared to cows synchronized with EB. Furthermore, treatment with GnRH as an ovulation inducer reduced and delayed the estrous detection rate, increased the diameter of the ovulatory follicle, and delayed the time of ovulation when compared to cows treated with EC.

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# Impacts of postpartum length at initiation of the FTAI protocol on pregnancy rates of Nelore beef cows

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## Resumo

The shortest interval between calving and initiation of FTAI protocols adopted by Brazilian cow-calf systems is 30 days (Vasconcelos et al., *Animal Reproduction* 14:547-57, 2014). This practice is largely based on research that characterized uterine involution and incidence of uterine disorders in *Bos taurus* females, including lactating Holstein cows (Crowe et al., *Animal* 8:40-53, 2014). Recent research in Nelore (*B. indicus*) cows reported limited prevalence of subclinical endometritis as early as 28 days after calving (Oliveira Filho et al., *Animal Reproduction Science* 237:106928, 2022). Hence, we hypothesized that Nelore cows can be assigned to an FTAI protocol prior to 30 days postpartum (DPP) and still experience satisfactory reproductive results. To investigate this theory, this experiment evaluated pregnancy rates in 5,258 Nelore cows ( $n = 1,703$  primiparous and 3,555 multiparous) according to DPP at initiation of the FTAI protocol (Meneghetti et al., *Theriogenology* 72:179-89, 2009). Cow body condition score (BCS; 1-9 scale) was recorded at FTAI, and pregnancy diagnosis performed ~30 days after FTAI. To facilitate statistical analyses, cows were ranked by DPP at initiation of the FTAI protocol and grouped based on 5-day intervals (total of 12 groups; from  $\leq 15$  DPP and 16-20 DPP to  $\geq 76$  DPP). Data were analyzed within parity and with cow as experimental unit, using the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC, USA) and polynomial contrasts to evaluate the impacts of DPP on BCS and pregnancy rates to FTAI (linear, quadratic, or cubic). In both parities, cow BCS at FTAI decreased linearly ( $P \leq 0.01$ ) with the advance of DPP (e.g., 4.79, 4.00, and 3.73 in primiparous, and 4.95, 3.70, and 3.23 in multiparous cows classified as  $\leq 15$  DPP, 36-40 DPP,  $\geq 76$  DPP, respectively). Pregnancy rate to FTAI was affected by DPP quadratically ( $P < 0.01$ ) for both parities. In primiparous cows, pregnancy rate increased until 36-40 DPP (60%) and then decreased with the advance of DPP, whereas cows classified as 21-25 DPP already expressed satisfactory results (42%). In multiparous cows, pregnancy rate increased until 46-50 DPP (71%) and decreased with the advance of DPP, and cows classified as 21-25 DPP also expressed satisfactory results (63%). Collectively, primiparous and multiparous Nelore cows evaluated herein expressed acceptable pregnancy rates when the FTAI protocol was initiated as early as 21 DPP. These results can be associated, at least partially, with hastened uterine recovery after parturition (Oliveira Filho et al., *Animal Reproduction Science* 237:106928, 2022) and adequate BCS levels shortly after calving (Vasconcelos et al., *Animal Reproduction* 14:547-57, 2014). Hence, the interval between calving and initiation of the FTAI protocol can be shortened by 10 days in Nelore females without major detriments to pregnancy rates, which can be of great value for cows that calve immediately prior or during the annual breeding season.

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FTAI/FTET/AI

## PGF2 $\alpha$ at the moment of AI may increase the fertility of suckled Nelore cows with reduced estrus behavior at timed-AI using sex-sorted or conventional semen

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### Resumo

This work aimed to evaluate pregnancy rates (PR) and ovulatory characteristics of Nelore cows receiving PGF2 $\alpha$  at the time of AI in a P4/E2-based timed-AI protocol, and to compare the effects of the PGF at AI in cows inseminated with conventional or sex-sorted semen, with the absence or expression of estrus. In experiment 1, 701 suckled multiparous Nelore cows were submitted to a timed-AI protocol. All cows received 12.5mg (im) of dinoprost tromethamine (Lutalyse®) at D7 and D9 of the protocol. At 48h after P4 device removal (D11), independently of the semen type, AI was performed with conventional or X-sorted semen from two different sires (Bull 1: conventional and sexed semen, Bull 2: conventional and sexed semen; one semen batch for each bull) and estrus behavior was determined (Estrotect® device). Also, at AI, cows received an additional dose of 12.5 mg im of Lutalyse® (PGF treatment) or 2.5 mL im of sterile saline (Control). Logistic regression was used considering  $P < 0.05$  and statistical tendency was considered when  $P > 0.05$  and  $P < 0.10$ . In experiment 2, 29 cows were submitted to the same P4/E2-based protocol of exp.1 and randomly assigned to PGF treatment or Control protocol, but the AI was not performed. Rather, cows had the ovaries scanned at D9, D11, and at 16, 24, 32 and 40h after D11 with B-mode and Doppler ultrasound. Results were analyzed with ANOVA considering  $P < 0.05$ . Overall PR was 37.6% (264/703). No effect of Bull ( $P = 0.83$ ) or AI technician ( $P = 0.20$ ) were detected, whereas BCS ( $P = 0.02$ ), estrus behavior ( $P = 0.01$ ), and type of semen ( $P < 0.001$ ) were factors affecting PR. Conventional semen had a 2.73x greater chance of successful pregnancy than sex-sorted semen. No treatment effect ( $P = 0.67$ ) was detected in cows receiving conventional or sex-sorted semen. However, there was a tendency ( $P = 0.08$ ) for interaction between treatment (PGF or control) and estrus behavior (estrus or no estrus). PGF2 $\alpha$  at AI tended to increase PR of cows that did not display estrus (Control: 21.2%,  $n = 99$ ; PGF: 35.9%,  $n = 92$ ;  $P < 0.10$ ). Then, ANOVA contrast analysis showed that the influence of PGF treatment at AI on PR of cows with reduced estrus behavior was observed for both, conventional (estrus cows with control protocol: 53.2%<sub>a</sub>,  $n = 111$ ; estrus cows with PGF treatment: 51%<sub>a</sub>,  $n = 149$ ; no estrus control: 34.1%<sub>b</sub>,  $n = 41$ ; no estrus PFG: 45.5%<sub>ab</sub>;  $n = 55$ ;  $P < 0.02$ ) or sex-sorted semen (estrus control: 31.2%<sub>a</sub>,  $n = 138$ ; estrus PGF: 27.7%<sub>a</sub>,  $n = 112$ ; no estrus control: 12.1%<sub>b</sub>,  $n = 58$ ; no estrus PFG: 21.6%<sub>ab</sub>;  $n = 37$ ;  $P < 0.02$ ). In exp.2, no significant effects of PGF at D11 were observed in follicular characteristics and/or ovulation performance. It was concluded that fertility of sex-sorted semen was lower than conventional semen, regardless of the PGF2 $\alpha$  treatment. The 12.5 mg treatment of Dinoprost at AI did not accelerate the occurrence of ovulation; however, it seemed to increase the pregnancy success of suckled Nelore cows with reduced estrus behavior, regardless of the semen type.

### Acknowledgements

CAPES.

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)****FTAI/FTET/AI**

# Effects of dose and splitting eCG treatment on timed-AI responses in suckled Nelore cows

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## Resumo

We aimed in this study to test the hypothesis that splitting in two moments or increasing the eCG dose from 300 UI to 400 UI enhances the conception rate in suckled Nelore cows submitted to timed-AI (TAI). Multiparous (n=638; Experiment1) and primiparous (n=1,313; Experiment2) Nelore cows, with a mean BCS of 3 (scale 1 to 5), and between 30- and 60-days postpartum were submitted to a hormonal protocol for TAI. In both experiments, cows received 2mg of estradiol benzoate (i.m., Gonadiol<sup>®</sup>, Zoetis) and an insertion of a P4 intravaginal device (1.9g, CIDR<sup>®</sup>, Zoetis) on the first day of the protocol (Day 0). On Day 7, cows were divided into four groups: 300IU-7/9 group: received 150IU eCG (Novormon<sup>®</sup>, Zoetis) on Day 7 and 150IU on Day 9 (multiparous, n=153; primiparous, n=331); 400IU-7/9 group: received 200IU eCG on Day 7 and 200IU on Day 9 (multiparous, n=163; primiparous, n=331); 300IU-9 group: received a single dose of 300IU eCG on Day 9 (multiparous, n=162; primiparous, n=332) and 400IU-9 group received a single dose of 400IU eCG on Day 9 (multiparous, n=160; primiparous, n=319). On Day 9, the P4 device was removed, and 0.6mg estradiol cypionate (i.m., E.C.P.<sup>®</sup>, Zoetis) and 12.5mg of dinoprost tromethamine (i.m., Lutalyse<sup>®</sup>, Zoetis) were administered in all cows. On Day 11, animals were evaluated by B-mode ultrasonography for determination of dominant follicle (DF) diameter, and TAI and estrous detection were performed. Pregnancy diagnoses were done between days 30 and 40 after TAI. Variables were evaluated by ANOVA using PROC MIXED or logistic regression using PROC GLIMMIX of SAS software. No significant difference (P>0.1) in the DF size at TAI was detected in multiparous; whereas in primiparous, the DF was larger in the 400IU-7/9 group (10.4±0.02mm; P=0.04) compared to others (300IU-7/9: 9.8±0.2mm; 300IU-9: 9.5±0.2; and 400IU-9: 9.5±0.2mm). For conception rates in multiparous cows (300IU-9: 48%; 300IU-7/9: 56%; 400IU-9: 54% and 400IU-7/9: 47%), the main effects of splitting or dose of eCG were not detected, but conception rate tended to be greater in the 300IU-7/9 group than in the 400IU-7/9 group. In primiparous, the conception rate was greater (P=0.02) in cows receiving 400IU (44%) than 300IU (38%), regardless splitting the dose. Furthermore, splitting the dose in two moments tended (P=0.09) to increase the conception rate (43% vs. 40%), regardless the eCG dose. Also, regardless the eCG treatment group, the conception rate was positively associated with DF size at TAI in primiparous cows. In conclusion, splitting or increasing the eCG dose had no noteworthy impact on DF, estrous expression and conception rate in multiparous Nelore cows. In primiparous, splitting or increasing eCG dose to 400IU has a positive impact, but the association of both factors was the only treatment that increased the DF size at TAI and had the greatest improvement on conception rates.



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# Effect of pre-synchronization with 300mg of Sincrogest Injetável® seven days before E2-P4 TAI protocol in dairy cows

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## Resumo

The aim of this study was to evaluate the effect of pre-synchronization with 300mg of long-acting injectable progesterone (P4i; Sincrogest Injetável®) seven days before E2-P4 based protocol on cyclicity rate at the beginning of the TAI protocol, dominant follicle size (DF), estrus, ovulation and conception rate. The experiment included 41 Holstein cows (34 lactating; 7 non-lactating) with BCS=2.72±0.04. D0 was considered the beginning of the TAI protocol. Seven days before beginning of TAI protocol (D-7) the cows were randomly according cyclicity assigned to one of two groups: CTRL (n=19): without treatment [CL\_D-7 = 68,4% (13/19)]; LA-P4i (n=22): pre-synchronization with 300mg of P4i [CL\_D-7 = 63,6% (14/22)]. Ultrasound exams were performed to evaluate the cyclicity (D-7 and D0), DF (D0, D8 and D10), ovulation rate to GnRH treatment (D8) and protocol end (D17). TAI protocol included 2mg of estradiol benzoate (Sincrodiol®), associate to 10.5µg of busereline acetate (GnRH; Sincroforte®) and insertion of a P4 intravaginal device (Sincrogest®) on D0. On D8, all cows received 530µg (PGF-2α; Sincrocio®) and 1mg of estradiol cypionate (SincroCP®) concomitant with P4 device withdrawal. At the same time, cows were painted with chalk on their tailheads, and removal of chalk on D10 was used as an indication of estrus. TAI occurred 48h after P4 device withdrawal (D10). Statistical analyses were performed using Glimmix procedure of SAS (9.4). No differences were found between treatments for DF (CTR=11.9±1.0mm; LA-P4i=12.9±0.9mm; P=0.13) or CL on D0 [CTRL=84.2%(16/19); LA-P4i= 72.7%(16/22); P=0.20]. However, ovulation rate after GnRH treatment evaluated on D8 tended to be greater on cows pre-synchronized with LA-P4 [CTRL=21.1% (4/19); LA-P4i=40.9% (9/22); P=0.07]. The DF did not differ between treatments on D8 (CTRL=12.0±0.9mm; LA-P4= 11.6±0.7mm; P=0.32) or D10 (CTRL=14.2±0.8mm; LA-P4i=14.1±0.6mm; P=0.93). However, cows with CL on D8 presented lower DF on D8 (No\_CL=13.2±0.84mm; CL=10.1±0.56mm; P=0.003), but similar DF on D10 (No\_CL=14.4±0.78mm; CL=13.4±0.53mm; P=0.19). No interaction between treatment and CL was observed for DF on D8 (P=0.44) or D10 (P=0.55). The ovulation rate after protocol tended to be greater on cows pre-synchronized with LA-P4i [CTRL=73.7% (14/19); LA-P4i=90.9%(20/22); P=0.07]. Estrus rate was greater on LA-P4i [CTRL= 36.8% (7/19); LA-P4i=68.2%(15/22); P=0.05]. Conception rate was similar between groups [CTRL=31.6%(6/19); LA-P4i=40.9%(9/22); P=0.38]. In conclusion, dairy cows pre-synchronized with 300mg of long-acting injectable P4 seven days before E2-P4 TAI protocol increase estrus rate, tended to be greater ovulation rate after GnRH treatment and TAI protocol end; however, no difference were found on cyclicity on D0, DF and conception rate.

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FTAI/FTET/AI

# Adjustment of FTAI protocols for *Bos taurus* beef heifers with prostaglandin administered at different times

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The aim was to evaluate the effect of endogenous progesterone (P4) concentration and time of a single use P4 device on the dominant follicle (FD) size, estrus and pregnancy rates in heifers submitted FTAI protocol. Pubertal *Bos taurus* and *Bos taurus* x *Bos indicus* beef heifers (n=540) with a mean BCS of  $3.53 \pm 0.15$  (1=thin and 5=obese) were randomly distributed in 2 experiments in a 2 x 2 factorial design: duration (D) of P4 device insert and level of endogenous P4 low (l or L) and normal (n or N). Experiment 1 groups were formed, ID7: P4 7d (0-7-9) 2PGF (n=70); nD7: P4 7d 1PGF (n=66); ID8: P4 8d (0-8-10) 2PGF (n=72); nD8: P4 8d PGF (n=70). In experiment 2, LD8: P4 8d with four handling (0-6-8-10) 2PGF (n=66); ND8: P4 8d 1PGF (n=64); LD9: P4 9d with four handling (0-7-9-11) 2PGF (n=66); ND9: P4 9d 1PGF (n=66). In the groups of experiment 2, PGF was anticipated, it occurred 48h before the P4 device withdraw. The hormones used were estradiol benzoate (2mg; Gonadiol®) IM and P4 intravaginal device (0.5g; DIB®) at D0. Estradiol cypionate (0.6mg; ECP®), eCG (200 IU; Novormon®) and PGF (25 mg; Lutalyse®) IM. The basis of the tail was painted at P4 withdraw for observation of estrus. The FD was measured at moment of P4 removed and at moment of AI. AI was performed 48h after P4 removal using semen from a single sire. The pregnancy diagnosis was performed 30 d after AI using an ultrasound. Analyses of binomial outcome variables and continuous outcomes were performed using the PROC GLIMMIX of SAS on Demand. The FD (mean± SE mm) at the FTAI in experiment 1 [ID7 ( $14.71 \pm 0.2$ ), nD7 ( $13.49 \pm 0.1$ ), ID8 ( $15.2 \pm 0.3$ ), nD8 ( $14.6 \pm 0.2$ )]. There was no difference in the pregnancy rate among groups (nD7=63.6±5%, ID7=57.5±5%, ND8=55.7±5%, nD8=56.9±5%; P=0.78). However, heifer from ID8 group showed higher proportion of estrus detection (95.8%; P=0.02) at AI than the other groups (nD7=78.7%, nD8=85.7%) but like ID7 (91.4%). In ovulation rate there is no difference among groups (P=0.47). At AI nD7 manifest the smaller FD diameter in comparison with the other treatments (P=0.002). In the experiment 2, the FD were [LD8 ( $14.9 \pm 0.2$ ), ND8 ( $15.6 \pm 0.3$ ); LD9 ( $16.2 \pm 0.2$ ), ND9 ( $14.8 \pm 0.2$ )] (E1: P=0.002, E2: P=0.001) and there was no difference in the pregnancy rate (ND8=54.6±6.2%, LD8=57.6±6.1%, ND9=54.6±6.1%, LD9=50.1±6.2%; P=0.85). However, heifer from LD9 group showed higher proportion of estrus detection (97.5%; P=0.038) and ovulation rate (25.8%; P=0.013) at AI than the other groups [estrus (LD8=89.4%, ND8=81.2%, ND9=78.8%); ovulation rate (LD8=7.6%, ND8=9.4%, ND9=10.6%)]. Also, LD9 induced large FD at P4 withdraw than other treatments. At AI LD9 had large FD than LD8 and ND9, but like ND8. It is concluded that PGF administration at the beginning of the protocol was able to increase DF and estrus rate. There was no difference in conception rate between the different protocols. The exposition of *Bos taurus* beef heifers to 9 days of 0.5g P4 device increase anticipation of ovulation.

