



A070 FTAI, FTET and AI

## **Second TAI with an early resynchronization in nulliparous and multiparous Nelore females**

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**Keywords:** early resynchronization, Nelore, TAI.

The aim of this trial was to compare the use of an early resynchronization (before pregnancy diagnosis) in Nulliparous and Multiparous Nelore females. A total of 308 Nulliparous and 142 Multiparous Nelore cows, with an average BCS of  $2.84 \pm 0.01$  were used. In the first TAI protocol the animals were synchronized with the following protocol: insert of an intravaginal progesterone device (CIDR 1.9g; Zoetis, Sao Paulo, Brazil) and 2.0 mg im of estradiol benzoate (EB; Gonadiol, Zoetis) on Day -11, 12.5 mg im of dinoprost tromethamine (PGF; Lutalyse, Zoetis) on Day -4, on Day -2 CIDR was removed and 0.5 mg im of estradiol cypionate (ECP; Zoetis) and eCG (200 heifers or 300 cows; Novormon; Zoetis) were administered. The TAI was performed on Day 0, 48 h after CIDR withdrawal. On Day 23, all animals received the insertion of a CIDR and 1.0 mg im of EB. On Day 30, the pregnancy diagnosis was performed and the CIDR was removed. The nonpregnant females also received 12.5 mg im of PGF. On Day 32 CIDR was removed and were administered 0.5 mg im of ECP and eCG (200 Nulliparous or 300 Multiparous). The second TAI was performed on Day 34, 48 h after CIDR removal. Pregnancy rates to 1<sup>st</sup> and 2<sup>nd</sup> TAI were analyzed using PROC GLIMMIX from SAS, being included in the models the effects of category, BCS, AI tech and AI sire. For analysis of the final pregnancy rate (1<sup>st</sup>+2<sup>nd</sup> TAIs) was included in the model the effect of category. Results were reported as least square means. None of the variables tested were significant in pregnancy rates for 1st and 2nd TAI. For Nulliparous pregnancy rates was 63.6% (211/308) and 55.7% (54/97) for 1st and 2nd TAI respectively. Multiparous cows pregnancy rates was 54.1% (68/142) and 63.5% (47/74) for 1st and 2nd TAI, respectively. The final pregnancy rate did not differ between categories, being 86.0% (265/308) and 80.9% (115/142) for Nulliparous and Multiparous Nelore females, respectively. The utilization of a 2<sup>nd</sup> TAI protocol using early resynchronization, before pregnancy diagnosis, did not differ between both, Nulliparous and Multiparous Nelore females, indicating be a viable alternative to improve the pregnancy rate early in the breeding season.



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### **The addition of GnRH and/or anticipated PGF2 $\alpha$ in progestogen/estradiol TAI-based protocol does not affect dairy cows fertility**

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**Keywords:** lactating cow, pregnancy rate, synchronization.

The aim of the present study was to evaluate the reproductive performance of lactating dairy cows submitted to different synchronization protocols for TAI. A total of 422 lactating dairy cows (Holstein and Holstein x Jersey), reared in loose-housing system with milk yield of  $23.4 \pm 0.4$  L/cow/day, body condition score (BCS)  $3.0 \pm 0.02$  (range 1-5) and  $144.8 \pm 4.5$  days in milk (DIM) from five commercial dairy farms was enrolled in the experiment. Cows were homogeneously distributed in four groups (control, n = 102; GnRHD0, n = 109; PGFD7, n = 102 and; GnRHD0 + PGFD7, n = 109) in a factorial 2 x 2 design considering milk yield, BCS and DIM. On a random day of the estrous cycle all animals were submitted to a synchronization protocol for TAI [Day 0: 2 mg of estradiol benzoate (EB; Gonadiol®, MSD, Argentina) + norgestomet ear implant (Crestar®, MSD, Netherlands); Day 8: implant removal + 0.530 mg of sodium cloprostenol (PGF2 $\alpha$ ; Ciosin®, MSD, Brazil); Day 9: 1 mg EB (Gonadiol®, MSD, Argentina); Day 10 AM: TAI]. In addition to the described protocol, the GnRHD0 group received 100  $\mu$ g of gonadorelin (Fertagyl®, MSD, Netherlands) on Day 0, the group of PGFD7, 0.530 mg of sodium cloprostenol (Ciosin®, MSD, Brazil) on Day 7 and the GnRHD0+PGFD7 group, 100 $\mu$ g of gonadorelin on Day 0 and 0.530 mg of sodium cloprostenol on Day 7. The animals in the Control group received no additional treatment. On Day 40, females were evaluated to pregnancy diagnosis by ultrasound exam. Statistical analysis was performed by GLIMMIX the SAS®. No interaction was observed between treatment with GnRH on Day 0 and treatment with PGF2 $\alpha$  on Day 7 for pregnancy rate 30 days after TAI [Control: 40.2 (41/102); GnRHD0: 37.6 (49/109); PGFD7: 38.2 (39/102) and; GnRHD0+PGFD7: 42.2% (46/109); P = 0.47]. Also, no effect of treatment with GnRH on Day 0 [With GnRHD0: 39.9 (87/218) and No GnRHD0: 39.2% (80/204); P = 0.82] or PGF2 $\alpha$  on Day 7 [With PGFD7: 40.3 (85/211) and No PGFD7: 38.9% (82/211); P = 0.52] was verified. Additionally, although it was observed farm effect (P = 0.03), no interaction farm and treatment (P > 0.05) was established. Therefore, the addition of GnRH at the beginning of the protocol and/or PGF2 $\alpha$  on the day before of ear implant removal do not increase pregnancy rate in dairy cows (Holstein and Holstein x Jersey) reared in loose-housing system submitted to progestogen/estradiol TAI based protocol.

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### **Does the use of eCG in TAI protocols improves follicular and luteal function of pregnant and non pregnant crossbred dairy cows?**

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**Keywords:** corpus luteum, follicular diameter, progesterone.

This study aimed to determine the impact of the use of equine chorionic gonadotropin (eCG) on follicular diameter (DFOL), luteal (DCL) and concentration of progesterone (P4) in pregnant and non pregnant cows submitted to a synchronization protocol for timed AI (TAI). A total of 126 crossbred dairy cows were subjected to the following protocol: on a random day of the estrous cycle (Day 0) was applied an intravaginal progesterone device (CIDR®, Zoetis, São Paulo, Brazil) associated with 2 mg of estradiol benzoate IM (Gonadiol®, Zoetis). At Day 8 the P4 device was removed and were administered 25 mg of dinoprost trometamina (Lutalyse®, Zoetis) and 1 mg of estradiol cypionate (E.C.P.®, Zoetis). At this time, cows were randomly assigned to one of two treatments: eCG (eCG; n = 61) 300IU of eCG IM (NOVORMON®, Zoetis, São Paulo, Brazil) was administered and No eCG (n = 65) without treatment. On Day 10, immediately before the TAI, cows had their DFOL measured by transrectal ultrasonography. On Day 20, the DCL was measured and blood collected for P4 concentration determination using the chemiluminescence. Pregnancy diagnosis was performed 30 days after TAI by ultrasound. Thus, four groups were established: eCG pregnant (n = 25), eCG not pregnant (n = 30), No eCG pregnant (n = 21) and No eCG not pregnant (n = 37). Statistical analysis was performed using SPSS (version 19) program, considering 5% significance. The DFOL were similar among groups (eCG pregnant, eCG non-pregnant, No eCG pregnant and No eCG nonpregnant: 10.1±3.4; 11.2±3.1; 12.1±2.4 and 11.1±2.9mm, respectively). The DCL were respectively; 19.8±3.8; 21.4±4.2; 20.5±3.7 and 19.9±3.8mm, also did not differ. However, the eCG pregnant group showed significant difference (P = 0.0007) for P4 concentration (16.7±11.1 ng/mL) compared with other groups eCG nonpregnant (8.2±6.7 ng/mL), No eCG pregnant (8.2±5.7 ng/mL) and No eCG nonpregnant (8.7±6.7 ng/mL), these last three did not differ significantly. In conclusion, pregnant cows treated with eCG had better luteal function and consequently higher concentration of P4.



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### **Addition of a second dose of prostaglandin F2 $\alpha$ to a fixed-time AI protocol improves fertility of anestrus dairy cows and without hyperthermia**

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**Keywords:** anestrus, PGF2 $\alpha$ , TAI.

This study was conducted during January to December 2014 in two dairy farms in the Minas Gerais State - Brazil, the aim was to evaluate if addition of a second prostaglandin (PG) F2 $\alpha$  dose improves fertility of anestrus lactating Holstein cows submitted to an estradiol/progesterone (P4) based timed AI (TAI) protocol. Ovaries were scanned by ultrasound to determine if a corpus luteum (CL) was present at the protocol initiation (d-11) and on the day of PGF2 $\alpha$  (d-4). Cows without CL on d-11 and d-4 were classified as anestrus (n=436). Anestrus cows were submitted to the TAI protocol: d-11: two intravaginal P4 devices (1.9g P4, CIDR, Zoetis) and 2.0 mg of estradiol benzoate (EB) i.m. (Gonadiol, Zoetis); d-4, 25mg of PGF2 $\alpha$  i.m. (Lutalyse, Zoetis) and withdrawal of one CIDR; d-2 withdrawal of the second CIDR and 1.0 mg i.m. of estradiol cypionate (E.C.P., Zoetis); on d0 TAI. On d-4, cows were randomly assigned to one of two treatments: one dose of PGF2 $\alpha$  on d-4 (1PGF) or two doses of PGF2 $\alpha$  (2PGF), the first on d -4 and the second on d-2. Rectal temperature was measured on the day of TAI and 7 days later. The average rectal temperature was calculated and the cows were divided into two groups: without hyperthermia (<39.1°C) or with hyperthermia ( $\geq$ 39.1°C). Pregnancy was diagnosed 60 d after AI. Binomial variables were analyzed using the GLIMMIX and continuous using the MIXED procedures of SAS. The synchronization rate in this study was 76.4% (n = 436) and the percentage of cows with hyperthermia was 58% (n = 436). The 2PGF treatment tended to improve the synchronization rate (80.2% [n=218] vs. 72.4% [n=218] P=0.07). An interaction (P=0.05) between treatment and hyperthermia was observed for pregnancy per AI (P/AI) on d 60. For hyperthermic cows, P/AI were 9.1% (n=123) and 9.4% (n=130) for 1PGF and 2PGF, respectively. For cows without hyperthermia, P/AI were 16.3% (n=95) and 30.0% (n=88) in 1PGF and 2PGF, respectively. When only ovulated cows were included in the analysis, then an interaction (P=0.05) existed between treatment and hyperthermia on P/AI at 60d. For cows with hyperthermia, P/AI were 13.8% (n=80) and 12.1% (n=100) in 1PGF and 2PGF, respectively. For cows not suffering of hyperthermia, P/AI were 19.4% (n=78) and 34.8% (n=75) in 1PGF and 2PGF, respectively. The addition of a second PGF2 $\alpha$  dose during the TAI protocol increased fertility in anestrus dairy cows without hyperthermia on the first 7 days after AI.



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### **Adjustment of the estradiol benzoate dose in the resynchronization protocol with unknown pregnancy status in suckled beef cows**

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**Keywords:** bovine, fertility, pregnancy loss.

This research aimed evaluated two doses of estradiol benzoate (BE) administered early in the resynchronization protocol 22 days (Day 22) after the first timed-AI (TAI) on the pregnancy rate by TAI (P/AI), pregnancy loss (PL) and induction of new follicular wave emergence (FW) in suckled beef cows. After 1st TAI and regardless of pregnancy status, 1426 cows (768 *B. taurus* and 728 *B. indicus*) were randomly divided into 2 groups according to the dose of BE [1 mg (n = 721) or 2 mg (n = 705); *Bos taurus* (RIC-BE®) and zebu (Gonadiol®)]. On day 22, cows received an intravaginal progesterone device (P4; PRIMER® and DIB®, taurine and zebu respectively). After 8 days (Day 30), the P4 removed and pregnancy diagnosis accomplished by ultrasound. The non-pregnant females received prostaglandin analogue [*Bos taurus* (Estron®) or zebu (Ciosin®)] IM. The *Bos taurus* cows received 10 mg of FSH (Foltropin®) and 1 mg of EB, whereas Zebu were treated with 300 IU eCG (Novormon®) and 1 mg estradiol cypionate (ECP®). The TAI was performed 44h or 48h after removal of the P4, Zebu and *Bos taurus* cows, respectively. Pregnancy diagnosis conducted at 30 and 62 days after 1st TAI. The data submitted for analysis with GLIMMIX (SAS 9.3). There was no interaction between treatments and breeds on the P/AI after the 1st TAI (P = 0.85), P/AI after 2nd TAI (P = 0.31), PL between 30 and 62 days after 1st TAI (P = 0.50), as well as the cumulative pregnancy (1st TAI+2nd TAI, P = 0.75). The P/AI after 1st TAI was similar (P = 0.85) among treatments (1 mg and 2 mg = 44.0%). However, the ratio P/AI after resynchronization was higher (P = 0.0001) in cows treated with 2 mg (1 mg = 36.1% and 2 mg = 47.3%). The PL at 1st TAI was similar (P = 0.37) among treatments (1 mg=3.8% and 2 mg=5.5%), however, the cumulative pregnancy was higher (P = 0.01) in cows treated with 2 mg of EB (68.2%) than those treated with 1 mg of EB (62.8%). A total of 40 zebu cows had their ovarian dynamics evaluated by ultrasound to assess the induction of a new follicular wave emergence after the treatment with different doses (1mg vs. 2mg) of BE 22 days after 1st TAI. In non-pregnant cows (1mg n= 12 and 2mg n= 12), despite of similar interval from EB treatment to new follicular wave emergence (mean ± SEM; P = 0.13), the emergence of new wave was more dispersed (P = 0.03) in those cows treated with 1mg of EB (1.8 ± 1.3 days) compared to cows treated with 2mg of BE (2.3 ± 0.6 days). Therefore, the dose of 2mg of BE is more efficient to induce a synchronous new follicular wave emergence, greater pregnancy per AI after resynchronization, without compromise the pregnancy established from the 1st TAI in suckled beef cows resynchronized with unknown pregnancy status.

