

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET/ET

The use of estradiol benzoate in the cervical relaxation protocol for non-surgical embryo recovery in sheep does not impair embryo cryosurvival and gene expression

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Non-surgical embryo recovery (NSER) is becoming an important approach for embryo recovery procedures in sheep and its success relies on the efficiency of cervical relaxation treatment before NSER. The protocol usually involves the combination of prostaglandin F_{2α} (PGF), estradiol benzoate (EB), and oxytocin (OT). However, EB may have an embryotoxic action and its use is not allowed in several countries. This study aimed to assess the role of using different doses of EB for cervical relaxation on NSER efficiency and embryonic viability and cryosurvival. Sixty crossbred Lacaune X Santa Inês ewes were superovulated (333 IU of pFSH i.m., Pluset, Biogénesis Bagó, Curitiba, Brazil) and randomly assigned to receive either 0.0 (G0; n=23), 0.5 (G0.5; n=16), or 1.0 (G1; n=21) mg of EB i.m. (RIC-BE[®], Agener União, Taboão da Serra, Brazil) associated with 37.5 µg of PGF i.m. (Prolise, Agener União) at 16 h before NSER and 50 IU of OT i.m. (Ocitocina Forte UCB, UCBVet, Jaboticabal, Brazil) 20 min before NSER. All morulae recovered were subjected to slow freezing for later *in vitro* culture, while the blastocysts were freeze-dried for gene expression analysis by RT-qPCR. Parametric data were analyzed by one-way ANOVA followed by a posthoc Tukey test, while the non-parametric data were analyzed by the Chi-square test. The expression gene was normalized (GAPDH, H2AFZ, and B-ACTIN), and the groups were compared by the $2^{-\Delta\Delta CT}$ method. NSER procedure was successfully performed regardless ($P>0.05$) of the treatment used (G0: 80%, G0.5: 82%, or G1: 80% of ewes) and it took similar ($P>0.05$) time to be conducted (G0: 21.3 ± 0.8 , G0.5: 26.4 ± 3.6 , or G1: 22.4 ± 1.9 min); the average number of recovered structures (G0: 7.1 ± 1.6 , G0.5: 9.7 ± 1.5 , or G1: 9.8 ± 2.4) and viable embryos (G0: 5.3 ± 1.2 , G0.5: 6.9 ± 1.8 , or G1: 6.0 ± 2.1) did not differ ($P>0.05$) among the groups as well. There was no difference ($P>0.05$) in the *in vitro* embryonic survival after 48 h of culture (G0: 60%, G0.5: 54%, or G1: 58%). The treatments did not affect ($P>0.05$) the expression of BAX, NANOG, OCT4, PRDX1, and HSP90 genes. The BCL2 expression, however, was downregulated ($P=0.04$) in G1 in comparison to G0.5, while no difference ($P>0.05$) was observed between G0 and G0.5 as well as between G0 and G1. In conclusion, although EB did not seem to affect embryo quality, its use did not promote any extra benefit, since the cervical relaxation treatment without EB was efficient to allow successful NSER, making the protocol cheaper, simpler, and applicable worldwide.

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FTET/ET

The conception rate and pregnancy loss in a fixed-time embryo transfer program from beef cattle are related to the luteal blood perfusion, but not to the corpus luteum size