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## Effect of estradiol benzoate dose on day 0 (1 vs 2 mg) on the efficiency of FTAI protocol in Nelore heifers

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### Resumo

The objective was evaluate the effect of administration different doses (1 vs 2mg) of estradiol benzoate (EB) on day 0 (D0) of the ovulation synchronization protocol on the estrus detection rate and pregnancy rate of the 1st, 2nd and 3th fixed-time artificial insemination (FTAI) in grazing Nelore heifers (*Bos indicus*). A total of 3,515 heifers were homogeneously divided according age [14mo (n=1,183) and 24mo (n=2,332)], weight (315.0±1.10 Kg; P=0.77), body condition score (BCS=3.15±0.01; P=0.98), cyclicity on day 0 (46.0%; P=0.93) and treatments in two experimental groups: 1EB (received 1mg of EB on D0, n=1,734) and 2EB (received 2 mg of EB on D0, n=1,781). The animals were randomly divided between the two groups and the experiment was conducted on six commercial farms from Mato Grosso, Brazil. The reproductive program consisted of a synchronization protocol for FTAI. Heifers were synchronized with a used progesterone device (P4), 0.53 mg of prostaglandin and 1 (1EB) or 2 (2EB) mg of EB. After seven (n=1,221), or eight (n=1,782) or nine (n=512) days the device was removed, had the basis of the painted tail for reading the manifestation of estrus when inseminated and received 0.5 mg of estradiol cypionate, 200 UI of equine chorionic gonadotropin and 0.53 mg of prostaglandin. FTAI was performed 48 hours after P4 device removal and heifers were inseminated using semen from bulls distributed homogeneously within the groups and identifying with or without estrus. After 30 day diagnosis of pregnancy was performed by ultrasonography and the non-pregnant females were resynchronized. Data were analyzed using the GLIMMIX procedure of SAS (9.4) and the value  $P \leq 0.05$  was considered for effect. The fixed factor was the treatment and the random factors were age, farm, weight, reproductive program and FTAI service. Estrus detection rate and pregnancy rate (P/AI) were defined as binomial variables. There was no interaction treatment and age (P=0.44), farm (P=0.78), reproductive protocol (P=0.40), service FTAI (P=0.76) and weight (P=0.46) for estrus detection. Furthermore, there was no treatment effect for the rate of estrus detection [1EB: 80.5% (1303/1619); 2EB 79.9% (1331/1666); P=0.69]. There was no interaction treatment and age (P=0.43), farm (P=0.38), reproductive protocol (P=0.89) and service FTAI (P=0.83) for P/AI. However, there was a treatment\*weight interaction (P=0.04) for P/AI. The P/AI was higher for heavy heifers treated with 2EB [ $>300\text{kg}$ ; 1EB: 49.3% (352/714)b; 2EB: 54.4% (385/708)a], however no difference in EB treatment was observed in light heifers [ $\leq 300\text{kg}$ ; 1EB: 52.8% (218/413)ab; 2EB: 53.4% (242/453)ab]. The data from the present experiment support that the treatment with 2 mg of EB in heavy heifers ( $>300\text{kg}$ ) increases the pregnancy rate at FTAI in Nelore heifers. However, no differences in EB dose on P/AI was observed in light heifers ( $\leq 300\text{Kg}$ ).

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# Effect of postpartum period on pregnancy rate to AI and late embryonic loss in suckling *Bos taurus* beef cows submitted to FTAI.

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## Resumo

The goal of this study was to evaluate the effect of the interval between calving and the beginning of the Fixed-time AI (FTAI) protocol on pregnancy rates suckling *Bos taurus* beef cows. This study was performed in a farm located in the west of Rio Grande do Sul state. A total of 1,348 beef cows were allocated into 4 groups according to postpartum time at the beginning of the protocol, with less than 30 days postpartum (DPP) (Group 1: n=260); between 30-40 DPP (Group 2: n=319); between 40-50 DPP (Group 3: n=318) and more than 50 DPP (Group 4: n=451). On day 0 (D0) the uterus and ovaries were evaluated by ultrasound, only cows without uterine content were submitted to protocol. The FTAI protocol consisted of the application of 2 mg of estradiol benzoate (Gonadiol, Zoetis®) IM and an intravaginal progesterone device (P4; 1.9g, CIDR, Zoetis®) was inserted. On D9 the P4 device was removed and all cows also received 25 mg of dinoprost tromethamine IM (Lutalyse, Zoetis®), 400 IU of eCG IM (Novormon, Zoetis®) and 1 mg of estradiol cypionate IM (E.C.P, Zoetis®). The AI was performed 48 hours after P4 removal, using two sires of high fertility (Concept Plus, Alta Genetics Brasil). Pregnancy diagnosis (PG) was performed 30 (PG30) and 90 (PG90) days after AI by ultrasound. The data were analyzed using Minitab® 21.1. The presence of CL on D0 was 1.15% (3/260) G1; 5.02% (16/319) G2, 36.79% (117/318) G3 and 37.47% (169/451) G4 (P<0.0001). The estrus rate in groups G1, G2, G3 and G4 was 57.31% (149/260), 79.31% (253/319), 83.33% (265/318), 87.36% (394/451), respectively (P=0.0001). In PG30, the pregnancy rate was 25% (65/260), 46% (147/319), 51% (162/318) and 61% (271/451) for groups G1, G2, G3 and G4, respectively (P=0.0001). At 90 days post insemination, PG90 was performed to confirm pregnancy and to determine pregnancy losses at groups (G1: 24.62% (16/65), G2: 8.16% (12/147), G3: 4.94% (8/162) and G4: 2.58% (7/271); P<0.0001). The results this study suggest that an interval less than 30 days between calving and the beginning of the FTAI protocol leads to a lower pregnancy rate at 30 and 90 days post AI in suckling *Bos taurus* beef cows. However, more studies are needed to better understand the uterine involution and pregnancy loss in cows submitted to FTAI with DPP less 30 days.

**Keywords:** postpartum days, cyclicity, pregnancy

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# Evaluation of antral follicle count, GnRH, and estrus expression in Nelore cows submitted to TAI: preliminary data

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The objective of this work was to evaluate the use of GnRH in *Bos taurus indicus* females with high or low AFC according to the estrus expression in the TAI protocol. Nelore cows (n=140) with approximately 60 days of puerperium were submitted to a conventional program of TAI. The animals were divided into one of four groups: i) high CFA (n = 37); ii) high CFA GnRH (n = 37); iii) low CFA (n = 33); and iv) low CFA GnRH (n = 33). The groups were balanced in terms of ECC, age, and ovarian status (presence of DF and CL). On D0 (initial day of the experiment), a B-mode ultrasound (Sonoscape A5) with 8 MHz linear transducer was used to determine the CFA, classifying the cows into low CFA (5 to 15 follicles, n = 66) or high CFA (26 to 80 follicles, n = 74). The pharmacological protocol used began with 2 mg of estradiol benzoate (Fertilcare Sincronização®, MSD Saúde Animal Ltda, São Paulo, São Paulo, Brazil) and the insertion of an intravaginal device of 0.6 g of progesterone (Fertilcare Implante®, MSD Saúde Animal Ltda, São Paulo, São Paulo, Brazil). On D8, the device was removed, 1.5 mL mg of eCG (Folligon®, MSD Saúde Animal Ltda, São Paulo, São Paulo, Brazil), 2 mL of sodium cloprostenol (Ciosin®, MSD Saúde Animal Ltda, São Paulo) and 1 mL of estradiol cypionate (Fertilcare Ovulação®, MSD Saúde Animal Ltda, São Paulo) were applied. All animals were painted on the tail, and the estrus was assessed on D10: 1) absent; 2) weak and 3) strong. The GnRH groups (high and low CFA) received 2.5 mL of GnRH (Gonaxal®, Biogénesis Bagó Saúde Animal Ltda, Mercês Curitiba, Paraná, Brazil) at the moment of AI. For insemination, only one Veterinarian performed all the procedures, with semen from a single bull and a single batch. The diagnosis of pregnancy was performed by ultrasound at 30 days. Data were analyzed by ANOVA using GLM and Tukey's test. The conception rate was analyzed by binary logistic regression including the main treatment effect and covariates ( $P \leq 0.05$ ). The conception rate ranking was established by a 2x2 aspect ratio test. Of the 140 cows included in the study, the overall conception rate was 70.7% (99/140). Conception rate was influenced by treatment ( $P = 0.008$ ), with GnRH treated groups having higher conception rates than controls: [high CFA\*GnRH 78.4%a (29/37); low CFA\*GnRH 88%a (29/33); high CFA\*control 56.8%b (21/37) and low CFA\*control 60.6%b (20/33)]. Age ( $P = 0.63$ ), ECC ( $P = 0.48$ ) and estrus expression ( $P = 0.68$ ) did not affect the conception rate. We concluded that the use of GnRH on D10 increases the conception rate of cows with high and low CFA.

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# Metabolic imprinting in Nelore calves (*Bos indicus*) treated with creep-feeding during pre-weaning: impacts on performance, reproductive efficiency and metabolome profile

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## Resumo

This experiment compared the outcomes of creep-feeding supplementation on growth, reproductive performance, and serum metabolome profile of young Nelore heifers. A total of 330 females calves were distributed according to BW (113.5±3.2 kg) and age (70.1±3.8 days) and assigned to either one of the treatments: 1) Creep (n=190): heifers had ad libitum access to the nutritional supplement (20% CP and 62.5% TDN; 291.9 ± 49.6 g/day) through a creep-feeder from 70 to 220 days after birth (Day 0); 2) Control (n=140): without any supplement. After weaning, all heifers were randomly allocated to the same pastures until the end of the breeding season. BW was recorded on days 70, 132, 220 (weaning), 360 (cyclicity induction) and 408 (D0 of TAI protocol). On days 220 and 360, blood samples were collected and US for backfat thickness (BFAT) was performed. Heifers were synchronized with a P4/E2-based TAI protocol on day 408 and reproductive status (Cyclicity, uterine and follicle diameter) was verified. Creep-feeding supplementation increased BW on days 132 (Creep: 179.9±1.8 vs. Control: 169.9±2.7kg; P=0.001) and 220 (Creep: 221.9±1.7 vs. Control: 205.5±2.7kg; P=0.0001) and BFAT (Creep: 4.75±0.1 vs. Control: 4.24±0.1mm; P=0.03). However, no differences on days 360 and 408 were observed for BW (243.6±1.7 vs. 240.4±2.7; 303.2±2.4 vs. 301.5±3.9), BFAT or reproductive status (P>0.50) between creep and control, respectively. Nevertheless, the P/AI was 10.4 percentage points greater in creep than control group [46.8% (89/190) vs. 36.4% (51/140); P=0.05]. To understand metabolic imprinting associated with the nutritional treatment, 80 heifers (40 per group) had 84 metabolites evaluated using targeted LC-MS/MS analysis. Biomarker analyses based on receiver operating characteristic (ROC) curves were performed by MetaboAnalyst 5.0. Differences between treatments on day 220 were found (ROC-AUC=0.79; P<0.05). The top significant features were selected for Over Representation Analysis. Pathway analysis revealed creep-feeding had an effect (P<0.03) on the oxidation of branched chain fatty acids (L-carnitine; L-acetylcarnitine and propionylcarnitine), urea cycle (L-alanine, L-aspartic acid and ornithine) and beta oxidation of very long chain fatty acids (L-carnitine; L-acetylcarnitine). Additionally, differences between treatments on day 360 were observed (ROC-AUC=0.88; P<0.05). Pathway analysis revealed creep-feeding had an effect (P<0.02) on the malate-aspartate shuttle [L-glutamic acid (LGA) and malic acid], arginine and proline metabolism [Argininosuccinic acid (AA), LGA, succinic acid and L-arginine], urea cycle (AA, L-arginine, L-aspartic acid and LGA) and aspartate metabolism (AA, LGA and L-Arginine). The creep-feeding improved BW and BFAT at weaning, changing some metabolic pathways. Although same BW, BFAT and reproductive status at TAI protocol, heifers subjected to creep-feeding had greater P/AI. Interestingly, other metabolic pathways were different at that time.

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# Does combining estradiol cypionate and GnRH for ovulation induction in recipient cows increase pregnancy rate after timed embryo transfer?

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## Resumo

Estradiol cypionate (EC) or GnRH have been widely used for ovulation induction in timed embryo transfer (TET). EC administration increases the proportion of cows that show estrus, whereas GnRH promotes more synchronized ovulations. The study aimed to evaluate the effect of the association of EC and GnRH on luteal function and pregnancy rate after TET in taurine cows. In Experiment 1, 12 cyclic non-lactating cows (Jersey and Holstein) received a progesterone (P4) intravaginal device (IVD) (1g, Primer, Agener União, Brazil) and 2mg of estradiol benzoate (EB; Agener União, Brazil) im on D -11. On D -4, 482µg of sodium cloprostenol (Agener União, Brazil) and 300IU of eCG (Ourofino, Brazil) were administered im. On D -2, the IVDs were removed, and half of the cows received 0.6mg EC (Zoetis, Brazil) im (EC+GnRH group), while GnRH group was not treated. On D0, cows received 10µg of buserelin acetate (Ourofino, Brazil) im and the diameter of the preovulatory follicle was assessed by ultrasound. On D6 and D13, the presence of corpus luteum (CL) was confirmed and blood samples were collected to measure P4 levels. In Experiment 2, 184 suckling crossbred cows (predominantly Angus), between 35 and 80 days postpartum and with BCS 3 to 4 (1 to 5 scale) received an IVD (1g P4, GlobalGen, Jaboticabal, Brazil) and 2mg EB (Biogénesis Bagó, Brazil) im on the D-10. On D-2, the IVDs were removed and 150µg of d-cloprostenol (Biogénesis Bagó, Brazil) and 400IU of eCG (Biogénesis Bagó, Brazil) were administered im. On the same day, half of the cows received 0.6mg EC (Cipiotec, Agener União, São Paulo, Brazil) im (EC+GnRH group), while GnRH group was not treated. On D0, all cows received 10µg of buserelin acetate (Gonaxal, Biogénesis Bagó, Curitiba, Brazil). On D7, cows with a CL and absence of reproductive disorders received a fresh or frozen-thawed in vivo produced embryo (grade 1 or 2; morula or early blastocyst). The diagnosis of pregnancy was performed by transrectal ultrasound 56 days after embryo transfer (ET). For statistical analysis, the data were evaluated using paired Student's T test or logistic regression with the SAS package, considering  $p < 0.05$  as significant. In Experiment 1, there was no difference in P4 concentration on Days 6 and 13 after GnRH treatment between the GnRH and EC+GnRH groups ( $p = 0.09$ ). However, cows from EC+GnRH group had greater CL diameter ( $p < 0.05$ ) compared to GnRH group. In Experiment 2, the utilization rate of recipients did not differ ( $p = 0.55$ ) between GnRH (84.8%) and EC+GnRH (81.5%) groups. The pregnancy rate per ET did not differ ( $p = 0.46$ ) between GnRH (62.8%) and EC+GnRH (58.7%) groups and no significant effect of embryo source (fresh or thawed) was observed. Therefore, the association of EC+GnRH does not increase P4 production and pregnancy rate in taurine recipient cows after TET with fresh or frozen-thawed embryos.

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# Influence of equine and human chorionic gonatropins on corpus luteum development and pregnancy rate in bovine embryo recipients

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## Resumo

We aimed to compare the effects of different hCG doses on corpus luteum (CL) development and effects of splitting the eCG dose in two moments during the ovulation synchronization protocol on pregnancy per embryo transfer (P/ET). In Exp1, non-lactating Nelore cows at two days after spontaneous ovulation were assigned into four groups (n=12-13/group): C (no treatment), hCG-500 (im; 500IU hCG; Chorulon, MSD), hCG-1000 (1000IU hCG) and hCG-2000 (2000IU hCG). Luteal area (LA) and blood perfusion (BP) were determined by color-Doppler ultrasound and P4 assayed every 48 hours for 14 days. In Exp2, primiparous crossbred suckled cows (NelorexAngus) were submitted to a P4/E2-based protocol on Day 0 (D0). Cows were divided into four groups (2x2 factorial): control (300IU eCG on D9 [Folligon, MSD]; n=204), eCG/2x (150IU eCG on D7 and D9; n=196), hCG (300IU eCG on D9 and 1000IU hCG on D14; n=200) and eCG/2x+hCG (eCG on D7 and D9 and hCG; n=204). On D9, P4 device was removed, 0.53mg cloprostenol sodium and 1mg E2 cypionate were injected and the dominant follicle (DF) size was evaluated. On D18, cows with CL received a fresh expanded blastocyst, and the LA, BP and plasma P4 concentrations were determined. Data were analyzed by ANOVA, Fisher's exact test or logistic regression of SAS. In Exp1, rate of accessory CL (aCL) differed (P<0.05) among the groups (C, 0% C; hCG-500, 8% BC, 1/12; hCG-1000, 62% A, 8/13; hCG-2000, 42% AB, 5/12). The area of primary CL and the sum of primary + aCLs were greater (P<0.05) in the hCG-1000 and hCG-2000 groups than in the C, whereas the hCG-500 did not differ from the others. For BP, there was no difference (P>0.1). The P4 concentrations were greater (P=0.05) in hCG-2000 than in C and hCG-500 groups, whereas the hCG-1000 group did not differ from the others. In Exp2, DF size (mm) on D9 tended (P=0.07) to be larger in the groups receiving eCG/2x (11.1±0.2 vs. 10.6±0.2). The rate of cows with >1CL on D18 was greater in hCG-treated cows (6.2%, 21/340 vs. 0.3%, 1/337). P4 concentrations on D18 were greater in hCG-treated cows, regardless the eCG treatment. For LA, a greater increase in LA occurred by the association of hCG and eCG/2x treatments compared to its significant isolated effects. For BP, an interaction of eCG and hCG treatments was observed, as indicated by the no effect of hCG in cows receiving a single eCG dose and the reduction of BP by the hCG treatment in eCG/2x-treated cows. P/ET at 30 days post ET was greater in eCG/2x (55%, 90/164) than in control (42%, 73/173), but did not differ in hCG (51%, 87/170) and eCG/2x+hCG (46%, 79/170) groups compared to others. In conclusion, the administration of at least 1000IU hCG increases the LA and P4 concentrations at diestrus but does not enhance luteal BP and P/ET. The eCG administration in two moments during DF growth improves DF, LA and P/ET in primiparous recipient beef cows, but its association with hCG is not indicated as it potentially reduces luteal BP.