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## **Association between pin-bone angle, calving difficulty and fertility in lactating Holstein cows**

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**Keywords:** dairy cow, fertility, pin-bone angle.

The objective of this study was to assess the possible association between pin-bone angle and fertility in lactating Holstein cows. Pin-bone angle was measured in 557 Holstein cows producing  $38.2 \pm 2.8$  kg/dia and at  $151 \pm 12$  DIM, housed in two free-stall herds in USA (herd 1 in California, herd 2 in Wisconsin). Pin-bone angle was measured with a digital angle-meter (ADA Angle-meter®) by a single technician without previous knowledge of the reproductive history of the cows. Data regarding calving difficulty (CD; scale of 1 ease to 3 very difficult) and conception results to 1st post-partum AI (CR1) were retrieved from the herd's software (Dairy Comp-305), used in both studied herds. Information of pin-bone angle was divided into quartiles, as follows: Q1 = cows with pin-bone angle of -2.0 to 0.2; Q2 = 0.3 to 1.0; Q3 = 1.1 to 1.7; Q4 = 1.8 to 5.3. Data was analyzed with the proc Glimmix of SAS (Version 9.3). There was no herd effect on CR1 (herd 1 = 27.9%; herd 2 = 32.3%;  $P = 0.26$ ); however, herd 2 had a greater proportion of cows having a difficult calving  $CD > 1$  (herd 1 = 8.0%; herd 2 = 19.7%;  $P = 0.03$ ). Parity tended to affect CD ( $P=0.10$ ) and CR1 ( $P=0.09$ ), and calving month had no effect ( $P>0.10$ ) on CD or CR1. Unexpectedly, pin-bone angle did not influence any studied variables (CD:  $P = 0.53$ ; CR1:  $P = 0.68$ ). Thus, the average CD and CR1 for each quartile was, respectively: Q1 = 1.2 and 33.5%; Q2 = 1.2 and 26.5%; Q3 = 1.2 and 25.6%; Q4 = 1.3 and 29.8%. In contrast to our initial hypothesis, these results suggest little to no influence of the pin-bone angle on calving difficulty and conception results to 1st postpartum AI in high producing Holstein cows.



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### **Delayed insemination time in taurine heifers that did not display estrus after progesterone device removal**

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**Keywords:** GnRH, heat, heifers.

Occurrence of estrus after synchronization of ovulation protocol is associated with a greater pregnancy rate following timed artificial insemination (TAI) protocols. However females that do not display estrus, present delayed ovulation (Lopes, M.S.B., et al., Anim. reproduction, v.9, n.3, p.520, 2012.) and low pregnancy per AI (P/AI) (Sá Filho et al. Theriogenology, v. 76, p. 455-63, 2011). Thus, delaying the TAI moment associated with GnRH treatment can be an alternative to improve the P/AI (Thomas, et al. J. Anim. Sci. v. 92, p.4189–4197, 2014). A total of 478 beef heifers [*B. taurus* (n = 368) and crosses (n = 110)] was used to compare TAI moment using conventional protocol of AI (48 or 60h) in females that did not display estrus until 48h after progesterone (P4) device removal. Females belonged to three farms of central and western regions of Rio Grande do Sul State and presented body condition score (BCS) of  $3.2 \pm 0.5$  (range 1 to 5) at beginning of protocol (Day 0). On Day 0, heifers were classified according to the presence of corpus luteum (with CL; n=392 and without CL; n=85) and received the insertion of P4 device previously used for 16 days was done (PRIMER®, Tecnopec) plus 2mg of estradiol benzoate (BE, RIC-BE®, Tecnopec) IM. On Day 8, P4 device was removed and 0,482mg of sodic cloprostenol (ESTRON®, Tecnopec) IM and 1mg of estradiol cypionate (cypionate HC®, Animal HertapeCalierSaude) IM were administrated. At this moment, females were marked with a paint stick (Raidl-Maxi, RAIDEXGmbH, Dettingen / Erms, Germany) in the sacral region. Heifers with clean sacral region 48 hours after P4 device removal were considered as displayed estrus and were immediately inseminated (Estrus-TAI48h; n=311). Heifers without estrus received 10µg GnRH (buserelin; Prorelin®) IM and were randomly assigned to be inseminated at 48 hours (NEstrus-TAI48; n=87) or at 60 hours (NEstrus-TAI60; n=79) after P4 device removal. The pregnancy diagnosis was performed 30 days after TAI by ultrasound. Statistical analysis was performed using PROC GLIMMIX SAS (SAS 9.3). The overall P/AI was 47.8% (228/478). There was no difference (P = 0.31) on P/AI among groups [Estrus-TAI48h = 49.5% (154/311), NEstrus-TAI60h = 49.4% (39/79) e NEstrus-TAI48h = 40.2% (35/87)]. Despite of the similarity among treatments, these results suggest that it is possible to improve the P/AI in heifers that did not display estrus 48 hours after P4 device removal, delaying the TAI moment associated with GnRH. It is noteworthy that further studies including a larger number of animals are certainly required.

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A077 FTAI, FTET and AI

## Evaluation of the effect of administering different commercial eCG on follicle growth and conception, and validation of its need in TAI protocols for postpartum Nelore cows

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**Keywords:** beef cows, equine chorionic gonadotropin, postpartum anestrus.

This study evaluated the effect of two different commercial equine chorionic gonadotropin (eCG) on ovarian follicular growth, dominant follicle diameter (ØFD) and pregnancy rate of Nelore cows (*Bos indicus*, n = 943) with 30-50d postpartum and synchronized to timed artificial insemination (TAI). The study was conducted at Vera Cruz IV Farm, Barra do Garças - MT. At the beginning of the treatment (D0), cows received an intravaginal device with 1g progesterone (Cronipres®, Biogenesis Bagó, Brazil) and 2 mg of estradiol benzoate i.m. (Bioestrogen®, Biogenesis Bagó). On D8, the device was removed and cows were administered i.m. 150µg of D-cloprostenol (Croniben®, Biogenesis Bagó) i.m and 1 mg of estradiol cypionate (Croni-Cip®, Biogenesis-Bagó). At that time, cows were homogeneously allocated (according to body weight, BCS and ØFD on D8) to one of three experimental groups: Control (without eCG), Novormon (MSD Animal Health, Brazil, 300IU i.m.) or Ecegon (Biogénesis-Bagó, 300IU i.m.). The TAI was performed 54h after device removal. Ultrasound examinations were performed on D0 to verify uterine regression, on D8 and D10 to evaluate the ØFD (mm; n = 529), and on D40 for pregnancy diagnosis (n = 943). The daily follicular growth rate (mm/d) was calculated by the difference between the ØFD on D8 and D10 divided by two. Data were analyzed using PROC GLIMMIX from SAS. Cows average BCS was 2.80 ± 0.02. The ØFD on D8 was similar (P = 0.92) between control (11.3 ± 0.2; n = 178), Novormon (11.1 ± 0.2; n = 174) and Ecegon (11.3 ± 0.2; n = 177), demonstrating the homogeneity between groups immediately before the treatment. However, cows treated with Novormon (13.4 ± 0.2<sup>a</sup>; n = 174) or Ecegon (13.5 ± 0.2<sup>a</sup>; n = 177) had higher ØFD in D10 than control cows (12.8 ± 0.2<sup>b</sup>; n = 178; P = 0.001). Besides, the total (from D8 and D10) and daily follicular growth rates were higher in groups Novormon (2.4 ± 0.1<sup>a</sup> and 1.2 ± 0.05<sup>a</sup>; n = 174) and Ecegon (2.3 ± 0.1<sup>a</sup> mm and 1.2 ± 0.05<sup>a</sup>; n = 177) compared to control group (1.6 ± 0.1<sup>b</sup> and 0.8 ± 0.04<sup>b</sup>; n = 178; P = 0.001), respectively. Similarly, pregnancy rate 35d after TAI was greater (P = 0.03) in cows treated with Novormon (52.1%; 160/307) and Ecegon (52.8%; 181/343) in relation to control cows not treated with eCG (43.3%; 127/293). Therefore, the efficacy of eCG to increase ovarian follicular growth and the size of the dominant follicle in TAI protocols was demonstrated, and it has enabled the increase of pregnancy rate in postpartum Nelore cows. Also, both commercial eCG tested (Novormon<sup>®</sup> and Ecegon<sup>®</sup>) were equally efficient.

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### **Conception rates and pregnancy losses in embryo recipients treated with lecirrelin 13 days after FTET - preliminary results**

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**Keywords:** *Bos indicus*, GnRH, resynchronization.

The use of GnRH analogues for resynchronization of females previously submitted to FTAI has been previously reported (Vianna and Gois, 2014, Anim. Reprod. 11, 353). This study compared the pregnancy per embryo transfer (P/ET) and pregnancy loss of embryo recipients treated or not with Lecirelin. In study 1, retrospectively data of 4263 crossbreed (Angus x Nelore) *in vitro* produced embryo transfer in Nelore cows recipients (ECC: 3.0 to 2.5 range 1-5) were used. All recipients were previously synchronized with progesterone (P4) intravaginal device (CIDR®, Zoetis, Brazil) and 2 mg estradiol benzoate (Gonadiol®, Zoetis). Nine days later, they received 12.5 mg PGF2α (Lutalyse®, Zoetis), 300 IU eCG (Novormon®, Zoetis) and 1 mg estradiol cypionate (E.C.P®, Zoetis) IM. The timed embryo transfer (TET) was performed 18 d after the beginning of the hormonal treatment. Thirteen days later, they received (G-P4+GnRH; n=810) or no (G-P4; n=3453) 25 µg of lecirrelin (Gestran Plus, Tecnopec, Brazil) IM. Transrectal ultrasonography for pregnancy diagnosis was done 29 and 60 days of the gestation. In the study 2 was retrospectively analysed similar to the previous, however the produced embryos were from Nelore bred (n=2152, G-P4+GnRH, n=341, G-P4, n=1811). Data were analyzed by Chi-square test (p <0.05). In the study 1, the P/ET at 29 days (P≤0.001) were higher G-P4 [47.0%, (1622/3453) than G-P4+GnRH [33.6%, (285/810)]. However at 60 days, the P/ET (P=0.11) were similar [G-P4: 40.6% (1405/3453) and G-P4+GnRH: 33.2 (269/810)]. Pregnancy losses (P≤0.001) were lower in the G-P4+GnRH [5.6% 16/285] than G-P4 [13.3% (217/1622)]. In experiment 2, the P/ET at 29 and 60 days of G-P4 (P=0.001) were higher [29 days: 39.2% (710/1811) and 60 days: 33.1% (601/1811)] than G-P4+GnRH [29 days: 30.0% (102/341) and 60 days 26.6% (91/341)]. The pregnancy losses (P=0.28) were similar in groups [G-P4 (15.3% 109/710), G-P4 + GnRH: 10.7% (11/102)]. These preliminary results showed that the application of lecirrelin unexpectedly decreased P/ET in the two retrospectively analysis. However, the pregnancy loss was reduced after the GnRH analogue administration. Further prospectively studies are necessary to confirm these results.



A079 FTAI, FTET and AI

### **Pregnancy rates in nonsuckled or postpartum Nelore cows treated with lecirrelina 13 days after ftet**

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**Keywords:** beef cattle, embryo recipients, GnRH.