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We evaluated the associations between luteal blood perfusion (LBP) or corpus luteum (CL) area and the conception rate or occurrence of pregnancy loss during a large-scale fixed-time embryo transfer (FTET) program in cows. Multiparous Brangus cows ($n=1,465$; 45 days postpartum), with body condition scores (BCS) between 2.5 and 4.0 (3.0 ± 0.3) were used in this study. On a random day of the estrous cycle (Day 0), the females were subjected to the FTET protocol, that entailed an estradiol benzoate injection (2mg, i.m., Sincrodiol[®], Ourofino, Cravinhos, Brazil) and insertion of intravaginal progesterone (P4)-releasing device (1g, Sincrogest[®], Ourofino). On Day 8, the P4 device was removed, and the animals received an injection of 0.526mg cloprostenol (Sincrocio[®], Ourofino), 1mg of estradiol cypionate (SincroCP[®], Ourofino), and 400IU eCG (Sincro eCG[®], Ourofino). On Day 17, each recipient was evaluated by ultrasound (E2V Sonoscape[®], Domed, Valinhos, Brazil) for CL area (cm^2) and using color Doppler for LBP scores (I/Low - vascularization area <40% of CL; II/Medium - vascularization >45% and <50%; and III/High - vascularization >50%). Immediately after the CL evaluation, a single in-vitro produced, fresh embryo (*Bos indicus-taurus*, Grade I and II blastocyst), obtained from a commercial laboratory, was deposited in a uterine horn ipsilateral to the CL. The pregnancy diagnosis was performed 30 days after embryo transfer and repeated 60 days later. A single, experienced technician performed all ultrasound evaluations and transfers. For analysis, in addition to LBP groups, recipients were retrospectively ranked according to CL area into Small <3 cm^2 (2.63 ± 0.01), Medium >3 and <4 cm^2 (3.44 ± 0.01), and Large >4 cm^2 (4.77 ± 0.03). Data were analyzed by a logistic regression model, including LBP score, CL area, and BCS as continuous variables. In the presence of a significant main effect ($P<0.05$), the rate ranking was established by a 2x2 proportion test between blood perfusion score (Low, Medium and High) and CL area (Small, Medium and Large). The overall conception rate was 44.2% (648/1,465), and it was affected by LBP score [$P=0.03$; High 48.4%^a (134/277), Medium 44.6%^a (427/958) and, Low 37.8%^b (87/230)], but not by CL area [$P=0.37$; Large 41.8% (225/538), Medium 45.2% (276/610) and, Small 46.4% (147/317)]. There was no interaction between LBP score and CL area ranking ($P=0.81$) and BCS also did not affect the results of this study ($P=0.51$). Regarding pregnancy loss up to 90 days, there was no effect on the CL size ($P=0.77$), but the luteal blood flow score showed an effect [$P=0.03$; High 3.6%^b (5/139), Medium 9.3%^a (44/471) and Low 10.3%^a (10/97)]. In conclusion, the conception rate and incidence of pregnancy loss in beef cattle undergoing a TETF program are both related to luteal blood perfusion, but not to CL size. Thus, the use of Doppler ultrasound in large-scale embryo transfer programs is a valid strategy to increase reproductive efficiency of cattle.

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Factors that impact pregnancy loss of beef embryos produced *in vitro*

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The use of *in vitro* produced embryos (IVP) in recent years has increased, however, pregnancy loss in recipient cows is still a concern. Thus, this study aimed to evaluate the factors affecting pregnancy loss (PL) in recipient cows of bovine embryos produced *in vitro*. The study was conducted in a commercial IVP laboratory, located in Uberlândia, Minas Gerais, Brazil. Data from 1225 pregnancies from 2018 to 2020 were analyzed. Oocytes were collected by ultrasound-guided follicular aspiration in selected donors. Embryos were classified according to quality and stage of development 7 days after fertilization. Fresh embryos were individually loaded and transported at 36°C and the cryopreserved embryos were vitrified, thawed, and rehydrated before transfer. Heifers and crossbred cows used as recipients were subjected to epidural anesthesia (lidocaine 2%) and after identifying the corpus luteum by rectal palpation, the embryo transfer (ET) was performed in the ipsilateral uterine horn to the CL. The pregnancy diagnosis was performed by transrectal ultrasonography within 30 to 60 days after estrus, and again 30 days later for pregnancy confirmation. Pregnancy loss was considered to occur when a cow that was diagnosed as pregnant on the first exam failed to remain pregnant in a subsequent exam. Data were analyzed by logistic regression, considering the effects of the season at the time of ET (Spring/Summer vs. Fall/Winter), breed (Nelore vs. Senepol) and embryo type (fresh vs. frozen). Each pregnant animal was considered an experimental unit. Statistical differences were established by $P \leq 0.05$ and trend of $0.05 < P < 0.10$. The overall PL was 12.73% (156/1225). A tendency ($P = 0.068$) was observed for a higher rate of PL during Spring/Summer (13.97%) compared to Fall/Winter (10.56%). No effects of embryo breed (13.31% for Senepol vs. 10.55% for Nelore embryos; $P = 0.239$) on PL rates as well as embryo type (12.53% for fresh vs. 13.40% for vitrified embryos; $P = 0.696$) were observed. The transfers carried out in the spring/summer period showed a tendency for higher PL, but the beef embryo type and the breed did not influence the PL rates of recipient cows.