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## Increased conception rate in a large-scale FTET program using color-Doppler ultrasonography to assess luteal blood perfusion in beef cattle

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### Resumo

The aim of this study was to evaluate the effect of corpus luteum (CL) blood perfusion and CL area on the conception rates of recipients in a large-scale timed embryo transfer (FTET) program. Multiparous Brangus cows ( $n = 1,465$ ) with 45 days postpartum and body condition scores (BCS) between 2.5 and 4.0 ( $3.0 \pm 0.3$ ) were used in this study. At random days of the estrous cycle (Day 0), the females received a FTET protocol with estradiol benzoate (2 mg, i.m., Sincrodiol®, Ourofino, Cravinhos, Brazil) and an intravaginal P4 device (1 g, Sincrogest®, Ourofino). On Day 8, P4 device was removed, and the animals received i.m. 0.526 mg cloprostenol (Sincrocio®, Ourofino), 1 mg estradiol cypionate (SincroCP®, Ourofino), and 400 IU eCG (Sincro eCG®, Ourofino). On Day 17, each recipient was evaluated by ultrasound (E2V Sonoscape®, Domed, Valinhos, Brazil) in B-mode for CL area ( $\text{cm}^2$ ) or color-Doppler for luteal blood flow score (I/Low - vascularization area  $< 40\%$  of CL; II/Medium - vascularization  $> 45\%$  and  $< 50\%$ ; or III/High - vascularization  $> 50\%$ ). Immediately after the CL evaluation, each recipient received ipsilateral to the CL a single fresh in vitro produced embryo (blastocyst stage) from a commercial laboratory. The pregnancy diagnosis was performed at 60 days. A single technician performed the ultrasound evaluations and embryo transfers. For data analysis, in addition to luteal blood perfusion groups, recipients were retrospectively ranked according to CL area into Small  $< 3\text{cm}^2$  ( $2.63 \pm 0.01$ ), Medium  $> 3$  and  $< 4\text{cm}^2$  ( $3.44 \pm 0.01$ ), or Large  $> 4\text{cm}^2$  ( $4.77 \pm 0.03$ ). Data were analyzed by a logistic regression model, including luteal blood perfusion score, CL area and BCS as continuous variables. In the presence of a significant effect ( $P < 0.05$ ), the rate ranking was established by a 2x2 proportion test between blood perfusion score (I, II or III) and CL area (small, medium or large). The overall conception rate of the study was 44.23% (648/1,465), being influenced by luteal blood perfusion score ( $P < 0.05$ ), but not by CL area ranking ( $P > 0.1$ ). Recipients with CL blood perfusion scores III (48.4%; 134/277) and II (44.6%; 427/958) had a higher conception rate/FTET ( $P = 0.01$ ) than those with blood perfusion score I (37.8%; 87/230). The CL area ranking did not differ ( $P = 0.09$ ) among females with CL Small (46.4%; 147/317), Medium (45.2%; 276/610), and Large (41.8%; 225/538). There was no interaction between luteal blood perfusion score and CL area ranking ( $P = 0.92$ ) and BCS also did not affect the results of this study ( $P = 0.93$ ). Considering the conception rate, we conclude the luteal blood perfusion is more important than the CL area in large scale programs of in vitro embryo transfer.

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# Influence of estrus score at TAI on pregnancy rate of Nelore (*Bos indicus*) heifers, primiparous and multiparous cows

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## Resumo

Studies show that the occurrence of estrus, based on the removal of paint at the base of the tail, is an important indicator associated with larger follicular diameters at TAI (timed AI), greater ovulation rate, higher serum concentrations of P4 during the subsequent luteal phase, and greater P/AI after synchronization protocol (Sá Filho et al., 2011). The aim of this study was to evaluate the influence of the estrus score on the pregnancy rate at TAI in beef heifers and cows. Nelore females (n=6,264) were used in this experiment [1,602 heifers (24 months), 1,022 primiparous and 3,640 multiparous]. Heifers were pre-synchronized 24 days before the beginning of the TAI protocol (D0) only with 150mg of injectable P4 (Sincrogest injetável®, Ourofino, Brazil). On D0, all heifers received 2mg IM EB (Sincrodiol®, Ourofino), 0.53mg IM sodic cloprostenol (PGF2 $\alpha$ ; Sincrocio®, Ourofino) and an intravaginal P4 device (Sincrogest®, Ourofino) was inserted. Primiparous and multiparous received the same treatment, except the treatment with PGF2 $\alpha$  on D0. On D8, the P4 device was removed and 0.5mg (heifers) and 1.0mg (primiparous/multiparous) of EC (SincroCP®, Ourofino), 0.53mg sodic cloprostenol (PGF2 $\alpha$ ; Sincrocio®), 200UI (heifers) or 300IU (primiparous/multiparous) of eCG (Sincroecg®, Ourofino) were administered. All females were painted with chalk on their tailheads and removal of chalk on D10 was used as an indication of estrus. TAI was performed 48h after P4 device removal. Ultrasound assessments were performed at 30 days to assess pregnancy rate. Statistical analyzes were performed using the SAS® PROC GLIMMIX (v. 9.4). The estrus detection was classified in scores [(scores: 1 (presence of chalk, no estrus), 2 (partial chalk, partial estrus) and 3 (total absence of chalk, estrus)]. The distribution of estrus was 22% (score 1), 23% (score 2) and 55% (score 3). No interaction category\*estrus score for P/AI (P=0.65) was observed. The results showed increase in P/AI according to estrus score in heifers [score 1=32%*c* (n=322); score 2=39%*b* (n=444) and score 3=50%*a* (n=836); P=0.001], in primiparous [score 1=50%*c* (n=327); score 2=57%*b* (n=266) and score 3=66%*a* (n=429); P=0.001] and in multiparous [score 1=43%*c* (n=752); score 2=54%*b* (n=711) and score 3=65%*a* (n=2117); P=0.001]. Analyzing all the category, the P/AI increased (P=0.0001) according to the estrus score [score 1=42%*c* (n=1421); score 2=50%*b* (n=3442) and score 3=61%*a* (n=1401)], regardless of the category. It was concluded that as the estrus score increases, the pregnancy rate also increases, regardless of the animal category. This information about estrus occurrence can support the use of strategies that may improve P/IA outcomes in TAI protocols. Examples are the use of GnRH in animals without estrus response, the adjustment of time to AI performance, and the use of semen of bulls with superior genetics in females that showed estrus.

**Keywords:** pregnancy rate, showed estrus, TAI

## Acknowledgments

Ourofino Saúde Animal.

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)****FTAI/FTET/AI****RELATIONSHIP BETWEEN POST-PARTUM PERIOD AND THE BEGINNING OF TAI PROTOCOL ON PREGNANCY RATE AND CALVING INTERVAL IN NELORE COWS**

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**Resumo**

We aimed to evaluate the effect of the postpartum period at the beginning of the TAI protocol on the pregnancy rate (P/AI) at 1st, 2nd FTAI, final P/AI and calving interval of multiparous Nelore cows. A total of 1.435 cows were used, which were synchronized using a based P4/E2 protocol lasting 10 days. Females were classified accordingly days postpartum (DPP) up to D0 of the first TAI protocol in DPP-1: <25 days; DPP-2: 26-30 days; DPP-3: 31-40 days; DPP-4: 41-50 days; DPP-5: 51-65 days and DPP-6: 66-80 days. At 30 days after TAI cows were submitted to ultrasound pregnancy diagnosis (D40) and the non-pregnant females were submitted to the second TAI (D50). Pregnancy diagnosis of the second TAI was performed (D80), following to final pregnancy diagnosis (D120; 1st and 2nd TAI). Statistical analysis was performed using the GLIMMIX procedure from SAS ( $P < 0.05$ ). There was a difference ( $P = 0.0006$ ) in the P/AI at the 1st TAI according to the DPP [DPP-1: 39.8% (37/93)c; DPP-2: 45.8% (93/203)bc; DPP-3: 52.1% (136/261)ab; DPP-4: 60.7% (68/112)a; DPP-5: 62.7% (245/375)a and DPP-6: 54.5% (242/375)a]. There was no difference ( $P = 0.70$ ) in the P/AI at the 2nd TAI according to the DPP [DPP-1: 48.2% (27/56); DPP-2: 54.5% (30/110); DPP-3: 53.6% (67/125); DPP-4: 50.0% (22/44); DPP-5: 47.3% (69/146) and DPP-6: 51.1% (68/133)]. There was a difference ( $P = 0.04$ ) in the final pregnancy diagnosis according to the DPP [DPP-1: 68.8% (64/93)b; DPP-2: 75.4% (153/203)ab; DPP-3: 53.6% (203/261)ab; DPP-4: 80.4% (90/112)ab; DPP-5: 80.3% (314/391)a and DPP-6: 82.7% (310/375)a]. No significant pregnancy losses differences were observed for the 1st TAI ( $P = 0.15$ ; mean of 4.9%), 2nd TAI ( $P = 0.94$ ; mean of 1.9%) and for the overall pregnancy losses ( $P = 0.34$ ; 1st + 2nd TAI; mean of 4.1%). Thus, the final pregnancy rate (1st+2nd TAI) was different ( $P = 0.04$ ) according to the DPP [DPP-1: 67.7% (63/93)b; DPP-2: 70.9% (144/203)ab; DPP-3: 75.5% (197/261)ab; DPP-4: 75.9% (299/391)ab; DPP-5: 76.5% (299/391)ab and DPP-6: 80.0% (300/375)a]. The calving interval (months) from cows that conceived in the 1st TAI was different ( $P = 0.0001$ ) according to the DPP [DPP-1:  $10.6 \pm 0.1$ a; DPP-2:  $10.8 \pm 0.1$ b; DPP-3:  $11.0 \pm 0.1$ c; DPP-4:  $11.3 \pm 0.1$ d; DPP-5:  $11.8 \pm 0.1$ e and DPP-6:  $12.2 \pm 0.1$ f months]. The calving interval from cows that conceived in the 2nd TAI was different ( $P = 0.0001$ ) according to DPP [DPP-1:  $11.9 \pm 0.0$ a; DPP-2:  $12.1 \pm 0.1$ b; DPP-3:  $12.3 \pm 0.1$ c; DPP-4:  $12.7 \pm 0.1$ d; DPP-5:  $13.1 \pm 0.1$ e and DPP-6:  $13.5 \pm 0.1$ f months]. Likewise, the mean of calving interval from 1st and 2nd TAI was different according to DPP [DPP-1:  $11.2 \pm 0.7$ a; DPP-2:  $11.3 \pm 0.6$ b; DPP-3:  $11.4 \pm 0.6$ c; DPP-4:  $11.7 \pm 0.6$ d; DPP-5:  $12.1 \pm 0.6$ d and DPP-6:  $12.5 \pm 0.6$ d months]. In conclusion, cows with DPP <25 days had lower P/AI at 1st TAI and final pregnancy rate. However, no effect was observed at 2nd TAI. Furthermore, our results showed reduction of calving interval according to reduction of DPP to TAI. However, there was no difference in the rates of pregnancy losses according to DPP.

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# Induction of synchronized estrus with human intravaginal tampon impregnated with progesterone associated with lecirelin in Saanen goats in the outbreeding season

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## Resumo

Estrus induction and/or synchronization protocols have a significant role in the success of artificial insemination, allowing to improve reproductive efficiency and distribution of kidding season throughout the year. In this context, there is a growing demand for simpler and less costly methodologies. Thus, this study aimed to verify the efficiency of using a human intravaginal tampon embedded with progesterone (P4) associated with lecirelin in the induction of synchronized estrus in dairy goats. A total of 19 multiparous Saanen goats were submitted to a hormonal protocol which consisted of the insertion of an intravaginal tampon impregnated with P4 (60 mg; Progocio, Agener União, São Paulo) that remained in situ for six days (In ewes: Prates et al., Arq Bras Med Vet Zootec, 71:345-8, 2019). On the fifth day, eCG (200 IU, i.m.; Novormon, Zoetis, São Paulo) and cloprostenol sodium (125 µg, i.m.; Estron, Agener União, São Paulo) were administered. The animals were randomly divided into experimental groups: with the use of lecirelin (25 µg; i.m.; Gestran Plus, União Química, São Paulo) 34 h after the tampon removal (GGnRH; n = 10) or not (Gcontrol; n = 9). The evaluation of ovarian follicular dynamics was performed by transrectal B-mode ultrasonography every 12 h from the tampon removal until ovulation, which was considered as the disappearance of the largest follicle. Blood samples were collected 12 h before the tampon removal for the measurement of P4 values by radioimmunoassay. All animals were inseminated via transcervical route with the technique recommended by EMBRAPA (Fonseca et al., Reprod Biol, 17:363-9, 2017). The data obtained were analyzed for normality using the Lilliefors test, and then the Mann-Whitney test was performed considering  $P < 0.05$ . The F test was also used to compare variances. At the time of tampon removal, all goats maintained P4 concentrations lower than 1.0 ng/mL. The outcomes related to estrus manifestation (57.9% [11/19]), duration of estrus ( $29.3 \pm 13.3$  h), percentage (89.5% [17/19]) and number of ovulations ( $1.6 \pm 0.7$ ), time from tampon removal to ovulation ( $44.5 \pm 9.5$  h), time from estrus to ovulation ( $22.9 \pm 9.4$  h), time from artificial insemination to ovulation ( $2.5 \pm 7.7$  h), diameter of the largest follicle ( $7.7 \pm 0.3$  mm), cervical mucus ( $3.2 \pm 1.0$ ), depth in artificial insemination ( $4.7 \pm 0.9$ ) and P4 values (0.0 ng/ mL) were similar between groups. However, Gcontrol needed more time for estrus manifestation after the removal of the tampon than GGnRH (Gcontrol:  $29.1 \pm 5.4$  h vs GGnRH:  $12.4 \pm 5.9$  h,  $P < 0.05$ ). In conclusion, the use of human intravaginal tampon with 60 mg of P4 was not effective in maintaining P4 values above 1.0 ng/mL until the time of its withdrawal. Furthermore, the addition of lecirelin to the hormonal protocol did not benefit the studied indices.

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# Treatment with GnRH at the time of AI improves pregnancy rate regardless estrous expression in 7-day P4 device protocols in Nelore but not in crossbred heifers

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The experiment evaluated the administration of GnRH at the time of AI in heifers that expressed or not estrous in a 7 days P4 device protocol on P/AI and pregnancy loss. A total of 2,087 heifers, aged 14 to 22 months, were used. The study was performed in two independent steps according to breed [Nelore (n= 1,124) and Nelore x Aberdeen Angus (n= 963)]. On D0, heifers received a P4 intravaginal device (Sincrogest®, Ourofino), 1 mg of estradiol benzoate (EB; Sincrodiol®, Ourofino) and 0.53 mg of sodium cloprostenol (PGF-2-α; Sincrocio®, Ourofino), i.m. After seven days (D7), in addition to removing the P4 device, 0.5 mg of estradiol cypionate (EC; SincroCP®, Ourofino), 0.53 mg of PGF-2-α (Sincrocio®) and 200 UI of eCG (SincroCG®, Ourofino) were administered. At the same time, heifers were painted with chalk on their tailheads, and removal of chalk on D9 was used as an indication of estrus. TAI was performed 48 h later (D9). At this time, heifers were randomly allocated to receive or not treatment with 10.5 µg of buserelin acetate (GnRH; Sincroforte®, Ourofino) in a 2 x 2 factorial arrangement: G1) with estrous-without GnRH [Nelore (n= 359); F1 (n= 298)]; G2) with estrous-with GnRH [Nelore (n= 357); F1 (n= 306)]; G3) without estrous-without GnRH [Nelore (n= 201); F1 (n= 182)]; G4) without estrous-with GnRH [Nelore (n= 207); F1 (n= 177)]. The pregnancy diagnosis was performed by ultrasound at D40 and at the end of the breeding season. Statistical analyses were performed using GLIMMIX of SAS® 9.4. In Nelore heifers (Experiment 1), there was no interaction between estrous\*treatment for P/AI (P= 0.89) or pregnancy loss (P= 0.18). There was an estrous effect (P= 0.01), in which heifers that demonstrated estrous had a higher P/AI when compared to those without estrous [47.2% (338/716) vs. 42.2% (172/408)]. Furthermore, treatment with GnRH increased (P= 0.04) the pregnancy rate, regardless of whether or not heifers demonstrated estrous [49.0% (277/565) vs. 41.8% (234/559)]. Pregnancy loss did not differ between treatments (P= 0.20) or estrous (P= 0.14). In crossbred heifers (F1 Nelore x Angus), there was no interaction between estrous\*treatment for P/AI (P= 0.44) and pregnancy loss (P= 0.23). There was no GnRH treatment effect for P/AI [G1) 59.7% (178/298); G2) 61.4% (188/306); G3) 47.8% (87/182); G4) 49.2% (87/177); P=0.32]. However, there was an estrous effect (P<0.01), where heifers that showed estrous had a higher P/AI when compared to those that did not show estrous [60.6% (366/604) vs. 48.5% (174/359)]. Pregnancy loss did not differ between treatment (P= 0.26) or estrous (P= 0.47). It is concluded that the administration of GnRH in Nelore heifers increased the pregnancy rate, regardless of the estrous behavior. In crossbred heifers, treatment with GnRH did not increase the pregnancy rate.