The aim of this study was to compare the embryo transfer per synchronized females (ET/treated), conception (P/transferred) and pregnancy (P/treated) in nonsuckled and suckled Nelore cows and subjected to timed embryo transfer (TET) using resynchronization with unknown pregnancy status. Nelore cows (n=3652) with BCS from 3.0 to 3.5 (range 1 to 5) were used as embryo recipients and allocated into two experimental groups: 1062 suckled cow 40 to 90 days postpartum (G-S) and 2590 nonsuckled cows (G-NS). All recipients were previously treated with P4 intravaginal device (CIDR®, Zoetis, Brazil) and 2 mg estradiol benzoate (Gonadiol®, Zoetis). Nine days later, they received 12.5 mg PGF2α (Lutalyse®, Zoetis), 300 IU eCG (Novormon®, Zoetis) and 1 mg estradiol cypionate (E.C.P®, Zoetis) IM. The TET was performed 18 d after the beginning of the hormonal treatment. Thirteen days after TET, they received 25 µg of lecirelin (Gestran Plus, Tecnopec, Brazil) IM. Pregnancy diagnosis was done 39 days after the start of hormone treatment and non-pregnant recipients with CL received an embryo (second TET). Data were analyzed by Chi-square test (p<0.05). The P/transferred was 47.5% (1232/2590) and 58.1% (617/1062); (P≤0.001) G-NS and G-S, respectively. After treatment with lecirelin, the ET/treated of non-pregnant cows (P≤0.001), P/transferred (P=0.02) and P/treated (P≤0.001) were higher for G-S [87.8% (391/445); 39.3% (154/391) and 34.6% (154/445)] than for G-NS [78.7% (1070/1358), 33.0% (353/1070) and 26.0% (353/1358)]. Cumulative pregnancy rate at 21 days, considering both TETs was 72.5% (771/1062) for G-S cows and 61.1% (1585/2590) for G-NS (P≤0.001). In conclusion, the resynchronization with lecirelin provided adequate ET/treated, P/transferred and P/treated rates in suckled and nonsuckled cows. Nelore suckled cows presented higher ET/treated, P/transferred and P/treated rates in relation to nonsuckled cows. Thus, using postpartum suckled cows as embryo recipients for TET may be an interesting animal category option.



A080 FTAI, FTET and AI

## Comparison between performance data of yearlings and 2 years old Nelore heifers - preliminary data

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**Keywords:** Nelore, precocious pregnancy, yearling pregnancy.

Aiming to diminish the backgrounding of the heifers associated to usage of genetic selection to precocious calving (PC), yearling Nelore heifers have been breeding to become pregnant at this early age. The PC has positive correlation with economic characteristics that impact the beef operation system. The aim of this trial was to evaluate retrospectively the productive data of heifers that became pregnant between 12 to 16 months (Precocious = P) or 24 to 27 months (Not precocious = NP). A total of 305 heifers born between September and November of 2010 and weaned between May and July of 2011 received a 600 grams/animal/day protein mixture between May and December. At December of 2011, all heifers received an induction of puberty protocol following timed artificial insemination (TAI) according to Rodrigues et al., (Theriogenology, 82, 760, 2014). After the TAI, heifers were exposed to bulls in a proportion of one bull for 30 heifers until March 2012 (2011/2012 breeding season; BS). Heifers that become pregnant in the first BS were classified as P (n=164) and heifers that were not pregnant were classified as NP (n=141). The NP heifers went to the next BS (2012/2013) with 24 to 27 months of age. All heifers were evaluated in the subsequent BS (2012/2013 and 2013/2014). The data was retrospectively compared two groups (P vs. NP) of heifers: number of gestations, calving rate and number of weaned calves, total weight (Kg) of weaned calf, pregnancy loss, calf mortality, weight of heifer at weaning, weight of heifer at beginning of BS and mature weight. Mature weight was achieved at approximately 44 months of age. Data were analyzed using MIXED procedure from SAS. There was no difference between age of heifers of P and NP group (432 days = 14.1 mo). The P heifers presented higher number of gestations ( $2.3 \pm 0.05$  vs.  $1.2 \pm 0.06$ ), number of calvings ( $2.1 \pm 0.06$  vs.  $1.2 \pm 0.07$ ) and number of weaned calves ( $1.4 \pm 0.04$  vs.  $0.7 \pm 0.05$ ) compared to NP group ( $P < 0.05$ ), respectively. Total weight of calf weaned (Kg) was higher to P group ( $312.5 \pm 10$ ) compared to NP ( $146.8 \pm 11$ ). Pregnancy loss (%) were higher for P (14.0) group compared to NP (4.3). There was no difference in calf mortality (10.5%), weight of heifers at weaning ( $214.1 \pm 1.8$ ), at beginning of BS ( $280.0 \pm 1.7$ ) and the mature weight ( $445.1 \pm 3.6$ ) of P and NP heifers. The pregnancy rates (%) at TAI and at end of BS when primiparous was  $41.6^a$  and  $67.9^x$  for P and  $27.9^b$  and  $60.4^y$  for NP, respectively. Weight of calves at weaning (Kg) for first and second parurition of P group was  $211^a$  and  $233^b$ , although was  $206^a$  for primiparous cows of NP group. In conclusion, yearling Nelore heifers increase the total weight of calf weaned, even with higher pregnancy loss, and also not alter their mature weight. Further prospective studies certainly should be necessary to validate these results.



A081 FTAI, FTET and AI

### **Circulating concentration of AMH in Holstein and Jersey breeds and its relationship to fertility in lactating cows and heifers**

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**Keywords:** AMH, dairy cow, fertility.

The objective of this study was to verify whether the circulating concentration of the anti-Mullerian hormone (AMH) was associated with fertility in Holstein and Jersey lactating cows and nulliparous heifers. Blood samples were taken from the caudal vein/artery from lactating cows (n = 141 Holsteins and n = 82 Jerseys) and heifers (n = 407 Holsteins and n = 122 Jerseys) with evacuated tubes containing EDTA. Samples were immediately refrigerated and plasma harvested, placed into microcentrifuge tubes within 2h and frozen until AMH analysis. AMH analysis was performed with the MOFA Global (Bovine Fertility Assay®) kit. Lactating cows and heifers underwent daily estrus detection performed in the mornings and followed by AI. Non-pregnant animals received an Ovsynch-like timed AI protocol. Retrospective analysis of the reproductive parameters included: 1) interval from calving to conception for lactating cows; 2) age at conception for nulliparous heifers. Statistical analysis was performed with the Glimmix and Corr procedures of the SAS software (Version 9.3 for Windows). The circulating AMH concentration in lactating cows was 312 ± 47 and 318 ± 61 pg/ml for Jersey and Holstein respectively (P>0.10). However, Jersey heifers had greater circulating AMH than Holstein heifers (311 ± 31 pg/ml; 225 ± 24 pg/ml respectively; P<0.05). Despite of breed, circulating AMH was not associated with calving to conception intervals in lactating cows (r = -0.05, P>0.10) or age at conception in nulliparous heifers (r = -0.06, P>0.10). In conclusion, circulating AMH doesn't correlate with reproductive performance of lactating cows or heifers from Holstein and Jersey breeds. Further studies are needed using a larger number of animals to confirm these findings.

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A082 FTAI, FTET and AI

### **Progesterone concentration on pregnancy in Holstein cows enrolled in a TAI protocol based on P4/E2**

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**Keywords:** fertility, primiparous, progesterone.

The present study evaluated if the progesterone (P4) concentration during ovulatory follicle development in a timed artificial insemination (TAI) protocol based on P4/E2 affects pregnancy per AI in lactating Holstein cows. Our hypothesis was that cows presenting greater P4 concentration have also greater fertility. To alter P4 concentration, females (n=594) were randomly assigned to receive one (n=310) or two (n=284) intravaginal devices of P4 (CIDR®, Zoetis, SP, Brazil). The TAI protocol utilized was: d-11 intravaginal device of P4 (new or previously used by 9 days) + 2mg im EB (Gonadiol®, Zoetis) + 100mcg im GnRH (Cystorelin®, Merial, SP, Brazil); d-4, 25mg im Dinoprost (Lutalyse®, Zoetis); d-2, 25mg im Dinoprost + 1mg im ECP (ECP®, Zoetis) + CIDR removal; d0, TAI. Females in the group 2CIDR received an additional CIDR at d-11, which was removed at d-4. At d-11 (n=117), d-4 (n=351), d0 (n=214) and d10 (n=72), blood samples were taken from cows for P4 concentration determination. Pregnancy per AI was determined by ultrasound at d32 (DG1) and d60 (DG2). The binomial data were analyzed using PROC GLIMMIX and continuous data using PROC MIXED of SAS. An effect was considered significant when P<0.05 and tendency when P<0.1. P4 did not differ among treatments at d-11 (1CIDR=4.2±0.4 ng/ml; 2CIDR=4.5±0.4 ng/ml; P>0.1), and at d-4 (1CIDR=3.5±0.2 ng/ml; 2CIDR=3.8±0.2 ng/ml; P>0.1). An interaction was detected between treatment and CL presence at the beginning of TAI protocol in P4 at d-4 (without CL and 1CIDR=2.7±0.3 ng/ml; 2CIDR= 3.6±0.3 ng/ml; P<0.05). There was no difference among treatments in pregnancy per AI and pregnancy loss between DG1 and DG2 (DG1: 1CIDR=26.1% [81/310] vs. 2CIDR=22.9% [65/284]; DG2: 1CIDR=23.9% [74/310] vs. 2CIDR=20.1% [57/283]; Pregnancy loss: 1CIDR=8.6% [7/81] vs. 2CIDR=10.9% [7/64]; P>0.1). An interaction was detected between [P4] at d-4 and parity (Primiparous=PP; Multiparous=MP) on DG1 (P4 < 2.61 ng/ml: PP=12.1% [16/69] vs. MP=10.1% [21/107]; P4 ≥ 2.61 ng/ml: PP=37.7% [28/63] vs. MP=14.6% [30/113]; P<0.05), and on DG2 (P4<2.61ng/ml: PP=12.4% [14/69] vs. MP=8.4% [19/107]; P4 ≥ 2.61 ng/ml: PP=34.2% [27/63] vs. MP=11.2% [27/113]; P<0.05). Exogenous supplementation of P4 in lactating Holstein cows submitted to a TAI protocol based on P4/E2 with an injection of GnRH at its beginning did not alter the pregnancy. The increase of P4 concentration during follicular development increases the fertility particularly in primiparous cows, but not in multiparous.



A083 FTAI, FTET and AI

### **Progesterone concentrations and ovarian response after PGF2 $\alpha$ administration at beginning of TAI protocol in *Bos taurus* beef heifers**

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Fundación Irauy.

**Keywords:** monodose, progesterone concentrations, TAI.

This experiment was carried out to evaluate the effect of the administration of PGF2 $\alpha$  at different moments of a progesterone (P4) and estradiol based treatment, on serum P4 concentrations, ovulatory follicle diameter and time of ovulation in *Bos taurus* beef heifers. Hereford heifers (n=25) with corpus luteum detected by ultrasonography and body condition score 4.5 $\pm$ 0.1 (Mean $\pm$ SEM, 1 to 8 scale) were used. Heifers were randomly allocated to one of two experimental groups: 1) two half PGF2 $\alpha$  doses of 250  $\mu$ g of cloprostenol (DL Ciclase, Syntex, Buenos Aires, Argentina) administered in the insertion and progesterone device removal or 2) a single dose of 500  $\mu$ g of cloprostenol administered at the time of P4 device removal. Females received an intravaginal device during 7 days containing 0.5 g of P4 (DIB 0.5, Syntex) and 2 mg of estradiol benzoate (Gonadiol, Syntex). At the time of DIB removal, 0.5 mg estradiol cypionate (Cipiosyn, Syntex) and 400 IU of eCG (Novormon, Syntex), were intramuscularly administered. The P4 concentrations were determined daily from the beginning of the treatment until 24 hours after P4 device removal. Ovarian follicles were monitored every 12 hours by ultrasonography (WED-9618V, Well.D, Shenzhen, China) since DIB removal until ovulation time. Data were analyzed by GLM and presented as mean $\pm$ SEM. According to the results, PGF2 $\alpha$  administration at the beginning and at the end of treatment induced lower P4 concentrations during DIB treatment (3.9 $\pm$ 0.4 vs. 7.1 $\pm$ 1.1 ng/mL; P<0.05). Furthermore, a greater ovulatory follicular diameter at DIB removal (12.2 $\pm$ 0.5 mm) compared with the group that received only PGF2 $\alpha$  at the end of treatment (9.6 $\pm$ 0.5 mm; P<0.05) was observed. At ovulation, follicular diameter showed a tendency to be larger in heifers treated with PGF2 $\alpha$  at beginning and the end of treatment (14.4 $\pm$ 0.4 mm) compared to heifers treated only at the end of treatment (13.1 $\pm$ 0.5 mm; P<0.1). Ovulation rate were 100% (13/13) and 83.3% (10/12) for heifers with PGF2 $\alpha$  at the beginning and the end vs. PGF2 $\alpha$  those received only at the end of treatment (P=0.43). Heifers treated with PGF2 $\alpha$  at the beginning (64.6 $\pm$ 10.4 h) showed a shorter interval between the P4 device withdrawn and ovulation compared to those treated only on device removal (78.0 $\pm$ 8.5 h; P<0.05). In conclusion, PGF2 $\alpha$  administration at the beginning and progesterone device removal reduced the P4 concentrations during P4 device treatment, increased ovulatory follicular diameter at P4 device removal and reduces the interval between the withdrawal P4 device and ovulation in *Bos taurus* beef heifers.



A084 FTAI, FTET and AI

## **Endogenous progesterone concentrations affect progesterone release from intravaginal devices used for estrus synchronization in cattle**

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**Keywords:** artificial insemination, FTAI, progesterone.