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Exposure to injectable progesterone previous to timed embryo transfer protocol increases pregnancy rate in embryo recipient beef cows

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The objective was to evaluate the effect of exposure to injectable progesterone previous to timed embryo transfer (TET) protocol on fertility in embryo recipient beef cows. Recipient Nelore and crossbred cows (n=480) with body condition score of 2.75 ± 0.02 (scale of 1 – 5) were used. Ten days before TET protocol (D-10), cows were divided into 2 experimental groups (Control and P4i groups). In this moment, cows of P4i group received 150mg of injectable progesterone IM (Sincrogest Injetável®, Ouro Fino, Brazil). On day 0 (D0), all cows (Control and P4i groups) received 2mg of estradiol benzoate (Sincrodiol®, Ouro Fino, Brazil), 375µg of cloprostenol (cows with CL; Sincrocio®, Ouro Fino, Brazil) and a intravaginal device containing 1g of progesterone (P4; Sincrogest®, Ouro Fino, Brazil). On day 8 (D8), the P4 device was removed, and cows received 500µg of Cloprostenol (Sincrocio®, Ouro Fino, Brazil), 300 IU of eCG (SincroeCG®, Ouro Fino, Brazil) and 1 mg of estradiol cypionate (SincroCP, Ouro Fino, Brazil). The timed embryo transfer was performed in all cows on D17. Ultrasound exams were performed 23d and 53d after TET. Statistical analyses were performed by GLIMMIX procedure of SAS. The service rate [Control 76.1% (188/247) and P4i 79.0% (184/233); P=0.48], conception rate at 23d after TET [Control 44.7% (84/188) and P4i 52.7% (97/184); P=0.12], conception rate at 53d after TET [Control 34.6% (65/188) and P4i 39.1% (72/184); P=0.40] and pregnancy loss [Control 22.6% (19/84) and P4i 25.8% (25/97); P=0.51] was similar between groups. However, the P/ET at 23 days after embryo transfer was greater (tendency; P=0.08) in the cows of P4i group [Control 34.0% (84/247) and P4i 41.6% (97/233)]. In conclusion, exposure to injectable progesterone previous to TET protocol increases the pregnancy rate at 23 days after embryo in embryo recipient beef cows.

Effect of treatment with bST at the beginning of the protocol (D0) on FTAI and FTET

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This study evaluated the effect of bST (Posilac[®]) treatment at the beginning of the protocol (D0) on the pregnancy rate at FTAI and FTET programs. Two experiments were carried out. In Experiment 1, 834 Brangus primiparous (P) and multiparous (M) cows were submitted to two FTAI with resynchronization at D22, and in Experiment 2, 265 crossbred (Angus vs. Nellore) heifers (H) and primiparous cows were submitted to FTET. In the Experiment 1, the cows were synchronized with progesterone and estradiol-based protocol. At D0, cows received an intravaginal P4 device (Primer Monodose[®], Agener União) and 2 mg of estradiol benzoate (EB) i.m. At the same time, cows were randomized according to body condition score (BCS; 2.82±0.01) and allocated into two groups: 1) Control [n=394 (P=70 vs. M=324)]: no treatment and 2) bSTD0 [n=440 (P=71 vs. M=369)]: treatment with 325 mg of bST applied at the tailhead s.c. (Posilac[®], Agener União) on D0. Eight days later (D8), concomitant with P4 device removal, animals received 1 mg of estradiol cypionate (ECP), 0.150 mg of D-Cloprostenol (PGF), and 400 IU of equine chorionic gonadotropin (eCG) all administered i.m. FTAI was performed 48 hours after the removal of the P4 device. In Experiment 2, animals were submitted to the same protocol described previously. At D0, the females were randomized according to BCS (3.29±0.03) and allocated into two groups: 1) Control [n=137 (H=44 vs. P=93)]: no treatment and 2) bSTD0 [n=136 (H=43 vs. P=93)]: treatment of 325 mg of bST s.c. (Posilac[®], Agener União) on D0. In both experiments, pregnancy diagnosis was performed at D30 via ultrasound evaluation. Statistical analyses were performed using GLIMMIX of SAS 9.4. Factors included in Experiment 1 were: pregnancy rate (P/AI) at D30 of 1ST and 2ND FTAI and the final P/AI (D60 of 1ST FTAI and D30 of 2ND FTAI). Factors included in Experiment 2 were: proportion of recipients transferred and pregnancy rate (P/ET) at D30. In Experiment 1, there was no effect of treatment (P=0.44) on the P/AI of the 1ST FTAI at D30. However, there were treatment*BCS interaction for the P/AI in the 2ND FTAI at D30. Cows with greater BCS treated with bST on D0 had a greater P/AI than cows with lower BCS [$\geq 3.00 + bSTD0 = 46.6\%$ (48/103)^A; $\geq 3.00 + Control = 31.8\%$ (28/88)^{AB}; $\leq 2.75 + bSTD0 = 28.1\%$ (56/199)^B; $\leq 2.75 + Control = 29.3\%$ (53/181)^B; P=0.03]. The final P/AI tended to be higher in the group treated with bST [bSTD0=53.0% (232/438) vs. Control=48.8% (190/389); P=0.08]. In Experiment 2, the treatment with bST did not affect the proportion of recipients transferred (P=0.13). However, recipients treated with bST showed a tendency to increase in P/ET [bSTD0=57.7% (60/104) vs. Control=48.2% (54/112); P=0.07]. In conclusion, there was no effect of the treatment with bST at D0 on the pregnancy rate of the 1ST FTAI. However, bST treatment increased the P/AI in cows with higher BCS at 2ND FTAI. Regarding TETF, recipients treated with bST at D0 presented a tendency to increase P/ET.