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# Fixed time artificial insemination (FTAI) in synchronous estrus induced dairy goats during non-breeding season: Preliminary results

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## Resumo

Fixed-time artificial insemination (FTAI) has been recommended in function of practical advantages such as its previous programming and for removing the handling of estrus behavior monitoring. FTAI is based on predictable average estrus onset and ovulation after a given estrous control protocol, allowing great number of goats being inseminated in closer time (Fonseca et al., *Reproductive Biology*, 17:268-273, 2017). For field applied implications, FTAI should consider management practices such as milking time and, necessarily, be adjusted for being performed in the morning (after) or in afternoon (before). The present study evaluated the efficiency of similar protocols of synchronous estrus induction for supporting FTAI done after (morning) or before (afternoon) milking time in acyclic multiparous dairy goats managed in tropical conditions. All animals received intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Progespon®; Zoetis, São Paulo, Brazil) for six days plus 37.5 µg of d-cloprostenol (Sincrocio®, Ouro Fino, Cravinhos, São Paulo, Brazil) and 200 IU of eCG (Novormon 5000®; Zoetis, São Paulo, Brazil) i.m. 24 hours before sponge removal. In T1 (n=13) all protocol procedures were performed 06:00 to 07:00 h and FTAI were done 54 h after sponge removal in the early afternoon. In T2 (n=12) all protocol procedures were performed 17:00 to 18:00 h p.m. and FTAI were done 62 h after sponge removal in the late morning. The following parameter were recorded: estrous response (%); interval to estrous onset (h); cervical mucus aspect (Fonseca et al., *Reproductive Biology*, 17:363-369, 2017) at FTAI time; intrauterine insemination (%); and conception rate. Quantitative data (mean±S.E.M.) were analyzed by one-way ANOVA, while qualitative data (%) were evaluated by Fisher exact test, both of then using 5% minimum level of significance. Estrous response (53.8 vs. 75.0%), interval to estrus onset (37.3 ± 4.1 vs. 37.3 ± 3.7 h), interval from estrus onset to FTAI (17.3 ± 4.1 vs. 25.5 ± 3.7 h) and conception rate (38.5 vs 58.3%) were similar (P>0.05) for both treatments. Cervical mucus varied from crystalline-striated (T2=8.3%), striated (T1=46.2 and T2=33.3%) and striated-caseous (T1=53.8 and T2=58.3%) (P>0.05). Intrauterine AI was performed 100.0% of goats. Preliminary results showed adequate cervical mucus for AI with frozen-thawed semen implicated in conception rates varying from 40 to 60% after FTAI in synchronous estrus induced acyclic dairy goats, encouraging the continuation of the study for searching the best protocol for FTAI in dairy goats in relation to daily management conditions.

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# Identification of factors that interfere on the pregnancy losses on suckled crossbred beef cows in the Paraguayan Chaco

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## Resumo

The aim of the present study was to identify factors associated with pregnancy loss in beef cows. The study was carried in 3 stations within a commercial cow-calf operation in Boquerón, Paraguay. A total of 1564 crossbred pregnant cows (*Bos taurus* x *Bos indicus*) were included in this study. Inclusion criteria were pregnant cows from the breeding season from September 2020 to March 2021 that were submitted to one fixed-time artificial insemination (FTAI) and natural service. The cows received a protocol based on estradiol, progesterone and eCG. Ten days after the FTAI, 4% (1:25) of clean-up bulls were introduced to the herd. All cows were evaluated by US (Aloka® SSD-500) 30 days after the FTAI to confirm the pregnancy. In order to measure the pregnancy of clean-up bulls and the pregnancy losses, a total of 7 US studies were performed with an interval of 30 days. Survival analyses were performed using the LIFETEST of SAS 9.4. Factors included for the analysis were: parity [multiparous (MUL), secundiparous (SEC) and primiparous (PRI)], station within the farm [Norte (NOR), Samu'u (SAM), Sur (SUR)], breeding method [FTAI (IA) and natural service (NS)] and conception month [September to October (SEP\_OCT), November to December (NOV\_DEC), January to February (JAN\_FEB) and March to April (MAR\_APR)]. Regarding the pregnant cows from the FTAI, sire [Hereadero (HER), Fuego (FUE) and CEO (CEO)], estrus expression [Yes (YES) and No (NO)], BCS at the FTAI and weight at the FTAI were considered for the analysis. Factors that influenced the pregnancy loss were parity [MUL=8.35% (99/1186), SEC=7.67% (22/287), PRI=3.3% (3/91); P=0.04], the station within the farm [NOR=7.78% (94/1208), SAM=6.55% (11/168), SUR=10.11% (19/188); P<0.001], the breeding method [AI=9.32% (59/633), NM=6.98% (65/931), P<0.001] and conception month [SEP\_OCT=11% (45/409), NOV\_DEC=8.04% (43/535), JAN\_FEB=6.2% (31/500), MAR\_APR=4.17% (5/120); P<0.001]. On the 633 cows pregnant from FTAI, the sire [HER=9.64% (51/529), FUE=8.11% (6/74), CEO=6.67% (2/30); P=0.58], BCS (P=0.17) and weight (P=0.70) at FTAI did not have an effect on the overall pregnancy losses of these cows, but estrus expression at the time of the FTAI influenced in the pregnancy losses [YES=7.43% (28/377), NO=12.11% (31/256); P=0.02]. This study demonstrated that parity, station within the farm, breeding method, conception time and estrus expression at the time of the FTAI are significantly associated with pregnancy losses in a commercial farm operation in Boquerón, Paraguay.

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)****FTAI/FTET/AI****Impact of 17 $\beta$ -estradiol (E2) addition at the moment of timed-AI (TAI) in Nelore cows**

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**Resumo**

We aimed in this study to evaluate the effects of E2 treatment at TAI moment on uterine gene expression, estrous expression rate (EER) and pregnancy rate (P/TAI) in Nelore cows with a small dominant follicle (DF) or not showing estrus. In Exp1 and Exp2, non-lactating cows were submitted to a P4/E2-based protocol starting at early diestrus (D0). On D7, 12.5mg dinoprost tromethamine and 1mg E2 cypionate were injected, and on D9 cows with DF<11.5mm were assigned into different groups. In Exp1 (n=16/group): Control (C, no treatment), 2-E2 (im, 2mg E2) and 4-E2 (im, 4mg E2); in Exp2: C (no treatment; n=12); E2 (2mg E2, n=14); GnRH (0.1mg gonadorelin acetate, n=13); and E2+GnRH (association of GnRH and E2, n=13). In experiments, between D9-D11, cows were submitted to transrectal ultrasonography every 12 (Exp1) or 6 (Exp2) hours (h) for determination of ovulation, endometrial thickness (ET) and for EER. In Exp1, a uterine cytological sample was collected 4h after treatment to evaluate the transcript expression of receptors for E2 (ESR1 and ESR2), oxytocin (OXTR) and P4 (PGR). In Exp3, 3,829 suckled cows, BCS of 2.9 (scale 1 to 5), were submitted to a TAI. On D0, cows received a P4-device (0.6g) and 2mg E2 benzoate. On D9, devices were removed, received 300IU eCG, 0.39mg sodium cloprostenol, 1mg E2 cypionate and marker stick at the base of the tail for EER. On D11, DF diameter was determined, TAI was performed and cows that without manifestation of estrus received 0.1mg gonadorelin acetate and were allocated into 2 groups: GnRH (n=368) and E2+GnRH (2mg E2; n=363). In Exp1, data were separated by orthogonal contrasts (C1; C vs. E2; and C2: 2-E2 vs. 4-E2) and Exp2 in a 2x2 factorial. Variables were evaluated by ANOVA or logistic regression using SAS software. In Exp1, ET reduced (P<0.05) 12h after treatment in E2-treated cows. The E2-treated cows had greater (P<0.05) transcript abundance for OXTR and lesser for ESR1 and ESR2. For C2 contrast, no significant difference was observed (P>0.1). In Exp2, the EER did not differ (P>0.1), but for the interval from treatment to ovulation (h), a significant interaction (P<0.05) reflected no E2 effect in cows treated with GnRH, whereas the interval was longer for E2 group (40 $\pm$ 1.6) than others (C: 31 $\pm$ 2; GnRH: 28 $\pm$ 0.9; E2+GnRH: 29 $\pm$ 0.1). The ovulation rate was greater (P<0.05) in the GnRH (100%) than in E2 (64%), but did not differ (P>0.1) in the C (83%) and E2+GnRH (84%) compared to others. In Exp3, the P/TAI was 55% for cows in estrus. For those without estrous signals, a greater (P<0.05) P/TAI was observed in the GnRH (34%) than in the E2+GnRH (31%). Regardless the E2 treatment, cows with a DF $\geq$ 11mm (n=192) had a greater (P<0.05) P/TAI (49%) than those with DF<11mm (n=377; 29%). In conclusion, E2 administration in the moment of TAI modulates expression of uterine receptors, but retards ovulation and decreases P/TAI when associated to GnRH treatment in suckled Nelore cows with a small DF or not showing estrus at TAI.



**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Impacts on pregnancy outcomes of anticipating resynchronization of ovulation by early detection of non-pregnant dairy cows

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## Resumo

We tested in dairy lactating cows the hypotheses: 1) that starting resynchronization (Resynch) of ovulation 17 d after TAI (Super-Early) improves the reproductive efficiency compared to the Conventional Resynch starting at 31 d after TAI; and 2) the treatment with 1mg estradiol benzoate (EB) at beginning of Super-Early Resynch does not impact on the pre-existing pregnancy and reduces false positive (FP) rate of Doppler-based pregnancy diagnosis (Doppler-US). Dairy cows from two commercial farms, with a body condition score between 2 and 4 (scale: 1-5), and after 52 days of voluntary wait period, were submitted to an EB/P4-based protocol for the first TAI post-partum (D0). On D17, cows were assigned to three experimental groups (n=150/group): Control (C); Super-Early Resynch (SE) and Super-Early Resynch + 1mg EB (SE+EB). On D31, non-pregnant (NP) cows of C group were submitted to the same hormonal protocol done in the first TAI and the second TAI on D42. In both Super-Early Resynch groups, all cows received on D17 a P4 intravaginal device (CIDR, Zoetis, Brazil) associated (SE+EB) or not (SE) to 1mg EB (i.m., Gonadiol, Zoetis). On D24, cows were evaluated by Doppler-US to determine luteal blood perfusion and those where luteolysis was detected were considered NP and received 25mg tromethamine dinoprost (PGF; i.m., Lutalyse, Zoetis). On D26, the P4 devices were removed and was administered 1mg estradiol cypionate (i.m., ECP, Zoetis) and second PGF dose in NP cows. On D28 a second TAI was performed. Both type of Resynch (Conventional or Super-Early) were repeated until D84, which allowed a maximal of 3 TAIs in the C group and 4 TAIs in the SE and SE+EB groups. Confirmatory pregnancy diagnoses were performed on days 31 and 66 after each TAI. Data were evaluated by ANOVA, Fisher's exact test or logistic regression of SAS. The pregnancy rate (PR) at first TAI did not differ ( $P>0.1$ ) among the C (44%), SE (47%) and SE+EB (47%) groups. Follicular diameter (mm) at resynchronized TAI was larger ( $P<0.05$ ) in the SE and SE+EB groups ( $17.5\pm 0.2$ ) than in C group ( $15.7\pm 0.2$ ). Overall PR at second, third and fourth TAIs did not differ ( $P>0.1$ ) among the C (30% [42/142]), SE (30% [44/145]) and SE+EB (30% [48/159]) groups; however, cumulative PR within the 84 days of experimental period was greater ( $P=0.05$ ) in cows submitted to Super-Early Resynch (79% [238/300]) than in C group (72% [108/150]). Furthermore, rate of FP for pregnancy results between D24 and D31 was lesser ( $P<0.05$ ) in the SE+EB group (18% [23/128]) than in SE (30% [48/160]). In conclusion, the Super-Early Resynch increases the PR during the first 84 days after voluntary wait period in dairy cows. The use of 1mg EB associated with a P4 device on D17 after TAI is preferable to increase the effectiveness of the Super-Early Resynch protocol, as it is not harmful to the previous pregnancy, and reduces proportion of FP results by Doppler-US at D24.

## Acknowledgments

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Splitting the eCG dose during synchronization of ovulation for TAI in suckled beef cows

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## Resumo

We aimed in this study to test the hypothesis that the splitting the administration of eCG in two moments (two days before the removal of the P4 device [Day 7] and on the day of removal [Day 9]) increases the conception rate in suckled Nelore cows submitted to timed-AI (TAI). Multiparous (n=800; Experiment 1) and primiparous (n=934; Experiment 2) Nelore cows, with a mean of 38 days postpartum and BCS of 3 (scale:1 to 5) were submitted to a hormonal protocol for TAI. On the first day of the protocol (Day 0), cows received 2mg of estradiol benzoate (i.m., Gonadiol, Zoetis) and an insertion of a P4 intravaginal device (0.5g, DIB, Zoetis). On Day 7, 12.5mg of dinoprost tromethamine (i.m., Lutalyse, Zoetis) was administered, and cows were randomly divided into 2 groups: eCG-7/9 group: received 150IU eCG (Novormon, Zoetis) on Day 7 and 150IU on Day 9 (multiparous, n=404; primiparous, n=470) and eCG-9 group: received a single dose of 300IU eCG on Day 9 (multiparous, n=396; primiparous, n=464). On Day 9, the P4 device was removed and 0.6mg estradiol cypionate (i.m., E.C.P., Zoetis) was administered in all cows. A marker stick at the insertion of the animal's tail was used for estrous detection. A subgroup of animals (n=111-127/group) were evaluated by B-mode ultrasonography on Days 7, 9 and 11 for determination of dominant follicle diameter. TAI and estrous detection were performed on Day 11. Pregnancy diagnoses were determined between days 40 and 50, and between days 90 and 130 after TAI. Variables were evaluated by ANOVA using PROC MIXED or logistic regression using PROC GLIMMIX of SAS software. No significant difference (P>0.1) in the dominant follicle size on Days 7, 9 and 11 was detected between groups in multiparous; whereas in primiparous, the dominant follicle was larger (P=0.02) on Day 9 in the eCG-7/9 group (10.2±0.02 mm vs. 9.4±0.02 mm). No significant difference (P>0.1) in the rate of estrous detection was detected between groups in multiparous or primiparous. In multiparous cows, the conception rate observed at the first and second pregnancy diagnoses did not differ (P>0.1) between the eCG-7/9 group (59% and 55%) and eCG-9 group (57% and 54%). In primiparous, a greater conception rate was observed at the first (P=0.02) and second (P<0.01) pregnancy diagnoses in the eCG-7/9 group (63% and 60%) than in the eCG-9 group (59% and 55%). No difference (P>0.1) in the rate of pregnancy loss was observed between groups in multiparous (5% vs. 6%); whereas in primiparous, a lesser (P<0.01) rate was observed in the eCG-7/9 (4%) than in the eCG-D9 (7%). In conclusion, splitting the dose of 300IU eCG had no impact on follicle growth, estrous expression or conception rate in multiparous; but it is an interesting alternative to increase the size of dominant follicle at the P4 device removal as well as pregnancy success in primiparous Nelore cows submitted to TAI.