The increasing use of timed artificial insemination (TAI) observed in Brazil in the last years was mainly due to the possibility of anticipating time to pregnancy, including in noncycling cows. In this context, a number of studies support the use progesterone (P4) devices in synchronization programs, but it represents 43% of the total cost of the protocols. In order to facilitate the application of the technique and reduce hormone costs, several authors propose re-utilization of this progesterone release devices (DLP), but the results are controversial and do not describe with precision the amount of remaining steroid in these devices after being used in cows at different luteal status. Thirty cyclic crossbred heifers were divided into three groups (n=10/group). In the G1 and G2 the DLP (DIB, MSD – Brazil) was inserted (D0) seven days after the induced ovulation with estradiol benzoate (Benzoato HC, Hertape – Brazil). Furthermore, 0.15 mg of D-cloprostenol (Veteglan, Hertape – Brazil) was administered three days after the DLP insertion to promote luteolysis in G2. In the G3, the luteolysis was induced with 2 doses of D-cloprostenol administrated 4 and 3 days before DLP insert aiming to maintain only the exogenous P4 source. The three groups remained with DLP for 8 days, simulating a conventional TAI protocol. After treatment, each group of three DLP's were separated and subjected to removal and weighing of grinding silicon. An extra group using a new device (G4) was added. The P4 extraction was performed with methanol and the samples were analyzed in duplicate by RIA (IM 1188, Immunotech Inc., Prague, Czech Republic). The G4 samples were considered as standard and used for extraction technique validation. P4 means were compared between groups using the Tukey test. The P4 concentration that remained into the P4 devices was  $30.75 \pm 1.13$  ng/mL (a);  $14.65 \pm 1.65$  ng/mL (b);  $10.96 \pm 1.15$  ng/mL (c);  $7.91 \pm 1.22$  ng/ml (d) ( $P < 0.05$ ) for G4, G1, G2 and G3 respectively. In addition, the absolute value of the residue found were 1,000 mg (a); 478 mg (b); 349 mg (c) and 257 mg (d), for G4, G1, G2 and G3 ( $P < 0.05$ ), respectively. Thus, the amount of P4 releasing from DLP is influenced by endogenous concentration of this steroid. Animals with high levels of endogenous P4 determined greater residual of P4 into the DLP. Additionally, lower quantity of residual P4 is expected when noncycling females are treated.

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A085 FTAI, FTET and AI

### **Different doses of equine chorionic gonadotropin in TAI protocol on suckled *Bos taurus* beef cows: impact on follicle growth and occurrence of estrus**

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**Keywords:** *Bos Taurus*, eCG, IATF.

This study evaluated the use of different doses (0, 300 or 400 IU) of equine chorionic gonadotrophin (eCG) administered at the moment at progesterone (P4) device removal and their impact on follicular growth and estrus occurrence on suckled *Bos taurus* beef cows. Cows from five commercial beef farms located in three distinguished regions of Rio Grande do Sul state, Brazil were used. At the onset of the synchronization protocol (D0), 966 cows with body condition score  $2.77 \pm 0.02$  (score 1-5) received an intravaginal P4 device (CIDR®, Zoetis, SP, Brazil) and 2 mg of estradiol benzoate IM (Gonadiol®, Zoetis). On Day 9, the P4 device was removed, and were administrated 12,5 mg of dinoprost tromethamine IM (Lutalyse®, Zoetis) plus 1mg of estradiol cypionate IM (E.C.P.®, Zoetis). At this moment, cows were randomly assigned to one of three treatments: Control (n = 323), 300 IU (n = 326) or 400 IU (n = 317). The TAI was 48 h later P4 device removal. The females had their tail-heads painted with chalk paint (Raidl-Maxi, Raidex GmbH, Dettingen / Erms, Germany) at the time of removed of P4 device. Females without mark at the time of TAI were considered as displayed estrous. Additionally, 435 cows in anoestrus (no corpus luteum at the time of device insertion P4; Control: n= 146; 300UI: n= 153; 400 IU: n= 136) had follicular diameter at the time of the remove device P4 and the TAI evaluated to determine follicular growth after treatment. Statistical analyses were performed using the GLIMMIX procedure of SAS and the data compared by orthogonal contrast (C): C1 (eCG effect) and C2 (effect of eCG dose). The eCG treatment increased the estrus occurrence (Control = 53.7%, 300 IU = 70.6% and 400 IU = 77.0%; C1;  $P < 0.0004$ ), however, there was no difference between the doses used (C2;  $P = 0.58$ ). Also, because of the similarity of follicular diameter at the time of P4 device removal (Control =  $11.0 \pm 0.2$  mm, 300 IU =  $10.7 \pm 0.2$  mm and 400 IU =  $11.0 \pm 0.2$  mm; C1;  $P = 0.32$  and C2;  $P = 0.38$ ) and larger follicular diameter on the TAI of cows treated with eCG (Control =  $13.5 \pm 0.3$  mm, 300 IU =  $14.0 \pm 0.2$  mm and 400 IU =  $15.1 \pm 0.3$  mm; C1 ;  $P < 0.04$  and C2;  $P = 0.19$ ) the follicular growth (mm/day) was greater (Control =  $1.2 \pm 0.1$  mm, 300 IU =  $1.6 \pm 0.1$  mm and 400 IU =  $2.1 \pm 0.1$  mm; C1;  $P < 0.0001$ ) in cows treated with eCG, regardless of the administered doses (C2;  $P = 0.33$ ). According to the literature, most animals having estrus manifest follicle and have higher probability of pregnancy. Therefore, the treatment with eCG, regardless of the dose of 300 or 400 IU administered, increases the occurrence of estrus and follicular growth in suckled *Bos taurus* beef cows.

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A086 FTAI, FTET and AI

### **Different doses of equine chorionic gonadotropin on timed artificial insemination protocol in suckled *Bos taurus* beef cows: impact on fertility**

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**Keywords:** bovine, eCG, FTAI.

This study evaluated the use of different doses (0, 300, or 400 IU) of equine chorionic gonadotropin (eCG; Novormon®, Zoetis, Cravinhos, SP, Brazil) at the time of withdrawal of progesterone device (P4; CIDR®, Zoetis), and its impact on pregnancy per artificial insemination (P/AI) in suckled *Bos taurus* beef cows. Cows maintained on a native pasture in five commercial farms located in three distinguished regions of Rio Grande do Sul / Brazil (Southeast, Southwest and Western center). At the beginning of protocol (D0), a total of 996 cows with body condition score of  $2.77 \pm 0.02$  (scale of 1 to 5) received a P4 insert and 2.0 mg estradiol benzoate IM (EB; Gonadiol®, Zoetis). On D9, P4 insert was removed, 12.5 mg of dinoprost tromethamine (Lutalyse®; Zoetis) and 1 mg of estradiol cypionate (E.C.P.®; Zoetis) were administered via IM. At this moment, cows were randomly assigned to receive one of three treatments: Control (n = 323; no eCG), 300 IU (n = 326) or 400 IU (n = 317) of eCG. The timed artificial insemination (TAI) was performed 48h after removal of P4. Additionally, a subset of cows (n = 718) were subjected to ultrasound examination at the time of insertion and removal of the P4 for evaluating the presence of the corpus luteum (CL). Pregnancy diagnosis was performed by ultrasound 30d after TAI. Statistical analysis was performed using the PROC GLIMMIX of SAS®, and the data were analyzed by orthogonal contrasts (C): C1 (eCG effect) and C2 (eCG dose effect). The eCG treatment increased the P/IA (control = 29.7%; 300 IU = 44.8% or 400 IU = 47.6%; C1; P < 0.0001). However, no difference between eCG doses (C2; P = 0.55) effect was found. In the subset of cows (n = 718) that had presence of CL evaluated, an interaction between cyclic status and treatment on P/AI (P = 0.05) was observed. Overall cyclicity rate was 21.2% (152/718). There was no effect of eCG in the cyclic cows [Control = 55.4% (31/56); 300 IU = 54.2% (26/48) or 400 IU = 52.1% (25/48)]. However, non-cyclic cows showed positive effect of eCG treatment [Control = 25.8% (47/182); 300 IU = 41.6% (82/197) or 400 IU = 49.7% (93/187)]. The eCG treatment (300 or 400 IU) administered at the time of P4 removal increased P/AI in anestrous suckled *Bos taurus* beef cows.

**Acknowledgments:** Zoetis, CAPES, Agropecuária Posto Branco – Fazenda Nova Esperança, Cabanha Aguada, Agropecuária Odair Gonzáles, Fazenda Três Angicos e Estância Nova Aurora.



A087 FTAI, FTET and AI

### **Follicle diameter on which eCG induces effect over follicular growth and fertility of *Bos indicus* cows submitted to TAI**

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**Keywords:** eCG, fertility, zebu.

The objective of this study was to identify the diameter of the follicle, at the moment of eCG treatment, on which the eCG treatment increase on the final follicular growth and pregnancy rate in *Bos indicus* cows submitted to timed artificial insemination (TAI). The study enrolled 556 lactating cows, with post-partum between 30 and 60 days and body condition score (BCS) of  $2.89 \pm 0.02$  (1 to 5 scale). In a unknow stage of the estrus cycle (D0) all animals received 2 mg of estradiol benzoate (Sincrodiol®, Ourofino, Brazil) and an intravaginal progesterone device (Sincrogest®, Ourofino). On D8 all cows received 500µg of cloprostenol (Sincrocio®, Ourofino), 1 mg of estradiol cypionate (E.C.P.®, Pfizer, Brazil) and the progesterone devices were removed. At this point, cows were assigned, according to the follicular diameter, in one of two treatments (Control or eCG). Animals from eCG (n=277) received 300IU of eCG (Folligon®, MSD, Brazil) and cows from Control (n=279) received no additional treatment. Cows received a TAI 48 hours after progesterone device removal. On the study, ultrasound exams were performed on D8 and D10 to measure the follicular diameter and on D40 for pregnancy diagnosis. All data were analyzed by GLIMMIX procedure of SAS. After evaluation of different follicular diameters at progesterone device removal, it was observed that eCG showed effect on follicular growth and pregnancy rate of cows with follicles smaller than 10.6mm and 9.4mm, respectively. Thus, in cows with follicles <10.6 mm, the eCG Group ( $1.35 \pm 0.09$  mm/day) showed greater follicular growth when compared to Control Group ( $0.79 \pm 0.07$  mm/day;  $P=0.001$ ) and in cows with follicles <9.4 mm, eCG Group [37.4% (34/91)] presented higher pregnancy rate than the Control Group [15.7% (17/108);  $P=0,001$ ]. In conclusion, treatment with eCG in TAI protocols increases final follicular growth and pregnancy rate in cows with follicles <10.6 mm and <9.4 mm at progesterone device removal, respectively.



A088 FTAI, FTET and AI

### **eCG increases fertility of crossbred dairy cows submitted to FTAI early in postpartum**

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**Keywords:** dairy cow, eCG, FTAI.

The aim of this study was to evaluate the effect of eCG on fertility of crossbred (*Bos taurus* x *Bos indicus*) lactating dairy cows submitted to a fixed time AI (FTAI) protocol between October 2013 and July 2014. A total of 679 cows, 492 multiparous and 187 primiparous, with an average milk production of  $21.4 \pm 7.6$  kg/d and average of  $124.6 \pm 97.0$  DIM was used. At a random day of the estrous cycle all cows received an intravaginal progesterone device (CIDR®, Zoetis, Brazil), 2 mg estradiol benzoate im (Gonadiol®, MSD, Brazil) and 100 µg gonadorelin (Fertagyl®, MSD, Brazil) in the morning (D0). At D7 morning, all cows received 150 µg D-Cloprostenol im (Sincrocio®, Ouro Fino, Brazil) and were randomly assigned into two groups: eCG (n = 340) – received 400 IU eCG im (Novormon®, MSD); and Control (n = 339) – did not receive eCG. At the afternoon of D8, devices were removed and all cows received 150 µg D-Cloprostenol im and 1 mg estradiol benzoate im. The FTAI was performed at D10 morning. Diagnosis and confirmation of pregnancy were performed 30 and 60 days after AI. Data were analyzed by logistic regression using the PROC GLIMMIX of SAS and the results are presented following the group order eCG and Control. On pregnancy per AI (P/AI), the eCG improved fertility at 30 (37.8% vs. 30.2%; P = 0.06) and 60 (31.9% vs. 25.1%; P = 0.08) days. However, pregnancy loss between 30 and 60 days (9.4% vs. 14.4%; P = 0.3) and 60 days to calving (9.2% vs. 12.2%; P = 0.5) did not differ between groups. In addition, no difference was observed in the twin calving between groups (4.1% vs. 2.4%; P = 0.5). Furthermore, there was interaction (P = 0.09) between the use of eCG and DIM. Cows treated with eCG and inseminated up to 70 days postpartum had higher P/AI at 30 (39.0% vs. 25.2%; P = 0.02) and 60 (32.8% vs. 21.3%; P = 0.05) days after FTAI. However, in cows with more than 70 DIM this did not happen at 30 (36.6% vs. 35.7%; P = 0.8) nor at 60 (31.1% vs. 29.9%; P = 0.7) days. It was concluded that the use of eCG on D7 of the protocol increased fertility of crossbred lactating dairy cows submitted to FTAI due to improvement in the P/AI of cows inseminated until 70 DIM. Additionally, the use of eCG on D7 of the protocol did not increase twinning rate.