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Treatment with bST in embryo recipients does not increase birth weight

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This study evaluated the effect of treatment with Posilac® (Posilac®, Agener União) of embryo recipients on the calf's body weight at birth time. A total of 95 Nelore (*Bos indicus*) calves were used. Calves were born from heifers crossbred recipients (Angus x Nelore) that received one of the following treatments (2x2 factorial design) during the synchronization protocol for FTET (D0 = 2mg estradiol benzoate and intravaginal P4 device; D9 = P4 device removal. 0.5 mg estradiol cypionate, 300UI eCG and 12.5 mg de dinoprost). The recipients were divided according to the treatments: 1) Control (n = 18): no treatment; 2) bSTD9 (n = 26): treatment with 325 mg of bST on D9 (P4 device removal); 3) bSTD18 (n = 24): treatment with 325 mg of bST on D18 (day of the embryo transfer) and 4) bSTD9/D18 (n = 27): treatment with 325 mg of bST on D9 and D18. The calves body weight was measured at birth time. Statistical analyses were performed using GLIMMIX of SAS 9.4. Factors included in this experiment were the recipient's treatment and the calves' body weight at birth time. No interaction bST treatment D9*D18 on calf's body weight at the birth time was observed (Control = 38.9 kg, bSTD9 = 38.9 kg, bSTD18 = 37.5 kg, bSTD9D18 = 39.2 kg; P = 0.30) and data was presented by main effects. No bST treatment effect was found on the birth weight, neither on D9 [Control = 38.7 kg (n = 42) vs. bSTD9 = 39.1 kg (n = 53); P = 0.43] nor on D18 [Control = 38.9 kg (n = 51) vs. bSTD18 = 38.4 kg (n = 44); P = 0.44]. Furthermore, there was no correlation between the weight of the recipient at the beginning of the synchronization protocol (D0) and the birth weight of the calf ($R^2 = 0.16$; P = 0.11). In conclusion, bST treatment in embryo recipients does not affect calf body weight at birth.

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Does the cavity of the corpus luteum affect circulating steroid concentrations or fertility in embryo transfer recipient Holstein cattle?