## Acknowledgments

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

**Effect of injectable progesterone (Sincrogest injetável® or Progecio®) on puberty induction in Nelore heifers**Clodo Aldo Rodriguez Machuca <sup>1</sup>; Villalba, M. M.R. <sup>1</sup>; Ibarra, J.J.M. <sup>2</sup>; Ortiz, T. J. <sup>2</sup><sup>1</sup> RG- Reprogen Reproducción y genética (4TO CANAL COTOCA SANTA CRUZ BOLIVIA),<sup>2</sup> Facultad de Ciencias Veterinarias UAGRM SCZ-BOLIVIA**Resumo**

The aim of this study was to compare the effect of pre-synchronization (Pre-Synch) with two distinct commercial injectable progesterone (P4i) thirty days before E2-P4 based protocol on cyclicity rate at the beginning of TAI protocol (puberty induction) and conception rate. Non-cycling Nelore heifers (n = 606; anestrus) from two commercial herds localized in Santa Cruz / Bolivia, with 22 months, 3.0 BCS (1-5 scale) and 350.9 ± 35.2 kg were randomly assigned to one of two groups: 1) Sincrogest Injectable : heifers were treated with 150mg of Sincrogest injetável® (i.m.; Ourofino Saúde Animal, Brazil; n=304); 2) Progecio: Heifer were treated with 140mg of Progecio® (i.m.; Agener União Saúde Animal, Brazil; n=301). D-30 was considered the beginning of the Pre-Synch program. On D-30, ultrasound examination (SonoScape S6V, Shenzhen, China) was carried out to identify the cyclic and non-cyclic heifers, based on the presence of corpus luteum (CL). Cyclic heifers on D-30 evaluation were excluded of the experiment. On D-20 (10 days after performing the pre-synchronization), all heifers were treated with 0.5 mg of EC [(Estradiol Cypionate); Cipiotec®, Agener União, Brazil]. On D0, TAI protocol has been started, and all heifers received an intravaginal P4 device [(0.5g of progesterone); Primer® Monodose, Agener União, Brazil] and 2 mg of EB [(Estradiol Benzoate); RIC-BE®, Agener União). On D8, 1 mg of EC, 530mg of sodic cloprostenol [(PGF-2α); Estron®, Agener União, Brazil] and 250IU of eCG (Novormon®, Zoetis, São Paulo, Brazil) concomitant to P4 device withdrawal. FTAI was performed 48h after P4 device withdrawal and at the same time all animal received 10µg of busserelin acetate [(GnRH), Gestar®, Over, Santa Fe, Argentina)]. Ultrasonography was performed on D-30 and D0 for cyclicity evaluation. Pregnancy diagnosis was evaluated 30 days after of artificial insemination. Statistical analyses were performed using Chi-Square Test Calculator (Software Social Science Statistics). The overall cyclicity rate was 45.2% (274/606). Sincrogest injectable group heifers showed higher cyclicity rate at beginning of TAI protocol [Sincrogest injectable=50.1% (155/304); Progecio=39.4% (119/302); P=0.004]. The overall pregnancy rate was 52.3% (292/558). Similar pregnancy rate between groups was observed 30 days after TAI [Sincrogest=51,6% (145/281); Progecio=53.1% (147/277); P=0.73]. In conclusion, the pre-synchronization with 150mg of Sincrogest injetável® thirty days before E2-P4 based TAI protocol demonstrated higher cyclicity rate, however similar fertility in Non-cycling Nelore heifers.

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Pre-synchronization before E2-P4 based protocol: fertility of high production Holstein cows previously exposed to long-acting injectable progesterone or intravaginal progesterone device

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## Resumo

The hypothesis was that different pre-synchronization programs including exposure to progesterone before the TAI protocol do not differ on fertility outcome. The aim of this study was to evaluate the fertility of high producing dairy cows submitted to two different programs of pre-synch before E2-P4 based protocol. Holstein cows (n=330) with daily production of 39.7±9.6Kg and 47.7±2.1 DIM were enrolled at 1<sup>st</sup> post-partum AI. D0 was considered the beginning of the TAI protocol. Fourteen days before the beginning of TAI protocol (D-14), the cows were randomly assigned to one of two groups: PreS-14d-P4D (n=166): pre-synch with 2mg of estradiol benzoate (Sincrodiol®) and P4 intravaginal device (Sincrogest®) of third use fourteen days before TAI protocol. On D-7 all devices were withdrawal and 1mg of estradiol cypionate (SincroCP®) and 530µg PGF-2α (Sincrocio®) were administered; PreS-7d-LAP4 (n=164): pre-synch with 300mg of P4i (Sincrogest Injetável®) performed seven days before TAI protocol. On D0, animals received an intravaginal P4 device (1g; Sincrogest®), followed by administration of 2mg of estradiol benzoate (Sincrodiol®) and 10.5µg of busereline acetate (GnRH; Sincroforte®); on day 7, animals received 530µg PGF-2α (Sincrocio®); on day 8 the P4 device was removed, 1mg of estradiol cypionate (SincroCP®) and 530µg PGF-2α (Sincrocio®) were given. TAI occurred 48h after P4 device withdrawal on D10. Ultrasonography was performed on D40 and D70 for pregnancy diagnosis and pregnancy losses (PL). Statistical analyses were performed using *Glimmix* procedure of SAS (v9.4). The overall pregnancy rate was 48,2% (159/330) on D40 and 37,2% (86/231) on D70. Overall PL was 6.67% (13/195). Similar P/AI was observed between groups on D40 [PreS-14d-P4D=45.8% (76/166); PreS-7d-LAP4=50.6% (83/164); P=0.41] and on D70 [PreS-14d-P4D=33.0% (37/112); PreS-7d-LAP4=41.2% (49/119); P=0.30]. Furthermore, similar PL were observed between D40 and D70 [PreS-14d-P4D=7.29% (7/96); PreS-7d-LAP4=6.06% (6/99); P=0.77]. In conclusion, both pre-synch programs were similar regarding reproductive efficiency, supporting the use of the pre-synch with 300mg of long-acting injectable in dairy farms as an alternative to pre-synchronization, simplifying the farm's management and the reproductive schedule. Further studies need to be carried out using a larger number of cows to confirm the lack of difference is accurate and repeatable.

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Ourofino Animal Health.

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Impact of early weaning at 150 days on the reproductive performance of Nelore cows and weaned female calves

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## Resumo

We aimed to evaluate the effects of early weaning on the reproductive performance of Nelore weaned calves and cows in the next breeding season (BS). Suckled cows that became pregnant by timed-AI (TAI) in the 2020 BS were submitted in 2021 to an early weaning (EW) at 150 days (27 primiparous [PRIMI] and 74 multiparous [MULTI]) or conventional weaning (CW) at 240 days post-partum (30 PRIMI and 77 MULTI). Body weight and condition score (BCS; 1-9) were determined at five moments: at 2020 BS, at 150 and 240 days post-partum, at pre-partum and at 2021 BS. Female calves weaned at EW (16 from PRIMI and 31 from MULTI) or CW (16 from PRIMI and 31 from MULTI) were evaluated. All calves received creep-feeding from 90 days of age until weaning. At 12 months (mo) of age, females' calves were evaluated for reproductive tract score (RTS) and body weight every 28±4 d. At 13 mo, heifers were allocated in a feedlot pen for 113 d. During the 2021 BS, dams and heifers (during feedlot period) were submitted to a P4/E2-based protocol (D-10) for TAI at Day 0 (D0), and a second TAI was performed at D22 in females detected with luteolysis by Doppler ultrasound. Presence of corpus luteum (CL) on D-10, and estrous expression and dominant follicle (DF) diameter and blood perfusion (BP) on D-2 and D0 were determined. Data were analyzed by ANOVA or logistic regression (SAS). An interaction of parity order and treatment was not observed ( $P>0.1$ ), but the weight (kg) and BCS was greater ( $P<0.05$ ) in MULTI cows at the five moments, and in dams from the EW than in the CW at 240 days post-partum (541 vs. 493; and 5.3 vs. 4.3), pre-partum (551 vs. 506; and 5.2 vs. 4.4) and 2021 BS (475 vs. 450; and 4.5 vs. 3.7). The proportion of cows with CL at D-10 was not affected ( $P>0.1$ ) by weaning but was greater ( $P<0.05$ ) in MULTI than PRIMI cows (40.4% vs. 15.7%). DF diameter and proportion of BP on D0 was greater ( $P<0.05$ ) in cows from EW group than CW group. The pregnancy rate (P/AI) at first TAI was greater ( $P<0.05$ ) in cows from EW group (60% vs. 45%), whereas no difference ( $P>0.1$ ) was observed at second TAI. Cumulative P/AI (two TAIs) were greater ( $P<0.05$ ) in cows from EW group (81% vs. 63%). For calves, the weaning did not affect ( $P>0.1$ ) BCS at TAI, but heifers from EW were lighter ( $P<0.05$ ) than CW at 13 mo (281±3.2 vs. 299±3.1kg) and 16 mo (354±5.1 vs. 372±4.2kg) of age (start of TAI protocol). Puberty, endometrium tonus and thickness and RTS at 16 mo did not differ ( $P>0.1$ ) between EW and CW groups. The DF diameter on D-2, and on D0 both DF size and BP were greater ( $P<0.05$ ) for heifers in the CW group than EW group, but P/AI at first and second TAI did not differ ( $P>0.1$ ) between groups. In conclusion, the weaning at 150 days in Nelore cattle is an interesting strategy to recover dam's body condition and to improve its pregnancy success in the next BS, without affecting the reproductive performance of female weaned calves.

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Use of PGF at the beginning of the protocol does not change the conception rate in *Bos taurus* multiparous beef cows

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The aim of the study was evaluating the dominant follicle size and rates of estrus expression and pregnancy in primiparous beef cows submitted to 7 days or 8 days FTAI protocol using a P4 device (0.5g). A total of 284 suckling *Bos taurus* and crossbred (*Bos taurus* x *Bos indicus*) multiparous beef cows with a mean body condition score (BCS) of  $2.82 \pm 0.15$  (1=lean and 5=obese). Females were distributed in experiment used a 2 x 2 factorial arrangement of four treatments. The two factors were: 1) time of exposure to progesterone (P4) (7 and 8 days); 2) prostaglandin treatment: one PGF (PGF at removal P4) and two PGF (PGF at D0 and moment of P4 removal). Thus, the treatments were PGFd0d7 (n=70), PGFd7 (n=70), PGFd0d8 (n=72) and PGFd8 (n=72). The hormones used were all from the same company (Zoetis) - estradiol benzoate (2mg) i.m. (Gonadiol®), P4 intravaginal device (DIB® 0.5mg), estradiol cypionate (0.6mg) i.m. (ECP®), equine chorionic gonadotropin (300 IU) i.m. (Novormon®) and PGF (25 mg Dinoprost Tromethamine) i.m. (Lutalyse®). at P4 device withdrawal (d7 or d8), at moment the tail was painted to estrus identification. The follicular diameter was measured at moment of P4 device removed and AI. Artificial insemination was performed 48h after P4 device removal using a semen of single sire. The pregnancy diagnosis was performed 30 d after AI using a transrectal B-mode ultrasound. Analyses of binomial outcome variables (expression of estrus and P/AI) and continuous outcomes (follicle diameter on P4 device withdrawal and FTAI) were performed using the Fit Mixed Effects Model procedure. Values are presented as percentage (%; binomial variables). The results of continuous outcome variables are expressed as means  $\pm$  standard error of the mean. The follicular diameter (mean  $\pm$  SE mm) at moment of device withdrawal (PGFd0d7 =  $11.4 \pm 0.19$ ; PGFd7 =  $10.7 \pm 0.18$ ; PGFd0d8 =  $12.8 \pm 0.26$ ; PGFd8 =  $11.56 \pm 0.26$ ; Protocol  $P < 0.0001$ ; PGF  $P = 0.0001$ ; Protocol\*PGF =  $0.0001$ ). The follicular diameter (mean  $\pm$  SE mm) at the FTAI (PGFd0d7 =  $13.6 \pm 0.2$ ; PGFd7 =  $13.1 \pm 0.18$ ; PGFd0d8 =  $15.02 \pm 0.3$ ; PGFd8 =  $14.38 \pm 0.23$ ; Protocol  $P = 0.0001$ ; PGF  $P = 0.001$ ; Protocol\*PGF =  $0.044$ ). The estrus rate (PGFd0d7 = 78.6%; PGFd7 = 62.8%; PGFd0d8 = 76.4%; PGFd8 = 66.6%; Protocol  $P = 0.84$ ; PGF  $P = 0.018$ ; Protocol\*PGF =  $0.1$ ). The pregnancy rate (PGFd0d7 = 47.1%; PGFd7 = 42.8%; PGFd0d8 = 55.6%; PGFd8 = 52.7%; Protocol  $P = 0.3$ ; PGF  $P = 0.55$ ; Protocol\*PGF =  $0.7$ ). The results indicate that prostaglandin analogue administration at the beginning of the protocol using 0.5g P4 device is associated with an increased dominant follicle size and estrous expression at FTAI in suckling *Bos taurus* and crossbred (*Bos taurus* x *Bos indicus*) multiparous beef cows. There was no difference in conception rate between the protocol of seven and eight days of exposure to P4 device (0.5g) with one or two PGF dosis, but further studies with a large number of animals are indicated.

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# What are the factors influencing the fertility of 527,093 timed-artificial insemination in Nelore beef cattle?

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## Resumo

The study investigated factors that may influence fertility of Nelore beef cattle submitted to timed-AI (TAI). A total of 527,093 records from 497 herds were included. Four classes of animals were established: young heifers (YH, n=13,823), heifers: (H, n=132,782), primiparous (P, n=91,855) and multiparous (M, n=288,633). Products (P4 implants, E2 esters, PGF, eCG and GnRH) from 8 companies were identified. The variables studied were: protocol length (7 [7D], 8 [8D], 9 [9D] and 9 days with 4 handlings [9D4H]), type of implant (monodose [MD], 1 g new [1g/1st], 1 g second use [1g/2nd] and 1 g third use [1g/3rd]), number of AI (first [1AI], second [2AI], ≥ third AI [≥3AI]) and dose of PGF on d0 and at the end of the protocol (half dose [Half-PGF] or full dose [Full-PGF]). For specific results, statistical analysis was carefully considered due to the effect of number of experimental units, which extremely influenced the P-value, making the interpretation of real biological differences risky or less meaningful. There was a difference on pregnancy per AI (P/AI) among classes of animals (YH: 42.2d, H: 47.2c, P: 50.3b and M: 56.1%a). Interestingly, we detected a more important effect of body condition score (BCS) on P than M cows. The P/AI of P with BCS <2.5 was 36.7% and M had 49.4%. The P and M with BCS 2.5 had 47.2% and 54.7% of P/AI. The P/AI of P and M with BCS 2.75 was 49.0% and 56.1%, and P and M with BCS 3.0-3.25 was 54.0% and 58.5%, respectively. However, P and M had similar fertility when BCS was 3.5-3.75 (57.9 vs. 58.8%) and ≥4.0 (58.4 vs. 59.1%). Results regarding protocol length revealed a similarity among protocols in all categories. The 7D, 8D, 9D, and 9D4H promoted similar P/AI in H (47.7, 47.7, 46.3 and 45.7%), P (50.0, 50.1, 51.6 and 50.4%) and M (56.8, 56.1, 55.2 and 56.1%). To avoid a confounding effect between use of implant and number of AI, we analyzed separately the effect of type of implant within class of animals and AI number. In H, the MD, 1g/1st, 1g/2nd, 1g/3rd promoted similar P/AI for the 1AI (48.1, 46.5, 47.6, and 47.7%) and 2AI (45.0, 46.3, 48.4 and 47.1%). For cows, the MD, 1g/1st, 1g/2nd promoted greater P/AI than 1g/3rd implant in 1AI (55.7, 56.7, 55.3 and 48.6%) and 2AI (52.8, 52.7, 54.3 and 43.7%). The Full-PGF on D0 promoted greater P/AI than Half-PGF in H (48.9 vs. 44.7%), P (52.0 vs. 46.6%) and M (58.3 vs. 55.8%). Similarly, Full-PGF at the end of the protocol increased P/AI than Half-PGF in H (47.4 vs. 45.8%), P (50.7 vs. 47.3%), and M (56.3 vs. 53.8%). In conclusion, the results are valuable to better understand factors that impact fertility of Nelore cattle. Remarkably, data suggest a flexibility in protocol length for all categories, moreover, primiparous with good BCS can achieve similar fertility than multiparous. Regarding specific protocol aspects, full dose of PGF may increase P/AI, and 1g/3rd implant may decrease fertility in cows.