**Acknowledgments:** FAPESP, CNPq, CAPES, MSD, Queima Ferro and São João Farms.



A089 FTAI, FTET and AI

### **Effect of progesterone concentration and the time GnRH administration on pregnancy rates in Holstein heifers treated with the J-synch protocol**

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**Keywords:** GnRH, Holstein, progesterone.

The aim of this study was to evaluate the effect of different concentrations of progesterone (P4) in the intravaginal devices (DIV) and different times of GnRH administration to induce ovulation in dairy heifers treated with the J-Synch protocol. In Experiment 1, Holstein heifers (n = 80) were used. On Day 0, all heifers received 2 mg of estradiol benzoate (BE, Proagro Argentina), while half were treated with DIV with 0.5 g of P4 (Synkro, Proagro, Argentina) and the other half with a DIV with 1 g of P4 (DIB, Syntex SA, Argentina). On Day 6, DIVs were removed and all heifers received 150 mg D + cloprostenol (PGF; Sincronil, Proagro). Heifers were again subdivided to receive 10.5 mg buserelin acetate (GnRH, Pro Buserelin, Proagro) 56 or 72 h after DIV removal and all were inseminated at fixed time (FTAI) at 72 h. Follicular dynamics, ovulation and pregnancy rates at 32 days were evaluated by ultrasonography (Honda 101 V, 5.0 MHz). When ovulation occurred earlier when the GnRH was administered at 56 h ( $91.9 \pm 3.2$  h) than when it was given at 72 h ( $98.8 \pm 3.2$  h;  $P = 0.035$ ). However, there was no difference in the time of ovulation between heifers treated with DIV 0.5 g ( $92.8 \pm 2.2$  h) or DIV 1 g P4 ( $97.9 \pm 2.8$  h;  $P = 0.126$ ). There was an interaction between DIV and GnRH time ( $P = 0.018$ ) in the diameter of the preovulatory follicle, being smaller for the subgroup DIV 1 g and GnRH 56 h ( $11.8 \pm 0.4$  mm) from the subgroup DIV 1 g and GnRH 72 h ( $13.4 \pm 0.4$  mm;  $p < 0.05$ ), while the other subgroups, DIV 0.5 g and GnRH 56 h ( $13.1 \pm 0.4$  mm) and DIV 0.5 g and GnRH 72 h ( $12.6 \pm 0.4$  mm) were intermediate. Finally, pregnancy rate was higher in animals treated with DIV 0.5 g (19/40; 72.5%) compared to animals receiving DIV 1 g (16/40; 40%;  $P = 0.006$ ). Although there was no difference between heifers treated with GnRH at 56 h (55.0%) or 72 h (57.8%;  $P = 0.9$ ). In Experiment 2, the pregnancy rate was determined in Holstein heifers (n = 417) that received DIV 0.5 g P4 and 2 mg EB on Day 0 and were divided into three groups. Heifers in the first two groups were treated with the J-Synch protocol, receiving GnRH at 56 or 72 h and received a FTAI at 72 h of DIV removal. Heifers in the control group were treated with a DIV for 7 days, PGF with the removal of DIV, 1 mg of EB on Day 8 and were FTAI at 56 h after DIV removal. There were no significant differences in pregnancy rates among heifers treated with the J-Synch protocol with GnRH at 56 h (82/137; 59.9%), those treated with the J-Synch protocol and GnRH at 72 h (89/140; 63.6%) and the control group (86/139; 61.9%;  $P = 0.816$ ). In conclusion, although although DIV with low P4 content improve pregnancy rates in heifers treated with the J-Synch protocol the time of GnRH administration did not affect pregnancy rates. Finally, pregnancy rates to the J-Synch protocol was not different to that in the conventional 7-day protocol.



A090 FTAI, FTET and AI

### **Effect of angiotensin-(1-7) on ovulation rate of goats submitted to fixed-time artificial insemination**

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**Keywords:** ACE, artificial insemination, progesterone.

A previous study showed an increase in estradiol production near ovulation in sheep subjected to estrus synchronization protocol when applying enalapril maleate (inhibitor of the Angiotensin Converting Enzyme-ACE) in the last three days of estrus synchronization protocol (Costa et al., *Reproduction in Domestic Animal*, v.49, p. e53-e55, 2014). The mechanism of this response may be due to the increase of angiotensin-(1-7), a new type peptide of the renin-angiotensin system, which occurs as a consequence of ACE inhibition (Brosnihan et al., *Brazilian Journal of Medical and Biological Research*, v.37, p.1255-1262, 2004). In this study was evaluated the effects of angiotensin-(1-7) in ovulation rate of goats subjected to a timed artificial insemination (TAI) protocol when applied near the ovulatory period. All animals were subjected to protocol synchronization of oestrus and ovulation, with 60mg of medroxyprogesterone acetate (Progespon-Syntex, Luis Guill on, Buenos Aires, Argentina) in intravaginal sponges for 11 days. On the 9th day were applied im, eCG 300UI (Novormon-Syntex) and 125µg cloprostenol (Sincrocio-Ouro Fino, Cravinhos, São Paulo, Brazil). On 11 day the sponges were removed and 12 and 13 days (24 and 48 hours after sponge removal, respectively) the animals received the treatments according to the experimental groups: control group received 30µg/kg of cyclodextrin in 2ml distilled water per animal subcutaneously and angiotensin group also received subcutaneously 50µg / kg of association Ang-(1- 7)+cyclodextrin, corresponding to 20µg/kg of angiotensin (1-7), diluted in 2ml of distilled water. Cyclodextrins are complex carbohydrates used in pharmaceuts preparations just to give solubility to the product. The AI was performed 38 and 50 hours after sponge removal with frozen semen, come from breeders approved in andrological examinations. Seven days after the last AI were collected blood from the jugular vein of 39 animals (20 angiotensin group and 19 in the control group) using vacuum tubes without anticoagulant and gel tab. The serum was frozen at -20°C until analysis. Progesterone concentrations were determined by ELISA test (Interkit-BioCheek, Foster City, CA, EUA). The results were analyzed using Fisher's exact test ( $p < 0.05$ ). The control group showed 73.68% (14/19) of ovulation and the group angiotensin- (1-7) showed 80% (16/20). There was no statistical difference between the two treatments ( $P = 0.72$ ). It is concluded that the application of angiotensin- (1-7) during the pre-ovulatory period, with only two applications, was not able to increase the ovulation rate of goats subjected to TAI.



A091 FTAI, FTET and AI

### **Effect of associating estradiol benzoate and GnRH at the beginning of the timed AI protocol on fertility of high producing Holstein cows**

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**Keywords:** dairy cow, estradiol benzoate , timed AI.

This study was designed to test the hypothesis that associating estradiol benzoate (EB) with GnRH at the beginning of the timed AI (TAI) protocol would improve conception results (P/AI) in high producing dairy cows. Holstein cows (n = 254) with average daily production of  $41.3 \pm 5.8$  kg, body condition score of  $2.9 \pm 0.1$  and at  $158.3 \pm 9.4$  DIM were synchronized at their first postpartum AI and if found open at pregnancy diagnosis. Cows were randomized according to parity and corpus luteum (CL) presence at D0 one of the following synchronization programs for TAI: 1) EBGnRH: D0 = 2mg of BE (Sigma-Aldrich® + insertion of P4 device (CIDR, Zoetis), D2 = GnRH (100 µg gonadorelin, Factrel, Zoetis), D7 = PGF2α (25mg dinoprost, Zoetis) and P4 device removal, D8 = second treatment with PGF2α, D10 = TAI performed simultaneously with a GnRH treatment, approximately 72h after P4 device removal; 2) NOEBGnRH: similar protocol, without the EB treatment on D0. Pregnancy diagnosis was performed by ultrasound 30 days after AI. Statistical analyses were performed with the procedure Glimmix of SAS, 9.3. Cows receiving EB associated with GnRH at the beginning of the TAI protocol had greater P/AI compared to cows that received only GnRH (42.0% vs 31.0%;  $P < 0.05$ ). In addition, there was no interaction between the type of protocol and parity (Primiparous: EBGnRH = 36.8% vs NOEBGnRH = 22.2%,  $P = 0.11$ ; Multiparous: EBGnRH = 43.5% vs NOEBGnRH = 41.2%,  $P = 0.80$ ) or CL presence (with CL: EBGnRH = 48.0% vs NOEBGnRH = 31.6%,  $P = 0.06$ ; without CL: EBGnRH = 32.7% vs NOEBGnRH = 30.0%,  $P = 0.79$ ). These results show a positive effect of associating EB and GnRH at the beginning of the TAI protocol; however, future experiments will be required to elucidate underlying physiological mechanisms linked to this improvement in fertility, possibly involved with an improved control of the follicle wave emergence.

**Acknowledgments:** Zoetis.



A092 FTAI, FTET and AI

### Effect of animal category on FTET efficiency in buffaloes

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**Keywords:** buffaloes, embryo, FTET.

The present study aimed to evaluate the effect of animal category (nulliparous, primiparous or multiparous) on fixed-time embryo transfer (FTET) efficiency in buffaloes. A total of 125 buffalo females was distributed according to the category into one of three groups: nulliparous (NG, n=42), primiparous (PG, n=27) and multiparous (MG, n=56). At a unknown stage of the estrous cycle (D0), all animals were submitted to the same FTET protocol, which consisted on the insertion of an intravaginal progesterone device (P4, 1g, Sincrogest®, Ourofino Agronegócio, Brazil) and the simultaneously administration of 2mg im (intramuscular) of Estradiol Benzoate (EB, Sincrodiol®, Ourofino Agronegócio). Nine days after (D9), the P4 device was removed and the animals received 530µg im of Cloprostenol sodic (Sincrocio®, Ourofino Agronegócio) plus 400 IU im of eCG (Folligon, MSD Saúde Animal, Brazil). On D11, all buffaloes received 20µg im of GnRH (Sincroforte®, Ourofino Agronegócio). The females were evaluated for the presence of CL eight days after the P4 devices removal (D17) and only the ones with CL were submitted to FTET. Ultrasonographic evaluations were performed (Mindray DP2200Vet, China) on the following days: D11, for measuring the diameter of the largest ovarian follicle; D17, to evaluate the diameter of the largest ovarian follicle, CL and ovulation rate and D40, for the pregnancy diagnosis. The binomial and continuous variables were analyzed by PROC GLIMMIX and MIXED of SAS, respectively. Significance was considered when  $P \leq 0.05$ . No differences were verified between categories (NG vs. PG vs. MG) regarding the diameter of the largest follicle on D11 (10.5±0.3 vs. 11.1±0.5 vs. 11.5±0.4 mm;  $P=0.25$ ) and on D17 (10.5±0.4 vs. 12.0±1.1 vs. 11.7±0.6 mm;  $P=0.30$ ), the CL diameter (16.1±0.3 vs. 15.4±0.9 vs. 16.6±0.4 mm;  $P=0.24$ ), the pregnancy per embryo transferred (38.5 vs. 40.0 vs. 27.5 %;  $P=0.56$ ) and the pregnancy per treated (23.8 vs. 14.8 vs. 19.6 %;  $P=0.65$ ). However, the ovulation rate differed between primiparous and multiparous (61.9<sup>ab</sup> vs. 42.3<sup>b</sup> vs. 71.4<sup>a</sup> %;  $P=0.001$ ). The results obtained in the present study allow concluding that is possible to use the FTET in the different categories (nulliparous, primiparous and multiparous) of buffalo females.



A093 FTAI, FTET and AI

### **Effect of the presence of corpus luteum in lactating buffaloes on the response to the Ovsynch protocol during the breeding season (preliminary results)**

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**Keywords:** buffaloes, cyclicity, Ovsynch.