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The aim was to evaluate the effect of a cavitory (CAV) or a compact (COMP) corpus luteum (CL) on circulating progesterone (P4) and estradiol (E2) concentrations in Holstein heifers, and on fertility of lactating Holstein cows submitted to embryo transfer. In study 1, Holstein heifers (n=27; 10 to 16 mo old) were evaluated daily by ultrasonography to identify a spontaneous ovulation (D0), and then, ovarian dynamics were assessed daily until the next ovulation. Heifers were classified according to the presence of a CAV-CL (n=8) or a COMP-CL (n=19) during this estrous cycle. Blood samples were collected daily to evaluate circulating P4 and E2 concentrations. The volume of the CL was calculated by the sphere formula ($V=4/3 \times \pi \times R^3$) based on the CL diameter measures. For CAV-CL, the volume of the cavity was equally calculated and subtracted from the total volume of the CL. In study 2, lactating Holstein cows (n=205; $\sim 35.3 \pm 0.6$ kg of milk/d) were synchronized to receive an embryo transfer (ET) 7 days (D7) after the second GnRH of an Ovsynch protocol (D0). Cows were classified as having a CAV-CL (n=57) or a COMP-CL (n=148) on D7. Circulating P4 concentrations, ovulatory follicle (OF) and CL diameter were evaluated on D0 and 7. Pregnancy diagnosis (P/ET) was performed on D30, 60 and 120. Pregnancy loss (PL) was determined between D30 and 120. For both studies CAV CL was considered when CAV was ≥ 10 mm. Circulating P4 analyses were performed by ELISA and E2 analyses were done by radioimmunoassay. Statistical analyses were done by PROC GLIMMIX of SAS 9.4 ($P \leq 0.05$). In study 1, there was no interaction between type of CL and time on CL volume during the cycle. Particularly on D7 and 10 of the cycle, circulating P4 concentrations were greater in heifers with CAV-CL than with COMP-CL (3.9 ± 0.5 vs 3.1 ± 0.2 and 5.6 ± 0.4 vs 4.0 ± 0.2 ng/mL). However, no differences were observed in the other days. Diameter of OF and E2 concentrations near luteolysis and ovulation were not affected by the type of CL. In study 2, there was no effect of type of CL on volume or circulating P4 concentrations on D7. However, the of the OF on D0 (classified as ≤ 18 or > 18 mm) affected the CL volume (7.4 ± 0.4 vs 9.2 ± 0.5 cm³) and circulating P4 on D7 (1.8 ± 0.6 vs 2.0 ± 0.1 ng/mL), regardless of the type of CL. Moreover, P/ET was similar between cows with CAV-CL and COMP-CL (D30: 42.1 vs 41.9%; D60: 29.1 vs 31.5%; D120: 25.9 vs 27.7%). In addition, there was no effect of CL type on PL (33.3 [7/21] vs 29.1% [16/55]). In conclusion, the presence of a cavity in a CL did not affect circulating hormone concentrations, follicle dynamics during the estrous cycle in Holstein heifers, nor P4 concentrations or fertility of lactating Holstein cows submitted to ET. These results support the awareness that recipient cows or heifers with a cavitory CL should not be discarded as ET recipients.

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FTET/ET

Effect of Sincroforte® (GnRH) or Sincrogest injetável® (injectable P4) treatments at the moment of embryo transfer on pregnancy rate of beef recipients