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## Fresh sexed semen reaches pregnancy rate like conventional frozen semen on timed AI

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### Resumo

The aim of this study was to evaluate the timed AI (TAI) pregnancy rate using fresh sexed semen (15 to 20°C). The work was carried out in MS state, where 567 zebu females (269 heifers and 298 cows similarly distributed among the groups) were synchronized for TAI. On D0, 2 mg of EB (Gonadiol®; Zoetis, São Paulo, SP, Brazil) was injected and an intravaginal P4 device (DIB®, 1g of P4; Zoetis) was inserted. At D9, the P4 device was removed and 0.6 mg of EC (ECP®, Zoetis, Brasil), 12.5 mg of Dinoprost (Lutalyse®; Zoetis) and 300 IU of eCG (Novormon®; Zoetis) on cows and 200IU of eCG on heifers was injected. On day 11, 48 hours (149 cows and 134 heifers) or 54 hours (149 cows and 135 heifers) after removal of the P4 device, the females were inseminated, using 6 Nellore bulls (similarly distributed among the groups) collected on the same day of P4 device removal (part of the ejaculate was frozen conventional and part sexed using flow cytometry technology for later cooling). At the time of AI (between 24 and 36 hours after leaving the semen from the laboratory), the animals were divided into four treatments in a 2x2 factorial design: conventional frozen semen 48h (CF48; N=143), conventional frozen 54h (CF54; N=128), fresh sexed semen 48h (FS48; N=140) and fresh sexed semen 54h (FS54; N=156). Storage during transport took place in a container developed for this purpose, with controlled temperature throughout the laboratory output and the TAI (15 to 20°C). The pregnancy rate was evaluated 30 days after AI, and gender proportion 80 days after TAI, by transrectal ultrasound. The data were analyzed using SAS PROC GLIMMIX (SAS/STAT® 9.2). There was no interaction bull\*treatment for P/AI (P=0.2689). Also, no interaction treatment\*moment of TAI was found for P/AI (P= 0.2777). The P/AI according to the groups was: CF48: 57.3%; CF54: 66.4%; FS48: 52.1%; FS54: 56.4%. There was no effect of the type of semen used in P/AI (Conventional: 61.6%; Fresh sexed: 54.4%; P= 0.0956). Furthermore, the TAI 48h or 54h after P4 device removal presented same P/AI (48h: 54.8%; 54h: 60.9%; P= 0.1130). However, there was difference between heifers; 52.8% and cows; 62.4% (P=0.0282). There was a difference in the proportion of pregnancies of the desired gender (females) according to the treatment (Conventional: 53.5%; Fresh sexed: 94.1%; P<0.001). However, there was no effect on the proportion of the desired gender according to AI time (P= 0.0809), nor in relation to cows x heifers (P=0.8072), and the bull used (P=0.6505). The results show that the fresh sexed semen, maintained under the temperature conditions described, reaches P/AI on TAI like conventional frozen semen when used 48h or 54h after P4 device removal (up to 36 hours after release in the laboratory). So, data suggests that it is possible to increase the number of pregnancies of calves of the desired gender without decrease the TAI P/AI and in a near future this technology can be commercially established in the market.



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**Buserelin treatment for timed artificial insemination in ewes.**

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**Resumo**

Follicular growth and estrus synchronization are normally satisfactory for fixed-time insemination (TAI) in ewes treated with intravaginal device (IVD) containing medroxyprogesterone acetate (MAP) and eCG treatment at IVD removal. However, as the timing LH peak caused by discharge of gonadotropin-releasing hormone (GnRH) can vary, ovulations are dispersed among animals. Thus, the addition of buserelin (GnRH analog) to the treatment, may increase the synchrony of ovulations and, consequently, pregnancy rate, replacing or improving the efficiency of eCG treatment. To test this hypothesis, a study was conducted during the reproductive season (March and April in Rio Grande do Sul farms). We used 357 ewes, kept in native field, with a minimum body condition score of 2.5 (0-5). All animals remained with polyurethane sponge containing 60 mg of MPA for seven days and received 250 mg of cloprostenol at the time of IVD removal (day 7 = D7). Considering the availability of animals on different farms, three different experiments were conducted. In Exp. 1, on D7, animals were randomly allocated into two groups: eCG (n=156), animals treated with 200 IU of eCG; or eCG-GnRH (n=116), animals treated with 200 IU of eCG on D7 and 4 mg of buserelin at the time of insemination. In Exp 2, also on D7, the animals were allocated to: eCG (n=45), 200 IU eCG at IVD removal; or GnRH (n=40), treated with 4 mg buserelin at insemination. In both experiments, on D9 (54h after removal of the IVD), ewes were submitted to cervical superficial TAI using 100 x 10<sup>6</sup> motile spermatozoa obtained from a semen pool collected from four rams with known fertility. Pregnancy was diagnosed by transrectal ultrasonography 24 days after TAI. Pregnancy data were analyzed using the Chi-square test, and P<0.05 was considered as significant. In Exp. 3, on D7, ewes were allocated to three groups: eCG (200 IU at IVD removal; n=10); eCG+GnRH (200 IU eCG at IVD removal and 4 mg of buserelin 36 h later; n=10); or GnRH (buserelin 36 h after IVD removal). Blood samples were collected 2, 6 and 12 days after TAI (54 h after IVD removal) for P4 analysis. Data were analyzed using mixed models for repeated data (P<0.05). In Exp 1, pregnancy rate tended (P=0.09) to be greater for eCG-GnRH (52.6%) compared to eCG (44.8%). In Exp. 2, pregnancy rate was significantly lower in GnRH (22.5%) compared to eCG (46.7%). In Exp. 3, there was no significant effect of group (P=0.66) or group x day (P=0.24) on P4 concentration, being observed a significant effect of day (P=0.001). The results indicate potential improvement on pregnancy rate with buserelin treatment at the time of TAI when eCG is administered at IVD removal, and this hypothesis should be tested on a larger number of animals. The use of buserelin alone, at the time of TAI, does not replace eCG treatment. Progesterone synthesis is not altered after eCG, eCG+GnRH or GnRH treatment.

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**Effect of different TAI protocols in 14-month-old Nelore (*Bos indicus*) heifers**

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**Resumo**

The aim of the present study was to evaluate the efficiency of two TAI protocols in precocious Nelore (*Bos indicus*) heifers aging 14 months. A total of 518 Nelore heifers from farms located in MS and BA (Agropecuária Jacarezinho), Brazil were used. At the beginning of the protocol, heifers were randomized by BCS ( $2.95 \pm 0.03$ ), BW ( $279.1 \pm 2.47$ ) and sire and divided into two treatments: 9dayP4 (n=259) and 7dayP4 (n=259). Heifers from 9dayP4 received on D0 a reused intravaginal P4 device (Previously used for 27 days; CIDR®; Zoetis, Guarulhos, SP-Brazil), 2 mg estradiol benzoate (EB; Gonadiol®, Zoetis) and 2.5 mg of dinoprost tromethamine (Lutalyse®, Zoetis). After 9 days (D9), P4 device was removed and heifers received 2.5 mg of dinoprost, 0.5mg of estradiol cypionate (EC; ECP®, Zoetis) and 200IU of eCG (Novormon®; Zoetis). Heifers from 7dayP4 received on D2 an intravaginal P4 device (0.36g of P4; PRIMER PR®; Agener União), 2 mg of EB (RIC BE®; Agener União, São Paulo, SP-Brazil) and 0.53mg of sodic cloprostenol (Estron®; Agener União). After 7 days (D9), P4 device was removed and heifers received 0.53 mg of sodic cloprostenol, 0.5mg of EC (Cipiotec®; Agener União) and 200 IU of eCG (Novormon®; Zoetis). At the same time, all heifers were painted with chalk on their tailheads, and removal of chalk on D11 was used as an indication of estrus. All heifers were inseminated on the same day (D11). A subset of heifers (n=66) was evaluated by US (Mindray® DP-2200Vet) in order to measure the diameter of the dominant follicle (DF) on D11. Statistical analyses were performed using GLIMMIX of SAS 9.4. There was a statistical difference in P4 device loss rate between groups [9dayP4=6.6% (17/259) vs. 7dayP4=0.4% (1/259);  $P < 0.001$ ]. The incidence of vaginitis at P4 removal was lower for 7dayP4 than 9dayP4 group [7.3% (19/249) vs. 39% (101/259);  $P < 0.001$ ]. Heifers treated with 7dayP4 group presented a lower proportion of arching of the column (1.8% vs 27.4%;  $P < 0.0001$ ) and elevated tail (49.6% vs 89.4%;  $P < 0.0001$ ). No difference was found for estrus detection [9dayP4=56.8% (147/259); 7dayP4=61.8% (160/259);  $P = 0.13$ ]. However, the DF was larger in 7dayP4 when compared to 9day P4 group ( $9.87 \pm 0.44$  vs.  $8.74 \pm 0.52$ ;  $P = 0.04$ ). It is not possible to verify an early ovulation, however it is an important and specific information for the paper. A tendency for greater pregnancy rate in 7dayP4 was observed [7dayP4= 44.0% (114/259) vs. 9dayP4= 37.8% (98/259);  $P = 0.07$ ]. In conclusion, the 7dayP4 group presented a lower P4 device loss rate and vaginitis incidence and larger DF on AI. The pregnancy rate tended to be greater in 7dayP4.

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# Efficiency of injectable P4 or EB at the beginning of resynchronization protocol (19 days after TAI) in lactating dairy cow

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## Resumo

The aim of this study was to evaluate the use of injectable progesterone (P4i) or estradiol benzoate (EB) to synchronize follicle wave in early resynchronization 19 days after 1st TAI on P/AI and false positive rates (FP) in *Bos taurus* cattle. In experiment 1, 366 lactating Holstein cows (40.4±0.3 Kg milk/d) from Brazil commercial farms were submitted to TAI (D0). Nineteen days after TAI, cows received an intravaginal P4 device (1g, Sincrogest®, Ourofino, Brazil) and were allocated into two groups: CTL (without treatment, n=184) and P4i (150mg i.m, Sincrogest injetável®, n=182). Seven days after (D26), the P4 device was removed and ultrasound evaluations of luteal vascularization were performed using Color Doppler (M5®, Myndray). Pregnant cows were reevaluated on D33 to confirm pregnancy, while non-pregnant cows were treated with 1 mg of estradiol cypionate (SincroCP®), 0,530g PGF-2α (Sincrocio®) on D26. At the same time, the dominant follicle (DF) was measured. The 2nd TAI was performed on D28, and the pregnancy was performed 32d after 2nd AI. In experiment 2, 234 Holstein lactating cows (40.6±0.2 Kg milk/d) were previously synchronized in the same way as Exp. 1 but were allocated into two groups: CTL (without treatment, n=117) or EB (EB 1mg, Sincrodio®, n=117). The evaluations and subsequent treatments were the same as described in Exp. 1. Statistical analyses were performed by SAS Glimmix procedure (v9.4). In Exp. 1, there was no difference between groups for P/AI at 26d [CTL=51.1% (94/184) vs. P4i=52.8% (96/182); P=0.73], FP [(CTL=31.9% (30/94) vs. P4i=32.3% (31/96); P=0.71)] and P/AI at 33d [(CTL=34.8% (64/184) vs. P4i=35.7% (65/182); P=0.64)]. Also, the DF in non-pregnant cows [(CTL=18.2mm vs. P4i=19.1mm; P=0.19)] and P/AI at the 2nd TAI [(CTL=32.0% (29/89) vs. P4i=30.2% (26/86); P=0.79)] was similar between groups. In Exp. 2, similar P/AI at 26d [(CTL=46.0% (44/117) vs. EB=49.6% (58/117); P=0.60)], FP [(CTL=26.0% (14/54) vs. EB=37.9% (22/58); P=0.17)] and P/AI at 33d [(CTL=34.2% (40/117) vs. EB=30.8% (36/117); P=0.58)] were observed between groups. Also, the DF in non-pregnant cows (CTL=20.4mm vs. EB=19.1mm; P=0.28) and P/AI at 2nd TAI [(CTL=31.7% (20/63) vs. EB=37.3% (22/59); P=0.52)] was similar between the groups. In conclusion, treatment with P4i or EB in the early resynchronization protocol did not affect the P/AI of 1st TAI at 26d and 33d, nor DF diameter at P4 device removal and nor the P/AI at 2nd TAI.

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# Factors that affect pregnancy rate and pregnancy loss in young Nelore heifers synchronized for TAI

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## Resumo

The aim of the present study was to evaluate the factors that affect the pregnancy rate (P/AI) and pregnancy loss (PL) of young Nelore heifers (10-15 months) submitted to TAI. A total of 5.431 heifers from a commercial farm in MT state (Brazil) were used. On D0, heifers received a reused intravaginal P4 device (CIDR®; Zoetis, Guarulhos, SP-Brazil) and 2mg of EB (Gonadiol®, Zoetis), i.m. On D7, heifers received 2.5 mg of dinoprost tromethamine (Lutalyse®; Zoetis) and on D9 200 IU of eCG (Novormon®; Zoetis), 0,5mg of EC (ECP®; Zoetis) i.m, concomitant with P4 device removal. At the same time, heifers were painted with chalk on their tailheads, and removal of chalk on D11 was used as an indication of estrus. The TAI was performed 48h (D11) after implant removal. After 30d (DG), the pregnancy diagnosis was performed by ultrasonography and non-pregnant heifers were resynchronized for TAI using the same protocol (until 3 TAI). At the end of the breeding season (EBS), heifers had their pregnancy confirmed and PL was verified. The data were analyzed through logistic regression by PROC GLIMMIX of SAS 9.4. Receiver operating Characteristic (ROC) curve analysis (MedCalc®) was used to select optimal cut-off points for the variables analyzed. The body weight (BW) influenced the P/AI (Linear effect; P=0.003). The BW cut-off points calculated by ROC curve was 288kg [ $\leq 288\text{kg} = 49.0\%$  (n=3,398) vs.  $> 288\text{kg} = 53.6\%$  (n=2,009); P=0.008]. The heifer age also impacted the P/AI (Quadratic effect; P=0.006). The age cut-off points calculated by ROC curve was 12.3 months [ $\leq 12.3\text{m} = 47.0\%$  (n=3,398) vs.  $> 12.3\text{m} = 52.3\%$  (n=2,009); P=0.008]. The pregnancy loss (DG/EBS) was influenced by the BW (linear effect; P=0.0003). The BW cut-off points calculated by ROC curve was 293kg [ $< 293\text{kg} = 13.0\%$  (n=2,304) vs  $> 293\text{kg} = 8.7\%$  (n=1,956); P<0.0001]. The PL was influenced by age (Quadratic effect; P=0.0003). The age cut-off points calculated by ROC curve was 14.7 months [ $< 14.7\text{m} = 13.0\%$  (n=3,130) vs.  $> 14.7\text{m} = 6.6\%$  (n=1,224); P=0.008]. Furthermore, the presence of CL at the beginning of TAI protocol impacted the P/AI [CLD0 = 47.0% (n=832) vs. NoCLD0 = 52.8% (n=2,144)]; P=0.003] and the PL [CLD0 = 11.7% (n=1,142) vs. NoCLD0 = 15.0% (n=395); P=0.01]. The estrus detection at TAI impacted the P/AI [Estrus = 51.7% (n=2,423) vs. NoEstrus = 42.0% (n=550); P=0.003] and the PL [Estrus = 10.3% (n=1,251) vs. NoEstrus = 12.0% (n=229); P=0.05]. Additionally, animals with higher BCS (ROC cut-off points 3.25; scale from 1 to 5) had lower PL [ $\leq 3.25 = 13.9\%$  (n=847) vs.  $> 3.25 = 10.4\%$  (n=3,382)]; P=0.003]. In conclusion, BW, age, cyclicity rate and presence of estrus impact the efficiency of P/AI and PL in young Nelore heifers submitted to TAI.

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Pregnancy loss after timed-artificial insemination in *Bos indicus* (Nelore) beef cows: effect of parity, body condition score, presence of corpus luteum, expression of estrus and GnRH treatment at the time of AI

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## Resumo

Pregnancy loss (PL) importantly impacts the profitability of livestock production systems, although it is not widely reported, particularly in *Bos indicus* cattle. The present study retrospectively evaluated PL after timed-artificial insemination (TAI) in *Bos indicus* (Nelore) beef cows according to several factors, such as parity, body condition score (BCS), presence of corpus luteum (CL) at the beginning of TAI protocols, and expression of estrus. Data from two experiments performed during three breeding seasons (BS) were retrieved, and both experiments included the evaluation of adding a GnRH treatment at the time of AI in a 7-d estradiol (E2) plus progesterone (P4)-based protocol, with intravaginal P4 implant removal on D7 and TAI on D9. In the first BS (Exp 1), cows received either GnRH or E2 benzoate (EB) on D0 and, on D9 cows were randomized to receive or not GnRH at TAI. During BS 2 and 3 (Exp 2), the TAI protocol initiated with EB and a P4 implant, and cows were randomized to receive or not a PGF treatment on D0. On D7, cows were treated with 0.5 or 1.0 mg E2 cypionate and, on D9 (TAI), cows were treated or not with GnRH. All cows received 300 IU eCG either on D6 or D7. All hormones were from GlobalGen vet science. In all BS, presence of CL and BCS were evaluated at the beginning of TAI protocols, expression of estrus was evaluated at TAI, and ovarian ultrasound evaluation was performed during the protocol on days 0, 7 and 9. The PL was assessed between the first pregnancy diagnosis (~35d) and parturition. Statistical analyses were done by PROC GLIMMIX of SAS 9.4 ( $P \leq 0.05$ ). There were no effects of hormonal manipulations within BS on PL. There was no interaction between GnRH treatment at TAI and the other variables within BS, and there was no main effect of GnRH treatment on PL (without = 10.1% [102/1,007] vs. with = 10.4% [114/1,100]). Considering all BS, primiparous had greater ( $P < 0.01$ ) PL than multiparous cows (14.0% [77/550] vs. 8.9% [139/1,557]), and cows not expressing estrus near TAI had greater ( $P = 0.05$ ) PL than those expressing estrus (13.5% [57/422] vs. 9.7% [156/1,617]). There was no interaction between follicle size at TAI and GnRH treatment on PL. However, the probability of PL linearly decreased as follicle size at TAI increased ( $P = 0.05$ ). Interestingly, there were no effects of service number (first TAI or resynchronization), BCS, or presence of CL on D0 on PL. In conclusion, primiparous and cows not expressing estrus near TAI had greater PL, while BCS, number of service and presence of CL on D0 did not affect PL. Finally, GnRH treatment at the time of AI had no effect on PL and did not interact with any of the variables, which is an exciting result, since GnRH at AI increases fertility in *Bos indicus* beef cows.