The aim of the present study was to evaluate the effect of the presence corpus luteum (CL) of lactating buffaloes in response to the Ovsynch protocol during the breeding season (autumn and winter). A total of 99 buffaloes, averaging  $5.3 \pm 0.2$  births, BCC of  $3.5 \pm 0.1$  and  $85.5 \pm 5.2$  days postpartum, was divided into two experimental groups: group without corpus luteum (CL, CG; n=50) and group with CL (CLG; n=49). At random stage of the estrous cycle (D0), all buffaloes received an intramuscular (im) injection of  $10 \mu\text{g}$  of GnRH (Buserelin acetate, Prorelinn<sup>TM</sup>, Inovare Inovare Biotecnologia e Saúde Animal, Monte Aprazível, Brazil). On D7, the animals received the administration im of  $0.53 \text{mg}$  of PGF2 $\alpha$  (Cloprostenol sodic, Cioprostinn<sup>TM</sup>, Inovare Inovare Biotecnologia e Saúde Animal, Monte Aprazível, Brazil). Two days after (D9),  $10 \mu\text{g}$  of GnRH (Prorelinn<sup>TM</sup>) was administered im and 16 hours later, all buffaloes were submitted to TAI (D10). Ultrasound evaluations (Mindray DP-2200Vet; Shenzhen, China) were performed to determine the cyclicity of the animals (presence of CL on D0), ovulation rate to the first GnRH (Ov-1<sup>st</sup>GnRH; presence of CL on D9), the diameter of the dominant follicle ( $\phi\text{DF}$ ) on D9 and D10, ovulation rate to the second GnRH (Ov-2<sup>nd</sup>GnRH, rating on D10 and D13) and conception rate (CR) on D40. The CL vascularization (central vascularization - CV, and peripheral vascularization - PV; 0 to 4 scale, where 0 represents the absence of vascularization and 4 the maximum vascularization) and the CL diameter ( $\phi\text{CL}$ ) were evaluated by color Doppler ultrasonography (Mindray M5Vet; Shenzhen, China) on Days 17, 21 and 25. The comparison between variables was performed by the analysis of variance (ANOVA), using the GLIMMIX procedures of SAS<sup>TM</sup>. The CV, PV and  $\phi\text{CL}$  variables were analyzed as repeated measurements, using the MIXED procedure of SAS<sup>TM</sup> through the effects of treatment, time and its interaction (Treat.\*Time). Difference was considered when  $P < 0.05$ . For all analyzed variables, the CLG showed superior results to CG: Ov-1<sup>st</sup>GnRH ( $89.8$  vs.  $42.0$  %;  $P < 0.01$ );  $\phi\text{DF}$  on D9 ( $13.4 \pm 0.3$  vs.  $12.1 \pm 0.3$  mm;  $P < 0.01$ );  $\phi\text{DF}$  on D10 ( $13.9 \pm 0.3$  vs.  $12.3 \pm 0.3$  mm;  $P < 0.01$ ); Ov-2<sup>nd</sup>GnRH ( $87.8$  vs.  $52.0$  %;  $P < 0.01$ ) and CR ( $65.3$  vs.  $20.0$  %;  $P < 0.01$ ). Similarly, for the CL vascularization data, greater results were observed for CLG on Days 17, 21 and 25, compared to CG: CV =  $2.8 \pm 0.2$ ,  $3.1 \pm 0.1$ ,  $3.3 \pm 0.1$  vs.  $1.8 \pm 0.3$ ,  $2.0 \pm 0.3$ ,  $2.2 \pm 0.3$  (Treat.:  $P < 0.01$ , Time:  $P = 0.02$ , Treat.\*Time:  $P = 0.93$ ); VP =  $3.2 \pm 0.2$ ,  $3.5 \pm 0.1$ ,  $3.6 \pm 0.1$  vs.  $2.3 \pm 0.3$ ,  $2.4 \pm 0.3$ ,  $2.4 \pm 0.3$  (Treat.:  $P < 0.01$ , Time:  $P = 0.14$ , Treat.\*Time:  $P = 0.83$ );  $\phi\text{CL}$  =  $17.3 \pm 0.5$ ,  $18.7 \pm 0.4$ ,  $19.2 \pm 0.5$  vs.  $15.2 \pm 0.8$ ,  $14.4 \pm 0.9$ ,  $14.1 \pm 0.9$  mm (Treat.:  $P < 0.01$ , Time:  $P = 0.02$ , Treat.\*Time:  $P < 0.01$ ). In conclusion, buffaloes cows presenting a CL at the beginning of the Ovsynch protocol have better ovarian responses compared to females without CL when performed during the breeding season.





A094 FTAI, FTET and AI

### **The effect of prostaglandin F2 $\alpha$ applied at the beginning of the estrus synchronization protocol with progesterone and estradiol in *Bos taurus taurus* heifers**

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**Keywords:** follicle diameter, progesterone device, TAI.

This research evaluated the effect of prostaglandin given at the start of timed artificial insemination (TAI) protocol on the follicle diameter at TAI and pregnancy rate. *Bos taurus taurus* beef heifers with 14 e 24 months old, body condition score with  $3.6 \pm 0.01$  (1 to 5), were maintained during the entire study in native pasture on western central region of Rio Grande do Sul/Brazil. At the beginning of the protocol (Day 0) the animals received 2 mg estradiol benzoate IM (RIC-BE®, Tecnopec, São Paulo, Brazil). The heifers were distributed in three treatments according to the use of different types of intravaginal progesterone devices (P4): T1 = new device (1g with P4, PRIMER®, Tecnopec), T2 = used device (PRIMER®, Tecnopec) and T3 = monodose device (0.75g of P4; PROCICLAR®, Hertape Calier, Juatuba, Brazil). Additionally, heifers were randomly distributed in six different treatments regarding if they received or not a single dose of PGF at D0: T1 (n=39), T1PGF (n=51), T2 (n=259), T2PGF (n=262), T3 (n=81) e T3PGF (n=70). In Day 8, it was administered 1 mg estradiol cypionate IM (EC; Cipionato HC®, Hertape Calier) and 0.150 mg d-Cloprostenol (PGF; Prolise®, Tecnopec) in all animals. All heifers were inseminated 48h (Day 10) after P4 removal. At the TAI moment, the follicular diameter was measured (FD). Bulls were introduced in the herd ten days after TAI and remained for 60 days. The ultrasound examination for pregnancy diagnosis was performed on D40. Follicular diameters were  $10.18 \pm 0.29$  (T1),  $10.65 \pm 0.30$  (T1PGF),  $14.03 \pm 0.27$  (T2),  $13.0 \pm 0.21$  (T2PGF),  $13.72 \pm 0.19$  (T3),  $13.03 \pm 0.25$  (T3PGF) (P=0.1). The pregnancy rate was lower on T1 and T1PGF (33.3% and 37.3%; respectively) when compared with the others treatments (57.4% (T2), 53.6% (T2PGF), 49.4% (T3), 47.1% (T3PGF) (P=0.019), respectively). The use of PGF at Day 0 did not influence the P/IA (P = 0.88). However the pregnancy rate was lower in T1 (35.6%; 32/90) when compared to T2 (55.5%; 289/521) and T3 (48.3%; 73/151) (P=0.0018). The P/AI did not differ between the 14 and 24 month-old heifers (50.2% and 50.6%, respectively) (P = 0.357). Besides that, the pregnancy rate at the end of breeding season was 86.7%, and was not affected by treatments or heifers' age. The use of PGF at the beginning on the protocol did not alter P/AI in beef heifers, and P/AI was satisfactory when used a monodose or previously used P4 device.



A095 FTAI, FTET and AI

### Effect of eCG (Syncro-Part) on follicle growth and P/AI of *Bos indicus* cows submitted to TAI

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

The objective of this study was to evaluate the effect of eCG (Syncro-part, CEVA, France) UI on follicular growth, diameter of the dominant follicle (ØFD), ovulation rate, CL diameter and P/AI of primiparous Nelore cows (*Bos indicus*), between 30 and 80 days post-partum, submitted to TAI. At unknown day of the estrous cycle (D0), all cows (n=587) received an intravaginal device with 1g progesterone and 2 mg of estradiol benzoate i.m. On D8, the device was removed and 500µg of D-cloprostenol and 1 mg of estradiol cypionate were administered intramuscularly. Simultaneously, cows were randomly distributed to one of three experimental groups: Negative Control (no eCG - administration i.m. of Syncro-Part injection vehicle only), Positive Control (administration i.m. of 300IU of eCG; Novormon®, Zoetis, Brazil) and Syncro-Part group (administration i.m. of 300IU of eCG; Syncro-Part, CEVA, France). Timed AI was performed 48h after progesterone device removal. A subset of cows (n=89) underwent ultrasound evaluations on D8 and D10 to assess the ØFD and follicular growth. On D18 another ultrasound evaluation was performed to measure CL diameter as well as to calculate ovulation rate. All cows received a further ultrasound evaluation on D40 for pregnancy diagnosis. The daily follicle growth rate was calculated by the difference between the ØFD on D8 and D10 divided by two. Data were analyzed using PROC GLIMMIX from SAS. The ØFD on D8 was similar (P = 0.82) among Negative Control (10.4±0.5 mm), Positive Control (11.1±0.2 mm) and Syncro-Part group (9.8±0.4 mm), showing that groups were homogenous before the treatment. As expected, cows treated with Syncro-Part had greater ØFD on D10 and ovulation rate (13.1±0.6<sup>a</sup> mm and 83.3%<sup>a</sup>) than Negative Control cows (11.7±0.7<sup>b</sup> mm and 56.7%<sup>b</sup>; P=0.03). In addition, on D10, the Positive Control group had intermediate ØFD and ovulation results (12.8±0.6<sup>ab</sup> mm and 75.9%<sup>ab</sup>) that were similar to both other groups. Moreover, daily follicle growth rate was greater in cows in the Positive Control (1.5±0.2<sup>a</sup> mm/day) and Syncro-Part (1.7±0.2<sup>a</sup> mm/day) than Negative Control group (0.7±0.1<sup>b</sup> mm/day; P=0.01). In contrast, there was no difference on CL diameter on D18 among experimental groups (Negative Control - 21.5±1.0 mm; Positive Control - 23.4±0.9 mm and Syncro-Part - 22.0±0.7 mm; P=0.32). The P/AI 30d after TAI was greater in Positive Control [54.7% (104/190)] and Syncro-Part [53.3% (106/199)] groups compared to cows in Negative Control group [37.9% (75/198)]. In conclusion, the Syncro-Part and Positive Control groups increased ovarian follicle growth, ovulation rate and P/AI of *Bos indicus* cows submitted to TAI in comparison to the Negative Control group.



A096 FTAI, FTET and AI

### **Efficiency of two progesterone implants containing different quantities of active principles**

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**Keywords:** cloprostenol, corpus luteum, progesterone.

This research evaluated the effect of two different intravaginal progesterone devices (IVP), with the same matrix, containing 1.2 and 0.6g of P4, used in Fixed-Time Artificial Insemination (FTAI) protocol, on ovarian and progesterone (P4) concentration. A total of 24 cyclic crossbreed heifers weighting between 270 and 450 kg, was divided into one of two treatments according to their weight. Females from G1 (n=12) received IVP with 1.2g of P4, while those from G2 (n=12) received IVP with 0.6 of P4. All animals were presynchronized to eliminate the presence or formation of corpus luteum (CL), avoiding the endogenous source of P4. For this, 10 days before the beginning of the synchronization, females received a norgestomet ear implant plus 5.0mg of Estradiol Valerate and 0.5mg of cloprostenol. A second dose of PGF was applied 3 days before the initiation of the synchronization protocol. The animals were evaluated by ultrasonography exam (Mindray – 2200 – 7.5 Mhz linear transrectal transducer) in order to confirm the absence of CL at Day 0. Also, other examination were daily performed from D2 to D10 to evaluate the follicular wave emergence and ovarian follicular dynamics. On the first day of synchronization (Day 0), IVP were inserted and 2 mg of Estradiol Benzoate (EB) were applied. On the Day 8, the IVP were removed and 1 mg of EB was administrated 24 h later. The P4 concentrations were determined by RIA (DPC Medlab® in the Unesp-Botucatu Endocrinology Laboratory) from blood collected before and after the IVP insertion. The P4 concentration and the follicular diameter were compared using the Shapiro-Wilk test. Data were analyzed by ANOVA. The differences between treatments were accessed by the Student “T” test and on different days by the Turkey test. The P values lower than 5% were considered significant. There were no differences in P4 concentration ( $P>0.05$ ) between treatments. Regardless of treatment, a significant effect of time was found ( $P<0.0001$ ). There was no difference on the day of new follicular wave emergency (G1 =  $3.3\pm 1.8$  and G2 =  $4.0\pm 1.2$ ) and on diameter of follicle on Day 10 (G1 =  $12.2\pm 0.6$  and G2 =  $12.9\pm 0.5$  mm). There was a similar follicular growth from Day 4 to Day 10 between groups. In conclusion, both IVP containing 1.2 and 0.6g of P4 are efficient to maintain satisfactory plasmatic P4 concentration, resulting in a quickly rise after insertion and sharp decline after its removal. Also, different amount of P4 in the device did not alter the plasmatic P4 profile, resulting in satisfactory control of follicular growth and synchronization of ovulation in cyclic heifers.



A097 FTAI, FTET and AI

## Reproductive efficiency of 24 months old primiparous Nelore cows and submitted to resynchronization programs after TAI

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**Keywords:** primiparous, synchronization, TAI.