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The objective was to evaluate the effect of administering GnRH (Sincroforte®, Ourofino Brazil) or injectable progesterone (iP4; Sincrogest injetável®, Ourofino, Brazil) on the day of embryo transfer (FTET) on fertility in beef embryo recipient (Nelore and Angus). In this study, 679 females (heifers, n=230; primiparous, n=164 and multiparous, n=285); from a commercial farm (Agropecuária Jacarezinho, Brazil) were used. On the day of the embryo transfer, recipients were randomly assigned based on their weight (411.9±1.1Kg) and corpus luteum (CL) diameter (19.8±0.2mm) and divided into three treatments groups: 1) Control (n=228): no treatment; 2) GnRH (n= 226): received 10µg of buserellin acetate (Sincroforte®); 3) iP4 (n= 225): received 300mg of iP4 (Sincrogest injetável®). On day 0 (D0) all recipients received an intravaginal P4 device (Sincrogest®, Ourofino Saúde animal, Brazil) and 2mg EB (Sincrodiol®, Ourofino). On D8, the P4 device was removed and all recipients received 0.53mg of PGF (Sincrocio®, Ourofino), 1mg EC (Sincrocip®, Ourofino) and 300IU of eCG (SincroecG®, Ourofino). On D17, fresh and vitrified embryos were transferred, and treatments was administered. Pregnancy diagnosis was performed on 23 and 53 days after ET. Statistical analyses were performed using GLIMMIX of SAS 9.4. The P/ET on D30 [Control=49.5% (113/228); GnRH=47.3% (107/226) and iP4=47.1% (106/225); P=0.86], on D60 [Control=37.4% (85/228); GnRH=37.2% (84/226) and iP4=39.6% (89/225); P=0.85] and pregnancy loss [Control=24.8% (28/113); GnRH=21.5% (23/107) and iP4=16% (17/106); P=0.53] was similar between groups. For P/ET on D30, no interaction treatment*category was observed (P=0.59). However, there was a tendency (P=0.06) of interaction treatment*category for P/ET on D60, which primiparous that received iP4 had greater P/ET [52.8% (28/53) than primiparous of Control [35.6% (21/59)] and GnRH [32.7% (17/52)] groups. There was no difference between treatments for heifer [33.5% (77/230)] and multiparous recipients [40.5% (115/284)]. No interaction treatments*recipient weight (412 or >412 Kg) was observed for P/ET at 30 (P=0.42) and 60 days (P=0.15) and for pregnancy loss (P=0.24). However, P/ET at 30 days was greater (P=0.02) in recipients with >412 Kg [50.3% (149/296) vs. 46.2% (177/383)]. No interaction treatments*embryo type (fresh and vitrified) was observed for P/ET at 30 (P=0.50) and 60 days (P=0.90) and for pregnancy loss (P=0.84). However, P/ET at 30 days and 60 days was greater (P=0.003 and P=0.005) in the fresh embryo [57.5% (122/212); and 45.8% (97/212)] vs. [43.7% (204/467); and 34.5% (161/467) than a vitrified embryo, respectively. We conclude that in primiparous recipients the administration of 300mg of iP4 may be a strategy to increase P/TE at 60 days.

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Effect of Sincrogest injetavel® (injectable progesterone) at different timepoints on fixed time embryo transfer (FTET) success in beef cows

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This study evaluated the impact of administering injectable progesterone (iP4; Sincrogest injetável, Ourofino, Brazil) either 10 days before the start of FTET protocol and/or on the day of embryo transfer in Nelore cows (*Bos indicus*) on fertility. In Experiment 1, 930 cows from a commercial farm (Agropecuária Jacarezinho, Brazil) were used. Ten days before the beginning of FTET protocol, cows were randomly assigned based on their BCS (2.56 ± 0.01) and divided into four groups using a 2x2 factorial design: 1) Control (n=239): no treatment; 2) iP4 D-10 (n=239): received 150mg of iP4 on D-10; 3) iP4 D17 (n=237): received 150mg of iP4 on D17; 4) iP4 D-10/ D17 (n=238): received 150mg of iP4 on D-10 and D17. All cows received on D0 an intravaginal P4 device (CIDR®, Zoetis, Brazil) and 2 mg EB (Gonadiol®, Zoetis). After 8 days (D8), the P4 device was removed and cows received 16.8mg of dinoprost, 0.3mg of EC (ECP®, Zoetis), and 300IU of eCG (Novormon®, Zoetis). On D17, the embryo was transferred, and pregnancy diagnosis was conducted on D40 and D53. In Experiment 2, 461 recipients' cows were used. Ten days before the start of the FTET protocol, cows were randomly assigned based on their BCS (2.89 ± 0.01) and divided into two treatments: 1) Control (n=232): no treatment or 2) iP4 (n=229): received 150 mg of iP4. The FTET protocol was the same as described in Experiment 1, adding the estroject® (Estroject, United States) to check estrus expression. Statistical analyses were performed using GLIMMIX of SAS 9.4. In Experiment 1, no interaction treatment*moment was observed for any variable and data was presented by main effects [D-10 (Control or iP4) or D17 (Control or iP4)]. Administration of iP4 on D-10 resulted in a greater proportion of recipients transferred than the Control group [75.7% (361/477) vs. 69.1% (329/476) P=0.03]. An interaction between treatment on D-10 and BCS has been observed in the proportion of recipients transferred [BCS ≤ 2.50 = Control: 64.9% (235/362) b vs. iP4: 74.9% (283/378); BCS > 2.50 = Control: 82.5% (94/114)a vs. iP4: 78.8% (78/99)a; P=0.04]. However, there were no differences for P/ET at 40 days (P=0.36) between Control [49.4% (157/318)] and iP4 [48.3% (170/352)] at D-10. Also, no significant differences for P/ET at 40 days were observed (P=0.09) between control [51.2% (176/344)] and iP4 [46.3% (151/326)] on D17. Likewise, there were no significant differences in pregnancy loss between the control and iP4 on D-10 (P=0.87) or on D17 (P=0.73). In Experiment 2, the proportion of recipients transferred did not differ between groups (P=0.40). The estrus expression (81.2 vs. 83.4%; P=0.55) and the P/ET (55.1 vs. 54.3%; P=0.87) were similar between the control and iP4 groups, respectively. In conclusion, the results indicate that administering iP4 10 days before starting the FTET protocol can improve the proportion of recipients transferred. However, no significant effect was observed on P/ET neither for treatment on D-10 nor D17.