## Acknowledgments

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Effect of the follicular stage and progesterone concentrations at the beginning of an estradiol/progesterone-based timed-AI protocol on emergence and development of a new follicular wave in *Bos indicus* heifers

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## Resumo

The study evaluated the effect of the follicular stage (pre- or post-deviation) at the onset of a synchronization protocol and the effect of higher or lower circulating progesterone (P4) from intravaginal devices on emergence and development of a new follicular wave (NFW) in *Bos indicus* heifers. Cycling Nelore heifers (n = 42; 24.0 ± 2.7 months old; BCS = 3.3 ± 0.1) were submitted to a presync protocol starting with 1.5 mg estradiol benzoate (EB), a P4 device and 2 doses of PGF (0.5 mg), 24 h apart. In half of heifers, the presync started on D-10, to result in a post-deviation 7-d old dominant follicle (DF) on D0. In the other half, the presync started on D-5, to result in a pre-deviation 2-d old follicle on D0. On D0, heifers received 1.5 mg EB and were randomized into a 2×2 factorial arrangement, composed by the follicular stage (pre- vs. post-deviation) at the onset of the protocol, and the P4 treatment with a new intravaginal device with 2 g (High P4) vs. a 1 g device previously used for 14 d (Low P4). Ultrasound evaluations were performed on D-10, -5, -2, and daily from D0 to 7, to evaluate follicular dynamics. Blood samples were taken daily from D0 to 7. On D7, the P4 devices were removed and heifers were reassigned to the presync, in a cross-over model. Statistical analyses were done by the PROC GLIMMIX of SAS 9.4 (P ≤ 0.05). Only data from heifers that had emergence of a NFW after D0 were analyzed (n = 58). On D0, before treatments, circulating P4 was similar between groups (0.4 ng/mL). As expected, on D0, the diameter of follicle was greater (11.2 ± 0.3 vs. 7.1 ± 0.2 mm), the number of follicles ≥ 5 mm was smaller (2.7 ± 0.3 vs. 5.2 ± 0.2) and the circulating estradiol was greater (5.6 ± 0.9 vs. 2.5 ± 0.4 pg/mL) in post-deviation than in pre-deviation group. Circulating P4 over time was greater in High P4 than in Low P4 group, especially during the first 3 d (3.3 ± 0.2 vs. 1.9 ± 0.1 ng/mL). Neither follicular stage nor P4 treatments affected day of emergence (2.6 ± 0.1 d), day of deviation (5.0 ± 0.1 d), or follicular diameter at deviation (6.6 ± 0.1 mm). The number of follicles ≥ 5 mm at deviation was not affected by follicular stage but was lower in High P4 than in Low P4 group (6.9 ± 0.5 vs. 8.6 ± 0.6). Follicular stage did not affect the NFW development until D7. However, follicular growth rate from emergence to D7 was greater in Low P4 than in High P4 group (1.12 ± 0.06 vs. 0.98 ± 0.04 mm/d), resulting in a greater DF on D7 (9.3 ± 0.3 vs. 8.5 ± 0.2 mm). Notwithstanding, P4 treatments did not affect the growth rate from emergence to deviation. In conclusion, neither the follicular stage at the onset of an E2/P4 protocol, nor treatment with distinct P4 devices affected the DF development during the common growth phase. However, lower circulating P4 resulted in more subordinate follicles at deviation and greater follicular growth after deviation.

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

# Variation in body condition score between synchronization of ovulation to timed artificial insemination influences fertility of Nelore cows

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## Resumo

The present study aimed to evaluate the body condition score (BCS) variation on the estrous manifestation and pregnancy rate of primiparous and multiparous cows. Five hundred and eighty-two multiparous and 261 Nelore primiparous cows, 30 to 45 days post-partum with BCS of  $3.45 \pm 0.45$ , in the region of El Carmen Rivero, Santa Cruz, Bolivia were used. BCS was performed by visual observation (scale 1 -5) and females received an intramuscular (IM) application of 2 mg estradiol benzoate (Sincrodiol, Ouro Fino, Brazil) and an intravaginal device containing 1.0 g of progesterone (Sincrogest, Ouro Fino, Brazil); (D0). Tail chalk application (Raidex, Alemanha) was made on D8 and the progesterone device was removed and 300 IU of eCG (SincroeCG, Ouro Fino®, Brazil), 1 mg of estradiol cypionate (SincroCP, Ouro Fino, Brazil) and 0,526 mg de cloprostenol (SincroCio, Ouro Fino, Brazil) IM was administered. TAI was performed 48 hours after the removal of the intravaginal device. Complete removal of chalk cows was classified in estrus and cows that had no or partial chalk removal, received a 0,01 mg buserelin acetate (Gestar®, Over, Argentina) application IM. Thirty days after TAI, pregnancy diagnosis was performed by transrectal ultrasound (Mindray, 7.5 Mhz, China) and a new BCS evaluation was performed (D40). Data were analyzed by logistic regression ( $p < 0.05$ ). Estrus manifestation was 50.7% (428/843) and there was interaction between BCS (D0) and category ( $P < 0.05$ ) [BCS $\geq$ 3.5 multiparous: 62.1% (241/388) vs. primiparous: 37.6% (49/130)  $P < 0.001$ ; BCS=3.0 multiparous: 54.0% (74/137) vs. primiparous: 38.2% (34/89),  $p = 0.029$  and BCS $\leq$ 2.5 multiparous: 29.8(17/57) vs. primiparous: 30.9(13/42),  $P = 0.92$ ]. The pregnancy rate was lower ( $p = 0.02$ ) in cows with BCS  $\leq$ 2.5 [44.4% (44/99)] than cows with BCS=3.0 [58.8% (133/226)] and BCS $\geq$ 3.5 [57.7% (299/518)]. Cows that expressed estrus had a higher pregnancy rate ( $p < 0.001$ ) [63.0 (270/428)] than cows that did not express estrus [17.0% (75/143)] or showed partial removal of chalk on D8 [48.1% (131/272)]. The variation of BCS between D0 and D40 was not influenced by the category ( $p > 0.05$ ), 267 gained [primiparous: 32.5% (85/261) and multiparous: 31.2% (182/582)], 352 kept [primiparous: 44.4% (116/261) and multiparous: 40.5% (236/582)] and 224 cows lost BCS [primiparous: 23.0% (60/261) and multiparous: 31.2% (182/582)]. The pregnancy rate was influenced by the variation of BCS and category, which showed interaction ( $p < 0.05$ ). Primiparous cows had a lower pregnancy rate ( $P = 0.01$ ) [28.7% (21/73)] than multiparous cows [53.6% (81/151)], other primiparous cows [maintenance: 55.1% (64/116) and gain [65.8% (56/85)] and multiparous cows [maintenance: 59.7% (141/236) and gain: 60.4% (110/182)], which have similar pregnancy rates ( $P > 0.05$ ). In conclusion, the maintenance or gain of ECC in primiparous cows during the postpartum period is highlighted as a relevant factor to improve fertility rates during the breeding season.

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

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# Effect of dose of estradiol cypionate as ovulation inducer on expression of estrus and fertility of Nelore (*Bos indicus*) heifers submitted to a 7d-timed artificial insemination protocol

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## Resumo

The study evaluated the effect of the dose of estradiol cypionate (EC; 0.5 vs. 1.0 mg) on expression of estrus and pregnancy per AI (P/AI) in Nelore (*Bos indicus*) heifers submitted to a 7-d estradiol (E2)/progesterone (P4)-based synchronization protocol for timed-artificial insemination (TAI). A total of 200 heifers (Age =  $26 \pm 2$  mo.; BW =  $308.3 \pm 1.8$  kg; BCS =  $3.1 \pm 0.02$ ) were enrolled in this experiment and the ovaries were previously examined by transrectal ultrasound (US) to confirm the presence or absence of CL. Heifers were submitted to a cyclicity induction protocol with 150 mg of injectable long-acting P4 on D-24, and 0.5 mg EC on D-12. At the beginning of the TAI protocol (D0), an intravaginal P4 device (0.5 g) was placed and 2 mg of estradiol benzoate, and 0.53 mg of cloprostenol sodium (PGF) were given im. On D7, concomitant with P4 device withdraw, heifers received im another PGF treatment, 200 IU of eCG, and were enrolled in a completely randomized design to receive 0.5 (EC0.5) or 1.0 mg of EC (EC1.0). In addition, heifers were painted with chalk on their tailheads for later evaluation of expression of estrus. On D9, the removal of chalk was evaluated and TAI was performed. Moreover, all heifers were treated im with 8.4  $\mu$ g of buserelin acetate (GnRH). Pregnancy diagnosis was performed by US 30 d after TAI, and heifers that were diagnosed not pregnant were reenrolled in the experiment for further resynchronization, following the same TAI protocol. Combining data from the first service and resynchronizations, the total TAI performed was 302 (CE0.5 = 150 and CE1.0 = 152). Statistical analyses were performed by the GLIMMIX procedure of SAS 9.4 ( $P \leq 0.05$ ). On D-24, 23.0% (46/200) of heifers had a CL, but there was no effect of the presence of CL on expression of estrus ( $P = 0.25$ ) or P/AI ( $P = 0.68$ ). Moreover, there was no effect of service on expression of estrus (first service = 79.0% [158/200]; second service = 83.8% [57/68]; third service = 79.4% [27/34];  $P = 0.64$ ) or on P/AI (first service = 54.0% [108/200]; second service = 48.5% [33/68]; third service = 47.1% [16/34];  $P = 0.61$ ). The EC treatment did not affect the expression of estrus (CE0.5 = 78.0% [117/150] vs. CE1.0 = 82.2% [125/152];  $P = 0.36$ ), neither P/AI (CE0.5 = 50.7% [76/150] vs. CE1.0 = 53.3% [81/152];  $P = 0.85$ ). There was no interaction between EC doses and expression of estrus ( $P = 0.22$ ), and, regardless of treatment, expression of estrus did not affect P/AI (with estrus = 52.1% [126/242] vs. without estrus = 51.7% [31/60];  $P = 0.81$ ). In conclusion, treatment with 0.5 or 1.0 mg of EC at the time of P4 device removal in a 7-d E2/P4-based TAI protocol resulted in similar expression of estrus and P/AI in Nelore heifers treated with GnRH at the time of AI.

**Keywords:** synchronization; TAI protocol, Nelore, beef cattle

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

FTAI/FTET/AI

**USE OF DIFFERENT PROGESTERONE DEVICES IN FERTILITY OF LACTATING DAIRY COWS SUBMITTED TO THE OVULATION SYNCHRONIZATION PROTOCOL**

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**Resumo**

The objective was to evaluate the fertility of lactating dairy cows submitted to ovulation synchronization protocols using different intravaginal progesterone (P4) devices. In experiment 1, 386 lactating Holstein cows were used. On a random day of the estrous cycle (D0), the cows were divided into two experimental groups to receive different intravaginal P4 devices (Control Group, n = 193, CIDR®, Zoetis, Brazil; Crestar Group, n = 193, Crestar Device 1.3 g, MSD, Brazil). Simultaneously, in all cows were administered intramuscularly (i.m.) 2 mg of estradiol benzoate (Fertilcare sincronização® - MSD, São Paulo, Brazil) and 0.250 mg of Gonadorelin (Fertagyl®, MSD, São Paulo, Brazil). Seven days later (D7), were administered 0.530 µg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil). On nine day (D9), the device was removed and 0.530 µg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil) and 1.0 mg of estradiol cypionate (Fertilcare ovulação® - MSD, São Paulo, Brazil) were administered. TAI was performed 48h after removal of the progesterone device only in cows expressed estrus. In experiment 2, 354 lactating Girolando cows were divided into two groups to receive different intravaginal P4 devices (Control Group, n = 177, FertilCare 1200®, MSD, Brazil; Crestar Group, n = 177, Crestar Device 1.3 g, MSD, Brazil). At the same time, 2 mg of estradiol benzoate (Fertilcare Synchronization® - MSD, São Paulo, Brazil) and 0.100 mg of Gonadorelin (Fertagyl®, MSD, São Paulo, Brazil) were administered. Seven days later, 0.530 µg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil) was administered. On day 9 (D9), the intravaginal devices were removed and were administered 0.530 µg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil), 300 IU of eCG (Folligon®, MSD, São Paulo, Brazil) and 1 mg of estradiol cypionate (Fertilcare Ovulation® - MSD, São Paulo, Brazil). The TAI was performed 48 hours after removal of the P4 device concomitantly with the administration of 0.100 mg of Gonadorelin (Fertagyl®, MSD, São Paulo, Brazil). The pregnancy diagnosis was performed 30 days after the TAI. Statistical analyses were performed using SAS. In Holstein cows, no differences were observed regarding device loss (Control 2.4% and Crestar 2.4%; P = 0.98), service rate (Control 87.3% and Crestar 84.7%; P = 0.47), conception rate (Control 38.8% and Crestar 44.4%; P = 0.51) and pregnancy rate (Control 33.9% and Crestar 37.6%; P = 0.76). In Girolando cows, animals in the Crestar Group had a higher incidence of P4 device losses (Control 6.2% and Crestar 11.9%; P = 0.005). However, there was no difference regarding the pregnancy rate in the different experimental groups (Control 49.4% and Crestar 47.4%; P = 0.85). It is concluded that the different P4 Control and CRESTAR IVG 1.3 g devices used in TAI protocols have similar fertility in lactating *Bos taurus* (Holstein) and *Bos taurus* x *Bos indicus* (Girolando) cows.

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)****FTAI/FTET/AI**

# Effect of GnRH treatment at the time of artificial insemination on fertility of *Bos indicus* (Nelore) beef cows

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## Resumo

The study evaluated the effect of a GnRH treatment at the time of timed-artificial insemination (TAI) on fertility of *Bos indicus* (Nelore) beef cows. We hypothesized that GnRH treatment at the time of TAI would increase pregnancy per AI (P/AI). Primiparous and multiparous cows from two farms (A: n = 699; B: n = 749), over two breeding seasons (BS), were submitted to a 7-d estradiol (E2)/progesterone (P4)-based TAI protocol. In farm A, cows were enrolled in this study only at the second TAI (resynchronization), whereas cows from farm B were enrolled at the first and second TAI. In both farms, on D0, body condition score (BCS) was evaluated and all cows received a 1 g intravaginal P4 device, 2 mg E2 benzoate (EB), and 0.53 mg cloprostenol sodium (PGF). On D7, concomitant with P4 device removal, cows received 0.53 mg PGF, 1 mg E2 cypionate and 300 IU eCG. On D9, expression of estrus was evaluated, TAI was performed and cows were randomly assigned to receive (G1) or not (G0) a treatment with GnRH at the time of TAI (farm A: 8,4 µg buserelin acetate [G0 = 347 and G1 = 352]; farm B: 25 µg lecorelin [G0 = 364 and G1 = 385]). All products were from GlobalGen, except for lecorelin that was from Agener. Pregnancy diagnosis was performed by ultrasound 30 d after TAI. Statistical analyses were performed by the GLIMMIX procedure of SAS 9.4 ( $P \leq 0.05$ ). In farm A, presence of CL on D0 was greater in multiparous than primiparous cows (72.6% [336/463] vs. 39.0% [92/236];  $P < 0.0001$ ). Presence of CL on D0 was greater in cows with  $BCS \geq 3$  (66.9 [238/356] vs. 55.4% [190/343];  $P = 0.02$ ), and those cows had greater expression of estrus than cows with  $BCS < 3$  (84.8 [302/356] vs. 73.8% [253/343];  $P = 0.002$ ). Moreover, more multiparous expressed estrus than primiparous cows (86.0 [398/463] vs. 66.5% [157/236];  $P < 0.0001$ ). Cows with CL on D0 had greater P/AI than cows without CL (65.4 [280/428] vs. 50.6% [137/271];  $P = 0.05$ ). Besides, P/AI was greater in cows that expressed estrus in comparison with cows that did not express estrus (64.0% [355/555] vs. 43.1% [62/144];  $P = 0.0009$ ). There were no interactions between GnRH treatment at TAI and CL on D0, BCS, or parity. Cows receiving GnRH had greater overall P/AI than cows that did not receive (62.5% [220/352] vs. 56.8% [197/347];  $P = 0.05$ ) and GnRH treatment at TAI increased P/AI of cows not expressing estrus (52.1% [37/71] vs. 34.3% [25/73];  $P = 0.05$ ). In farm B, GnRH at TAI did not interact with parity, number of AI, BCS, or estrus. Likewise, the GnRH treatment increased P/AI (G1 = 54.3% [209/385] vs. G0 = 49.7% [181/364];  $P = 0.02$ ). In conclusion, our hypothesis was supported, and the study reinforced the benefit of a GnRH treatment at TAI on increasing P/AI of *Bos indicus* (Nelore) beef cows.