The present study evaluated the reproductive performance of Nelore (*Bos indicus*) primiparous cows having the first parturition at 24 months (mo) and submitted to two consecutive timed artificial insemination (TAI) programs with 32 days interval between inseminations. The trial was performed in one commercial beef farm, located in Camapua – MS, Brazil. Nelore females (n = 1,028) from three different categories [cows with the first parturition at 24 mo (Primi24; n = 115); cows with the first parturition at 36 mo (Primi36; n = 475) and cows with second parturition at 48 mo (Second48; n = 438)] were submitted to the same reproductive program. During early postpartum (30 to 60 days) females were synchronized to receive a TAI. All females received, on the first day of the treatment (Day 0) an intravaginal progesterone device (P4; Cronipress Monodose M-24®, Biogenesis Bagó) and 2 mg of estradiol benzoate (EB; Bioestrogen®, Biogenesis Bagó) IM. On Day 8, the P4 devices were removed and it was administered 300 IU of eCG (Novormon®, Zoetis) IM, 1 mg of estradiol cypionate (EC; ECP®, Zoetis) IM and 0.75 mg of cloprostenol (PGF; Croniben®, Biogenesis Bagó) IM. All females were inseminated 48h after P4 device removal (1st TAI). On Day 32, all females were resynchronized at unknown pregnancy status, by the insertion of a P4 device and the administration of 1 mg of EB IM. On Day 40, the P4 device was removed and the pregnancy diagnosis was performed by transrectal ultrasonography (Chison 9300VET, Kylumax, Brazil). Non-pregnant females received 300 IU of eCG IM, 1 mg of EC IM and 0.75 mg of PGF IM and they received a TAI 48 h later (2nd TAI). The pregnancy diagnosis of the second TAI was performed on Day 72. Statistical analysis was performed using the GLIMMIX procedure of SAS (SAS® 9.3 Institute Inc., Cary, NC, USA, 2003). Pregnancy rate after 1st TAI was similar between the different animal categories (Primi24 = 53.0 vs. Primi36 = 54.3 vs. Second48 = 56.6%; P = 0.62). Furthermore, similar pregnancy rate was also observed after the 2nd TAI (Primi24 = 38.9 vs. Primi36 = 41.5 vs. Second48 = 46.6%; P = 0.22). Additionally, no difference was observed regarding the cumulative pregnancy (1st+2nd TAI) between categories (Primi24 = 71.3 vs. Primi36 = 78.1 vs. Second48 = 78.3%; P = 0.70). Therefore, primiparous Nelore cows calving at 24 and 36 mo of age and second parturition cows aged 48 mo at calving had similar reproductive efficiency. The present data indicated the possibility of reducing the age at first parturition of heifers, without compromising the subsequent reproductive performance.

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A098 FTAI, FTET and AI

### **Efficacy of different drugs on ovulation induction of dairy cows submitted to timed artificial insemination**

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**Keywords:** Girolando, ovulation induction, TAI.

Among the main estradiol esters used in cattle timed artificial insemination (TAI) programs, estradiol benzoate (EB) and estradiol cypionate (EC) has been commonly used because they low cost. But the estradiol estres present differences regarding their indication due to display different half-lives. In order to reduce management in TAI protocols, this study evaluated the follicular dynamics in dairy cows treated with EB and EC as drugs for ovulation induction. Therefore, 44 lactating Girolando dairy cows with average body condition score of 3 (range 1-5) were used. At random stage of the estrous cycle, the cows received an intravaginal device containing 1g of progesterone (DIB®, MSD Animal Health, São Paulo, Brazil) and 2mg of EB intramuscular (im) of which was considered day 0 (D0). At D8, the intravaginal device was removed and 150µg cloprostenol (CIOSIN®, MSD Animal Health) were applied (im) in all females. At this time the animals were randomly assigned into one of two treatments: EB Group (n = 22) and EC group (n = 22). The EC group received 1mg of EC (im) (ECP®, Pfizer, São Paulo, Brazil) at the time of P4 device removal in order to induce ovulation, while the EB group received 1mg of EB (im) (Estrogin®, Agroline, São Paulo, Brazil) 24 hours later (D9). After P4 device removal, ultrasonographic evaluations were performed every 12 hours until the ovulation. The following parameters were evaluated: diameter of ovulatory follicle - OF (mm); ovulation rate - OR (%) and P4 device removal/ovulation interval - ROI (h). Statistical analysis was performed using chi-square test for OR and "t" test for OF and ROI, with a 5% significance level. The results for EB and EC groups were, respectively: OF: 13.82±0.41mm and 13.86±0.61mm; OR: 86.36 % and 81.82%; ROI: 69.16±1.80 h and 64.82±2:58 h. There was no statistical difference (p>0.05) between treatments for any evaluated parameters. The results of this study corroborate with Sales et al. (Theriogenology, v.78, p.510-516, 2012) that observed no difference between the benzoate and cypionate for synchronization of ovulation in Nellore cows. This study also found greater dispersion of ovulations in the group treated with estradiol cypionate as noted in Martins et al. data (Acta Scientiae Veterinariae, v.33, p.285, 2005). Preliminary results of this study allow us to conclude that both drugs tested for ovulation induction can be used in TAI of Girolando dairy cows with the same efficiency.



A099 FTAI, FTET and AI

### **Estradiol, GnRH or their association at the beginning of the resynchronization protocol results in similar reproductive efficiency in dairy cows**

**L.M. Vieira<sup>1</sup>, B.M. Guerreiro<sup>1</sup>, E.O.S. Batista<sup>1</sup>, B.G. Freitas<sup>1</sup>, M.F. Sá Filho<sup>1</sup>, G.S.F.M. Vasconcellos<sup>1</sup>, J.G. Soares<sup>1</sup>, L.G.M. Bragança<sup>2</sup>, S.S. Plá<sup>3</sup>, P.S. Baruselli<sup>1</sup>**

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**Keywords:** estradiol, lactating cow, resynchronization.

Two experiments were performed aiming to evaluate the reproductive efficiency of dairy cows undergoing resynchronization after TAI (Exp 1) and the effect of resynchronization on the corpus luteum (CL) blood flow (Exp 2). The Exp 1, enrolled 601 cows distributed into 3 groups, EB (n=200), GnRH (n=197) and EB+GnRH (n=204). Following previous TAI all females were resynchronized with the insertion of a P4 device (Primer®, Tecnopec) on Day 25 (EB and EB+GnRH groups) or on Day 26 (GnRH Group). Concomitantly with the P4 device insert, females of EB group received 2mg of EB (RIC-BE®, Tecnopec), GnRH group, 25µg of lecorelin (Gestran®, Tecnopec, Brazil) and group EB+GnRH, 2mg of EB and 25µg of lecorelin. On Day 33 all devices of P4 were removed and females of both experimental groups underwent an ultrasonography exam in order to assess pregnancy diagnosis from the 1<sup>st</sup> TAI. All animals classified as non-pregnant received 0.282 mg of sodium cloprostenol (Estron®, Tecnopec) on Day 33, 1mg of EB on Day 34 and TAI on Day 35 AM. The pregnancy diagnosis reconfirmation of the 1st TAI and pregnancy diagnosis of the resynchronization was performed on Day 65. Statistical analysis was performed using the GLIMMIX procedure of SAS®. The experimental groups had similar pregnancy rate following 1st TAI, both at 33 [BE: 33.0, GnRH: 35.0 and BE+GnRH: 34.3%; P = 0.61] and 65 days [BE: 26.0, GnRH: 28.9 and BE + GnRH: 26.5%; P = 0.26]. Additionally, similar pregnancy loss was also observed between 33 and 65 days of pregnancy after 1st TAI [BE: 21.2, GnRH: 17.4 and BE+GnRH: 22.9%; P = 0.47]. Finally, the pregnancy rate 30 days after TAI related to the resynchronization was also similar between groups [BE: 20.9, GnRH: 17.2 and BE+GnRH: 25.4 %; P=0.54]. For all analyzed variables it was observed farm effect (P<0.05), however with no farm and treatment interaction (P>0.05). In Exp 2, 42 lactating cows used in the previous experiment [EB: n=15; GnRH: n=12; EB+GnRH: n=15] were evaluated by Doppler ultrasonography every 48h, between Days 25 and 33, to access CL vascularization according to the resynchronization protocol. Statistical analysis was performed using the GLIMMIX procedure of SAS®. No treatment effect (P=0.77), time (P=0.11) and interaction (P=0.81) between treatment and time was observed for CL vascularization rate. Therefore, the CL vascularization rate remained similar between the experimental groups during the experimental period (Day 25: 76.1, Day 27: 79.1, Day 29: 77.5, Day 31: 76.3 and Day 33: 77.1%). In conclusion, the resynchronization using 2mg of EB, 25µg of lecorelin or a combination of both after TAI results in similar pregnancy rate after 1st TAI and after resynchronization. Still, EB administration at the beginning of resynchronization protocol does not compromise the CL vascularization of females previously inseminated.

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A100 FTAI, FTET and AI

### **Factors affecting vaginal temperature in high producing lactating Holstein cows**

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**Keywords:** milk production, pregnancy per AI, Heat stress.

The aim of this experiment was to evaluate the continuous collection of vaginal temperature for 72 h (every 10 min) in lactating Holstein cows (n= 480; 591 data collections of insemination) allocated in one single cross-ventilation barn. Cow were fitted with a thermometer attached to a progesterone intravaginal device (1.9g P4, CIDR, Zoetis) prior to timed artificial insemination (TAI). Milk production, DIM, skin thickness, coat color, body condition score (BCS), and pregnancy per AI (P/AI) data were collected and recorded for further analyses. Ambient temperature and humidity of barn was measured for 72 housing a data logger for calculation of the temperature and humidity index (THI). Data was analyzed using ANOVA and Pearson's correlations using proc GLM, Corr and Logistic of SAS. Maximum THI (MAX) and percentage of time above a vaginal temperature of 39.1°C (PCT) were used as data logger responses, class variables were created for MAX and PCT (High vs. Low) using the median threshold. There was a low correlation between THI and PCT ( $P < 0.05$ ) ( $r = 0.01$ ). Skin thickness was also poorly correlated with PCT ( $P < 0.05$ ) ( $r < 0.01$ ). Cows with black color coat spent less time with high vaginal temperatures ( $P = 0.05$ ). Primiparous ( $P = 0.04$ ) and cows with low BCS ( $P < 0.01$ ) had greater PCT. Milk production was affected by parity ( $P < 0.01$ ) and PCT ( $P = 0.02$ ; High = 43.5 vs. Low = 41.2 kg/d). There was a milk production by MAX interaction for PCT ( $P < 0.01$ ), whereas only cows in the highest milk production quartile ( $> 50.7$ kg/d) spent more time with high vaginal temperatures. Among the independent variables included in the model, parity ( $P < 0.01$ ), PCT ( $P = 0.03$ ; 26.1 vs. 17.4% for Low and High, respectively) and a PCT by milk production interaction ( $P = 0.05$ ) affected P/AI. The decrease in P/AI in cows with High PCT only occurred in cows with the highest production. In summary, there is a large variability in individual capacity of cows respond to heat stress. Parity, BCS, coat color and milk production affected PCT, particularly under high ambient temperatures. Selection of animals with efficient control of body temperature in spite of high milk production should be further approached as a strategy to maintain adequate fertility of dairy herds.



A101 FTAI, FTET and AI

### **Bacterial frequency of uterine samples from Mangalarga Marchador mares**

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**Keywords:** endometritis, microbiologic, susceptible mares.

The endometritis in mares is considered one of the most frequent causes of infertility, ranked as the third most important disease in this species. Etiological agents of endometritis in the equine species can be *Streptococcus equi* subsp. *zooepidemicus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, and others (LANGONI et al., *Revista Brasileira de Reprodução Animal*, 23, 44-51, 1999). However, according to LeBlanc (*Reproduction in Domestic Animals*, 45, 21-27, 2010), the therapy is based, among other aspects, in the neutralization of virulent bacteria in controlling inflammation after breeding, which makes the identification of the etiologic agent essential for successful treatment. Therefore, this study aimed to identify the frequency of the possible etiologic agents of endometritis in the studied group of mares. Thus, 22 uterine samples obtained by sterile swab kept in Stuart transport medium of mares Mangalarga Marchador aged three and 20 years, with a history of subfertility and positive endometrial cytology for endometritis were used. Results were analyzed using descriptive analysis to calculate the absolute and relative frequencies (SAMPALIO, *Estatística Aplicada à Experimentação Animal*, 1998). Of the 22 samples analyzed, 14 (63.6%) were positive for microbiological examination, results similar to that found by Oliveira et al. (*Veterinary Medicine*, 1, 19-25, 2007) who obtained 64.4% (41/64) of positive mares in their study. The most common microorganisms were *Enterococcus* sp (18.2%), *Escherichia coli* and *Staphylococcus* sp (13.6%), *Micrococcus* sp (9.1%), *Streptococcus* sp and *Serratia* sp (4.5%). The frequency of *Enterococcus* sp and *Escherichia coli* identified in this study may be due to anatomical defects in perineal and vulvar region in consequence to advanced age of some mares, predisposing to pneumovagina and fecal contamination. According to LeBlanc (*Proceedings of Reproductive Pathology*, 78-84, 1997), it provides greater frequency of bacteria commonly found in the intestine. In conclusion, it was possible to identify the frequency of causing agents for endometritis in the studied group of mares.

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A102 FTAI, FTET and AI

## **Pregnancy associated glycoprotein predicts pregnancy losses in dairy cattle**

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**Keywords:** dairy cows, glycoprotein, pregnancy loss.