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FTET/ET

Effect of estrus manifestation using ESTROTECT™ on reproductive efficiency of Nelore and crossbred recipients submitted to FTET

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This study evaluated the reproductive efficiency of Nelore and crossbred (*Bos taurus* x *Bos indicus*) recipients submitted to a fixed-time embryo transfer (FTET) program using ESTROTECT™ to evaluate the occurrence of estrus. A total of 522 Nelore (n=393) and crossbred recipients (n=129) from farms located in SP (Nelore Mocho CV, Brazil) and BA (Agropecuária Jacarezinho, Brazil) were used. These recipients were submitted to a progesterone and estradiol-based synchronization protocol. At D0, the recipients received an intravaginal P4 (Primer Monodose®, Agener União) device and 2 mg of estradiol benzoate (EB; RIC-BE®, Agener União) intramuscular (IM). Nine days later (D9), the P4 device was removed, and recipients received IM 0.526 mg of sodium cloprostenol (PGF; Estron®, Agener União), 1mg of estradiol cypionate (EC; ECP®, Zoetis), and 300 IU of eCG (Novormon®, Zoetis). At the same time, ESTROTECT™ was placed at the tailhead to evaluate the estrus response following to the FTET protocol. After nine days (D18), estrus expression was detected (ESTRUS and NO ESTRUS), and the embryo transfer was performed in recipients with a corpus luteum (CL), regardless of estrous occurrence. At this time, the diameter (mm) of the CL was performed using an ultrasound B mode (SonoScape® model S8V) in a subgroup of animals (n=221). On D18, 83.7% (437/522) of the recipients presented an activated ESTROTECT™. An ultrasound evaluation was performed 22 days later (D40) to confirm pregnancy per ET (P/ET). Statistical analyses were performed using GLIMMIX in SAS 9.4. Recipients that expressed estrus presented higher BCS [ESTRUS=3.29±0.02 (n=437) vs. NO ESTRUS=3.11±0.04 (n=85); P<0.001]. Recipients that expressed estrus had a greater proportion of recipients transferred [ESTRUS=88.1% (385/437) vs. NO ESTRUS=65.9% (56/85); P<0.001]. The breed of the recipients also influenced (P=0.009) the proportion of recipients transferred and no interaction between estrus occurrence and the breed was found (P=0.31). Nelore recipients had a greater transferred rate when compared with crossbred females [NELLORE=86.3% (339/393) vs. CROSSBRED=79.1% (102/129); P=0.009]. Also, recipients that expressed estrus had a greater CL diameter at ET [ESTRUS=19.7mm (n=191) vs. NO ESTRUS=18.1mm (n=30); P=0.035]. Crossbred recipients presented a greater CL diameter than Nelore recipients [CROSSBRED=21.5mm (n=84) vs. NELLORE=18.2mm (n=137); P=0.0005]. Recipients that expressed estrus had a greater P/TE when compared with recipients that did not express estrus [ESTRUS=60.8% (233/383) vs. NO ESTRUS=48.2% (27/57); P=0.049]. In conclusion, recipients that expressed estrus during the FTET protocol had a greater BCS proportion of recipients transferred, CL diameter, and P/TE. Nevertheless, Nelore recipients presented a greater proportion of recipients transferred, and the crossbred recipients had a greater CL diameter.