## Acknowledgments

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**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

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**EFFECT OF hCG AT THE TIME OF AI ON THE PREGNANCY RATE OF *Bos indicus* COWS SUBMITTED TO THE OVULATION SYNCHRONIZATION PROTOCOL**Leonardo Teixeira <sup>2</sup>, Matheus Guapo Pavarina <sup>2</sup>, Matheus Pedroso Vicente <sup>1</sup>, Patrick Santos Freitas <sup>3</sup>, Laís Reis Carvalho <sup>1</sup>, José Nélio de Sousa Sales <sup>1,4</sup><sup>1</sup> UFLA - UNIVERSIDADE FEDERAL DE LAVRAS (LAVRAS-MG), <sup>2</sup> SGP - SINCRONIZA GERENCIAMENTO PECUÁRIO (UBERABA-MG), <sup>3</sup> MGP - MGP ACESSORIA VETERINÁRIA (UBERABA-MG), <sup>4</sup> UFJF - UNIVERSIDADE FEDERAL DE JUIZ DE FORA (JUIZ DE FORA-MG)**Resumo**

The objective was to evaluate the effect of the administration hCG at the moment of TAI in *Bos indicus* cows that had not demonstrated estrous during the ovulation synchronization protocol. On a random day of the estrous cycle (D0), *Bos indicus* lactating cows (n=684) received 2mg of estradiol benzoate (Fertilcare Sincronização, MSD, Brazil) and an intravaginal progesterone device (Fertilcare 1200, MSD, Brazil). On day 8 (D8), the progesterone device was removed and cows received 500µg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil), 1.0 mg of estradiol cypionate (Fertilcare ovulação® - MSD, São Paulo, Brazil) and 300 UI of eCG (Folligon, MSD, Brazil). In addition, the tailhead of was marked with chalk for evaluate estrus expression between D8 and D10. On day 10 (D10) the cows that did not remain marked were considered in estrus and excluded from the experiment. Cows that remained with the marking on the tailhead were considered non-estrous and, simultaneously, were randomly distributed three experimental groups (Control group, GnRH group and hCG group). Control group (n=228) cows did not receive treatment. GnRH group cows received 100 µg of Gonadorelin (Fertagyl, MSD, Brazil; n=227) and hCG group cows received 1000 UI of hCG (Chorulon, MSD, Brazil; n=229). After the administration of treatments, all cows were inseminated. Pregnancy diagnosis were performed 30 days after TAI (D40). Cows were considered non-pregnant were kept with clean-up bulls for 42 days in proportion of one bull to five cows. Statistical analysis was performed by SAS. The pregnancy rate was higher in the hCG group compared to the control group [Control – 42.9% (98/228)b, GnRH – 46.7% (105/225)ab, hCG 53.3% (122/229)a; P=0.04]. In addition, there was a tendency (P=0.09) to have a higher pregnancy rate at the clean-up bulls on cows of the hCG group compared to the control group [Control – 13.9% (18/130)B, GnRH – 17.5% (21/120)AB, hCG 25.2% (27/107)A]. It is concluded that the use of hCG at the time of TAI in *Bos indicus* cows that did not manifest estrous during the ovulation synchronization protocol increases the pregnancy rate of TAI and clean-up bulls in lactating *Bos indicus* cows.

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# Effect of body condition score and antral follicle count on pregnancy rate of postpartum Nelore cows submitted to protocol for timed artificial insemination and resynchronization

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The present study aimed to evaluate the effect of antral follicles count  $\geq 3\text{mm}$  (AFC) and body condition score (BCS) on the pregnancy rate (P/AI, %) of 603 Nelore multiparous cows, 30 to 45 days post-partum. In exp. 1, 323 cows,  $\text{BCS}=3.0\pm 1.0$  were used. AFC was performed by transrectal ultrasound (Mindray, 7.5Mhz, China) and females received an IM application of 2 mg of estradiol benzoate (Gonadiol, Zoetis, Brazil) and 1.9g of progesterone intravaginal device (CIDR, Zoetis, Brazil, D-11). On D-2, device was removed and 300 IU of eCG (Novormon, Zoetis, Brazil), 1 mg of estradiol cypionate (ECP, Zoetis, Brazil), 12.5 mg of dinoprost (Lutalyse, Zoetis, Brazil) IM were administered. TAI was performed 48 hours after CIDR removal (D0). On D31, pregnancy diagnosis was performed and the non-pregnant cows were resynchronized by the same treatment (TAI D42). In exp. 2, 280 cows,  $\text{BCS}=2.8\pm 0.4$ , were synchronized by the same methodology, however, on D22 all cows received a CIDR and Gonadiol. Pregnancy diagnosis was performed on D31 and CIDR withdrawn from pregnant cows and non-pregnant cows were resynchronized according to the treatment described (TAI D33). Cows were distributed according to score into low ( $\leq 13$ ), intermediate (14-36) and high AFC ( $\geq 37$  follicles) groups. Data were analyzed using GLIMMIX ( $P < 0.05$ ). In exp. 1, in the first TAI, low AFC cows had a higher P/AI [67.5(73/108);  $P=0.03$ ] than high and intermediate AFC [51.8(56/108), 57.0(61/107)].  $\text{BCS} \leq 2.75$  cows showed lower P/AI (54.2(102/188) than  $\text{BCS} \geq 3.0$  [65.1(88/135)  $P=0.05$ ]. AFC didn't influence on resynchronization P/AI [low:37.1(13/35), intermediate:58.6(27/46), high:50.0(26/52)  $P=0.18$ ] however,  $\text{BCS} \leq 2.75$  cows had a low P/AI ( $P=0.04$ ) [46.5(40/86)] than  $\text{BCS} \geq 3.0$  [55.3(26/47)]. Considering the two TAI, the cumulative P/AI showed an interaction between AFC and BCS ( $P < 0.05$ ) [Low AFC and  $\text{BCS} \geq 3.0=91.6(44/48)$ ,  $\text{BCS} \leq 2.75=70.0(42/60)$   $P=0.006$ , intermediate AFC and  $\text{BCS} \geq 3.0=79.1(38/48)$ ,  $\text{BCS} \leq 2.75=84.7(50/59)$   $P=0.47$ , high AFC and  $\text{BCS} \geq 3.0=82.0(32/39)$ ,  $\text{BCS} \leq 2.75=72.4(50/69)$   $P=0.23$ ]. In exp. 2, in the first TAI, low AFC cows had a higher P/AI [60.2(56/93)  $P=0.03$ ] than intermediate [43.6(41/94)] and high [44.1(41/93)] AFC cows.  $\text{BCS} \leq 2.75$  cows showed tendency to lower P/AI ( $P=0.09$ ) 45,1(70/155) than  $\text{BCS} \geq 3.0=54.4$  (68/125)]. There was no effect of AFC and BCS on the resynchronization of P/AI [low AFC=40.5(15/37) intermediate AFC=35.8(19/53) high AFC=40.3(21/52)  $P=0.71$ ] [ $\text{BCS} \leq 2.75=37.6$  (32/85)  $\text{BCS} \geq 3.0=40.3(23/57)$   $P=0.80$ ]. Cumulative P/AI was influenced by AFC [low=76.3(71/93) intermediate=63.8(60/94); high=66.6(62/93)  $P=0.04$ ] regardless of BCS [ $\leq 2.75=65.8(102/155)$ ,  $\geq 3.0=72.8(91/125)$   $P=0.18$ ]. In conclusion, AFC influenced the first TAI so after exposure to exogenous progesterone, no further effect on resynchronization. Low AFC and  $\text{BCS} \geq 3.0$  cows showed better performance on 33 to 42 days of breeding season, possibly by increasing synchronization rates.

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## Progesterone (Injectable Sincrogest) pre-exposition to ovulation synchronization protocol tend to increase the conception rate at 30 days after TAI in high production *Bos taurus* dairy cows

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### Resumo

The objective was to evaluate the effect of pre-exposition of injectable progesterone to timed artificial insemination (TAI) protocol on conception rate of high production *Bos taurus* dairy cows. In the study, 293 Holstein and crossbred (Holstein X Jersey) dairy cows at  $57,0 \pm 0,5$  days in milk, body condition score of  $2,72 \pm 0,01$  (scale of 1 – 5) and production of  $35,0 \pm 0,6$  were used. Seven days before TAI protocol (D-7), cows were divided into two experimental groups (Control group and P4i group). In the Control group, cows received 2 mg of estradiol benzoate (RIC-BE, Agener, Brazil), 25 $\mu$ g de Lecirelina (Tec-relin, Agener, Brazil) and a progesterone intravaginal device (Primer, Agener, Brazil). After seven days (D7), cows received 500 $\mu$ g of Cloprostenol (Estron, Agener, Brazil). On day 8 (D8), the progesterone device was removed and cows received 500 $\mu$ g of Cloprostenol (Estron, Agener, Brazil) and 1 mg of estradiol cypionate (Cipiotec, Agener, Brazil). On the P4i group, cows received 300mg of injectable progesterone (Sincrogest Injetável®, Ouro Fino, Brazil) on D-7 and were submitted to the same synchronization protocol as the Control group. All cows were inseminated 48 hours after the removal of progesterone device. Ultrasound exams were performed 30 and 60 days after TAI to evaluate the conception rate at 30 and 60 days and pregnancy losses from 30 to 60 days after TAI. Statistical analyses were performed by GLIMMIX procedure of SAS and the binomial variables were presented by percentage. There was a statistical tendency in the conception rate at 30 days after TAI [Control 52.7% (77/146)B and P4i 61.2% (90/147)A; P= 0.10]. However, conception rate at 60 days after TAI [Control 50.9% (60/118) and P4i 58.5% (69/118); P= 0.23] and pregnancy losses [Control 4.7% (3/63) and P4i 2.8% (2/71); P= 0.45] were similar between groups. In conclusion, the pre-exposition to progesterone on TAI protocol tends to increase the conception rate at 30 days after TAI in high production *Bos taurus* dairy cows.

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**EXPOSURE TO PROGESTERONE PRIOR TO TAI DOES NOT INTERFERE WITH OOCYTE QUALITY AND GENE EXPRESSION OF CUMULUS CELLS OF *Bos indicus* IN ANESTRUS COWS**

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**Resumo**

The objective was to evaluate the oocyte and cumulus cell quality in *Bos indicus* cows supplemented with injectable progesterone prior to the FTAI protocol. *Bos indicus* multiparous cows (n=30) were randomly assigned to two experimental groups (Control and P4i). Control group cows did not receive treatment prior to the FTAI protocol. In the P4i group, cows received 150 mg of injectable progesterone ten days before the start of the follicular emergence synchronization protocol (D-10). On D0, the cows received 2mg of estradiol benzoate and an intravaginal progesterone device. Five days later (D5), the cows were submitted to follicular counting and aspiration (OPU). After follicular aspiration, oocytes were evaluated for quantity and quality (color, homogeneity and integrity of the cytoplasm, as well as the number and degree of compaction of cumulus cells) and stored for gene expression evaluation. The relative expression of target genes in oocytes (GDF9 and BMP15) and cumulus cells (BAX, BCL2 and HAS2) were evaluated by real-time PCR. Statistical analyses were performed by SAS. There was no difference among treatments in the number of aspirated follicles (Control=25.8±2.6; P4i= 27.4±5.4; P=0.63), in the total of retrieved oocytes (Control=15.4±1.6; P4i=13.1±1.8; P=0.15), in the number of grade 1 oocytes (Control=2.3±0.6; P4i=1.8±0.5; P=0.32), grade 2 (Control=4.1±0.4; P4i=3.7±0.9; P=0.70), grade 3 (Control=3.6±0.7; P4i=3.6±0.7; P=0.90), degenerate (Control=5.3±0.9; P4i=3.9±0.5; P=0.15), the oocyte quality index (Control=2.8±0.1; P4i=2.9±0.1; P=0.86) and the rate of viable oocytes (Control=65.3%; P4i=69.9%; P=0.36). Similarly, no differences were observed in the quantification of GDF9 transcripts among groups (P=0.52); as well as BMP15 (P=0.74), BAX (P=0.62), BCL2 (P=0.78), BAX/BCL2 (P=0.59) and HAS2 (P=0.55). It is concluded that injectable progesterone prior to the FTAI protocol does not improve oocyte quality and does not interfere with the metabolism of cumulus cells evaluated according to this methodology.

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## Beta-carotene, ADE vitamins and biotin supplementation in the feeding on reproductive performance of timed-fixed-inseminated beef cows

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### Resumo

Seasonal climatic variations mainly in tropical areas, is an import factor for decreasing of the reproductive performance. Thus, the necessity to developing management strategies to reduce the impact of fluctuations in forage availability and quality throughout the year, such as deferral of pastures and supplementation with additional sources of protein, energy, and minerals. This study aimed to evaluate the effect of supplementation with beta-carotene and vitamins (ADE and biotin) on the reproductive performance of lactating zebu cows submitted to FTAI. The experiment was conducted on a private property, during rainfall season, enrolling 395 cows blocked by body condition score, beta-carotene dosage, breed and sex calf, in two lots: Control (n = 195) and Treated (n = 200). The animals were kept on pastures and supplemented with mineral mix (no added vitamins; 150g / animal / day). For the treated group, the same product was added, plus a vitamin premix (500mg  $\beta$ -Carotene, 70,000 IU Vit. A, 10,000 IU Vit. D3, 500mg Vit E and 10mg Biotin, 200g / animal / day). The treated animals were supplemented by 90 days starting at D-30 until D60 (considering D0 the beginning of the protocol of synchronization of ovulation for FTAI). Blood samples were collected for serum levels of beta-carotene and beta-hydroxybutyrate, as well as a transrectal ultrasonographic examination to determine the cow's cyclicity, diameter of the dominant follicle and later to confirm pregnancy. Statistical analysis showed that serum betacarotene concentration was higher in the control group (P = 0.05; Control: 4.53 vs. Treated: 4.27) and that in this group there was also a higher rate of estrus manifestation (P < 0.001; Control: 89.92% vs. Treaty: 78.84%). Although there was no difference (p> 0.005) between the groups for the pregnancy rate (Control: 63.50% vs. Treated: 56.92%). The expression of estrus had a positive correlation with the pregnancy rate in the FTAI when disregarded the effect of the groups. Interaction of the pregnancy rate with the diameter of the dominant follicle was also demonstrated. Thus, beta-carotene and vitamins (ADE and biotin) do not impact on reproductive performance of zebu lactating cows in the rainfall breeding season. Maybe in the transition from dry-rainfall season it could present a positive effect.

**Keywords:** beef cattle, fertility, nutrition, reproduction

**Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)**

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# Impact of intravaginal progesterone devices on pregnancy per AI of lactating *Bos indicus* cows and heifers submitted to an ovulation synchronization protocol

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## Resumo

The objective was to evaluate the pregnancy rate of two new progesterone (P4) devices in lactating Nelore *Bos indicus* heifers and cows. In experiment 1, heifers with a BCS of  $3.2 \pm 0.01$  and 24 to 27 months old were randomly assigned to two groups to receive different P4 devices. The animals in the F600 group (n=151) received a new P4 intravaginal device (FertilCare 600® - Monodose - MSD, Brazil) and the heifers in the Crestar0.5 group (n=149) received a new P4 device (Crestartar 0.558®, MSD, Brazil). In experiment 2, lactating *Bos indicus* cows with a BCS of  $2.61 \pm 0.01$  were divided in two experimental groups. The animals in the F600 group (n=314) received a new intravaginal P4 device (FertilCare 600®, MSD, Brazil) and the cows in the Crestartar1.0 group (n=311) received a new P4 device (Crestartar 1.0® - MSD, Brazil). In experiments 1 and 2, concomitantly with the treatments, the animals received 2mg of EB (Fertilcare Sincronização®, MSD, Brazil) on D0. In addition, heifers received 265µg of Cloprostenol (Ciosin®, MSD, Brazil; Experiment 1). On D8, 0.5mg of EC (Fertilcare Ovulação®, MSD, Brazil) was administered to heifers and 1mg of EC to cows, 300IU of eCG (Folligon®, MSD, Brazil) and 265µg of Cloprostenol (Ciosin®, MSD, Brazil) in all animals of the two studies. In addition, the P4 intravaginal devices were removed and a wax marking at the base of the tail was performed to observe the occurrence of estrus between D8 and D10. In both experiments the females were inseminated 48 hours after removal of the P4 device, and the estrus occurrence reading was performed at that moment. Pregnancy diagnosis and cyclicity rate were performed 30 days after TAI (D40). Statistical analysis was performed with the aid of SAS. In experiment 1, the rates of estrus (P=0.24), cyclicity in pregnancy diagnosis (P=0.88) and pregnancy (P=0.56) were similar between groups (F600 group - 66.2%, 97.7% and 43.1% and Crestartar group 72.5%, 97.6% and 45.0%, respectively). In addition, the pregnancy per AI (P/AI) was higher (P=0.008) in heifers that expressed estrus during the TAI protocol (Non-estrus 32.6% and Estrus 49.0%). However, the presence of CL on D0 did not affect estrus (P=0.63) and P/AI (P=0.97). In experiment 2, the estrus rate (P=0.34) and P/AI (P=0.92) were similar between the experimental groups (Group F600 - 60.3% and 50.3% and Group Crestartar 54.0% and 48.6%, respectively). However, the P/AI was higher in cows that showed estrus during the TAI protocol (No-estrus 32.7% and Estrus 51.8%; P=0.008) and in multiparous cows (primiparous 39.9% and multiparous 55.7%; P=0.001). It is concluded that the P4 Crestartar 0.558® devices (heifers) and Crestartar 1.0® (cows) have fertility results similar to those observed in females that received the P4 Fertilcare 600® device in ovulation synchronization protocols.