The objective of this study was to determine the association between concentrations of pregnancy associated glycoprotein (PAGs) and pregnancy loss in lactating dairy cattle following fixed-timed artificial insemination (TAI) or timed embryo transfer (TET). Three experiments were performed, on experiment 01 (TAI - artificial insemination on d0) and 02 (TET - embryo transfer on d7) the pregnancy diagnosis was performed by ultrasonography at d31. In pregnant cows blood samples were collected and serum concentrations of PAG were determined by ELISA. On d59, a second pregnancy check was performed and the pregnancy loss was determined when a cow had a viable embryo on d31 of gestation but not on d59. In study 03 (TET - TE d7) cows were considered pregnant based on the presence of a vascularized corpus luteum on d24 (evaluated with a color doppler US) ipsilateral to the CL at TET, on days 31, 38, 45, 52 and 59 the cows were scanned to evaluate a viable embryo/fetus and blood samples were collected to evaluate the progesterone concentration (P4) and PAGs. One-way ANOVA (SAS 9.4) was used to evaluate differences of PAGs among d31, the LOGISTIC procedure was used to determine the probability of pregnancy maintenance. To test of the effectiveness of a single circulating PAG sample on d31 to predict pregnancy loss, a receiver-operating characteristic (ROC) curve was generated on MedCalc software package to determine PAG concentrations on d31 that should predict pregnancy loss with  $\geq 95$  accuracy in both TAI and TET. A total of 77 cows experienced pregnancy loss on TAI (exp 01/n=413) and 47 cows experienced pregnancy loss on TET (exp 02/n=238). Cows that were pregnant at d31 and maintained the pregnancy until d59 had higher ( $P<0.05$ ) circulating concentrations of PAGs (AI=9.58 $\pm$ 0.31; ET=8.61 $\pm$ 1.53) compared to cows that experienced pregnancy loss (AI=4.15 $\pm$ 0.33; ET=3.78 $\pm$ 0.58) between d31 and 59 of gestation in both TAI and TET. Lower PAGs concentration on d31 resulted in higher ( $P<0.05$ ) pregnancy loss until d59 of gestation (linear). Circulating concentration of PAGs below 1.4 ng/mL (TAI; minimal detectable level 0.28 ng/mL) and 1.85 ng/mL (ET) was 95 % accurate in predicting EM (between d31-d59) at d31 of gestation. On experiment 03, no effect was detected ( $P>0.05$ ) of PAGs and P4 concentration on d24 in pregnancy maintenance until d31. The P4 concentration at d31 not interfere ( $P>0.05$ ) in pregnancy maintenance; however, there was effect ( $P<0.05$ ) of PAGs at d31 in pregnancy loss, as observed in experiments 01 and 02. The majority of pregnancy losses occurred between 24 and 31 (n=20; 20%), followed by days 31 and 38 (n=8, 10%), 38 and 45 (n=6, 7.5 %) and 45 to 59 and (n=1, 1%). In conclusion, PAGs concentration on d31 of pregnancy can be successfully used to detect pregnancy losses occurring after d31.



A103 FTAI, FTET and AI

### **Timed artificial insemination according to diameter of the preovulatory follicle improves fertility in lactating beef cows**

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**Keywords:** cattle, fertility, TAI.

The objective of this study was to evaluate whether timed artificial insemination (TAI) according to the diameter of the dominant preovulatory follicle (POF) would improve pregnancy rates in beef cows. In this study, lactating Nelore cows (*Bos indicus*; n=413), with 430-640 kg body weight, which were submitted to an estradiol/progesterone-based TAI protocol. On Day 0, cows given a progesterone device (1.9g Progesterone; CIDR®, São Paulo, Brazil) plus 2 mg of oestradiol benzoate (Bioestrogen®, Curitiba, Brasil) im, to synchronize follicular waves. On Day 10 (07:00 a.m.), the diameter of the POF was assessed by ultrasonography, and cows were randomized into Control (n=209) and Block (n=204) Groups. Control-Group cows were inseminated 48 h after CIDR removal, soon after ultrasound examination (08:00 a.m. on Day 10), and Block-group cows were inseminated at four time points according to the diameter of the POF as described by Pfeifer et al. (Reproduction, Fertility and Development, abstract, p 97, 2015): B0 (POF  $\geq$  15 mm, TAI at 08:00 a.m. on Day 10, n=51), B1 (POF 13 to 14.9 mm, TAI at 02:00 p.m. on Day 10, n=70), B2 (POF 10.1 to 12.9 mm, TAI at 08:00 a.m. on Day 11, n=54), and B3 (POF  $\leq$  10 mm, TAI at 02:00 p.m. on Day 11, n=28). Pregnancy status was assessed 30 days post-AI by ultrasonography. The diameter of the pre-ovulatory follicles on Day 10 was analyzed by analysis of variance – Factor ANOVA and the means between groups were compared by Tukey’s post hoc test. Pregnancy rate was evaluated by Chi-square analysis. There was no difference in the diameter of the POF 48h after CIDR removal (P=0.4) between Groups. The pregnancy rate was greater in the Block-Group (63.2%, 129/204) than in the Control Group (48.8%, 102/209; P=0.002). In conclusion, our results demonstrate that TAI results can be improved when performed according to the diameter of the preovulatory follicle. This technique may represent an effective tool to improve fertility of lactating beef cows receiving TAI protocols utilizing estradiol as ovulation inducer.



A104 FTAI, FTET and AI

**Impact of equine chorionic gonadotropin associated with temporary weaning, estradiol benzoate, or estradiol cypionate on timed artificial insemination in primiparous *Bos indicus* cows**

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**Keywords:** beef cattle, calf removal, eCG, TAI.

This study aimed to determine the impact of equine chorionic gonadotropin (eCG) associated with different timed-artificial insemination (TAI) protocols on the pregnancy rate (PR) in *Bos taurus indicus* previously treated with progesterone (P4). Five hundred and fifty-seven primiparous cows received the following treatments: on day 0 (d0), GeCGTW (group eCG+Temporary Weaning(TW); n=178) received 0.558g intravaginal progesterone (P4)+1.0 mg of estradiol benzoate (EB)(IM); on d8 (P4 removal+0.075 mg D-cloprostenol+400 IU eCG+TW for 48h); on d10, TAI+calf return to dam; GeCGEB (group eCG+EB; n=176) the same as GeCGTW without TW+1.0 mg of EB on d9; GeCGEC (group eCG+EC;n=203), the same as GeCGTW without TW+1.5 mg estradiol cypionate (EC) (IM). On d35 after TAI pregnancy diagnosis (PD) was performed. Open cows remained with clean-up bulls for 90 days and then underwent pregnancy diagnosis by ultrasound. Drugs used were from Biogenesis Bagó (Curitiba, PR) except eCG (Foligon, MSD, SP) and the EC (Zoetis, SP). The statistical analysis was performed with Chi-square (level of P<0.05). The PR of TAI was 51.1, 47.1 and 47.8% for GeCGTW, GeCGEB, and GeCGEC (P>0.05) respectively. The PR following clean-up bulls was 88.3, 47.3, and 31.1% (P<0.05). The final PR (TAI+clean-up bulls) of the groups was 94.4, 72.1, and 64.0%, respectively (P<0.05). In conclusion, there were no detectable differences in PR among TAI protocols; PR in the GeCGTW protocol following clean-up bulls was greater compared to others (P<0.05); the overall PR of cows subjected to TAI+clean-up bulls was significantly greater in GeCGTW as compared to other groups.



A105 FTAI, FTET and AI

### **Impact of hormonal modulation of proestrus on uterine gene expression associated with cell proliferation of suckled anestrus beef cows**

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**Keywords:** equine chorionic gonadotrophin, estradiol cypionate, timed artificial insemination.

Previous studies demonstrated that estradiol cypionate (ECP) supplementation at the onset of proestrus alters uterine transcriptome of anestrus suckled beef cows (Sá Filho et al., IETS, 2015. p. 102 – abstract). Enriched pathways affected by the ECP supplementation described in this previous work identified genes associated with cell proliferation, suggesting a relationship with the modulation of uterine receptivity. Thus, the aim of the present study was to evaluate the impact of ECP and/or equine chorionic gonadotropin (eCG) supplementation at the onset of synchronized proestrus on endometrial expression of candidate genes associated with cell proliferation, in suckled anestrus beef cows. Evaluated genes were: progesterone receptor (PGR), oestrogen receptor 1 (ESR1), oestrogen receptor 2 (ESR2), epidermal growth factor receptor (EGFR), heparin-binding EGF-like growth factor (HB-EGF), collagen, type IV, alpha 1 (COL4A1), patched homolog 2 (PTCH2) and integrin, beta 3 (ITGB3). A total of 46 suckled cows was treated with 2 mg of intramuscular estradiol benzoate and received a P4 intravaginal device. Eight days later, P4 devices were removed, and cows received an intramuscular administration of 500 mg of cloprostenol. Cows were blocked by body condition score and diameter of largest follicle (LF) at the time of progesterone (P4) device removal. Simultaneously, animals were randomly assigned to one of the following groups: control (CON; n=11), ECP (1mg; n=11), eCG (400IU; n=12) and ECP+eCG (1mg and 400IU, respectively; n=11). At 48 h after the P4 device removal, all cows received 10 µg of buserelin acetate and were immediately artificially inseminated at fixed time. Six days after GnRH treatment, cows that presented a recently formed corpus luteum had uterine tissue collected by transcervical biopsy. The P values < 0.10 were considered as statically different. There were no interactions (P>0.10) between ECP and eCG on the expression of the evaluated transcripts. ECP treatment induced greater endometrial abundance of PTCH2 (P=0.07) and COL4A1 (P=0.02) genes, whereas suppressed EGFR (P=0.09) gene expression. ECP treatment did not affect the gene expression of ESR1 (P=0.90), ESR2 (P=0.61), HB-EGF (P=0.80) and ITGB3 (P=0.57). On the other hand, eCG treatment induced greater endometrial abundance of HB-EGF (P=0.06), ESR2 (P=0.09), and ITGB3 (P=0.05) genes, whereas reduced the gene expression of ESR1 (P=0.05). eCG supplementation did not alter the expression of EGFR (P=0.34), PTCH2 (P=0.31) and COL4A1 (P=0.19). Additionally, the expression of PGR was not altered by either ECP (P=0.51) or eCG (P=0.25) treatments. Therefore, the present results support the hypothesis that supplementation with ECP or eCG at onset of the proestrus in suckled anestrus beef cows acts on the endometrial tissue to alter the abundance of genes associated with cell proliferation during early diestrus.

**Acknowledgments:** FAPESP (2012/14731-4) and Empyreo Farm.



A106 FTAI, FTET and AI

### **Impact of bovine genetic group (*Bos indicus* vs. *Bos taurus*) and level of dry matter intake (high vs. low) on gene expression of liver enzymes related to progesterone metabolism**

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**Keywords:** liver, metabolism, progesterone.

The objective of this study was to evaluate the liver expression of six genes associated with hepatic progesterone (P4) metabolism (CYP2C19, CYP3A4, SRD5A1, SRD5A3, AKR1C4 and AKR1D1) in heifers of *B. indicus* (Nelore; n=16) and *B. taurus* (Holstein; n=16) breeds that were kept in a high (HDM, gaining 900 g/day) or low (LDM, maintenance, NRC 2001) levels of dry matter intake. Cycling heifers from both genetic groups and level of dry matter intake were randomized in a 2x2 factorial design. The diet was administered for 33 days prior to liver biopsy. Pre-synchronized heifers, on day seven after ovulation, received two doses of PGF2 $\alpha$  6 hours apart. Twelve hours after the last treatment all heifers received a new P4 device (CIDR®, Zoetis, Brazil), 2 mg of estradiol benzoate i.m. (EB, Sincrodiol®, Ourofino Agronegócio) and an additional PGF2 $\alpha$  treatment. Forty eight hours after device insertion, liver tissue was collected for later gene expression analysis, in addition to simultaneous blood sampling for assessment of circulating P4. Liver samples were immediately stored in 1 mL of RNA-stabilizing solution (RNAlater Ambio Inc., Austin, TX), and maintained at 4°C for 24h, and then at -80°C for further mRNA quantification by qPCR. Circulating P4 (mean $\pm$ SEM) and gene expression were analyzed with the MIXED and GLIMMIX procedures, respectively, from SAS. There was an interaction between genetic group and diet for circulating P4 (P=0.07). Zebu heifers receiving HDM had lower circulating P4 than LDM (2.3 $\pm$ 0.2 vs 3.4 $\pm$ 0.2 ng/mL, P=0.002). In contrast, diet did not influence circulating P4 in *B. taurus* heifers (2.0 $\pm$ 0.2 vs 2.3 $\pm$ 0.2 ng/mL, P=0.4). In addition, *B. taurus* and *B. indicus* heifers receiving HDM had similar P4 concentrations, respectively (2.0 $\pm$ 0.2 vs. 2.3 $\pm$ 0.2 ng/mL, P=0.40). However, Nelore heifers on LDM had greater circulating P4 compared to *B. taurus* heifers on the diet (3.4 $\pm$ 0.2 vs. 2.3 $\pm$ 0.2 ng/mL, P=0.006). There were no interactions between genetic group and diet (genetic group\*diet=P>0.10) for any of the evaluated genes. Interestingly, *B. taurus* heifers had greater expression of SRD5A1 (P<0.0001), AKR1C4 (P<0.0001) and AKR1D1 (P=0.002) than zebu heifers. However, the expression of CYP3A4 was greater in zebu than in *B. taurus* heifers (P<0.0001). Independently from genetic group, expression of AKR1D1 in heifers fed HDM was greater compared to LDM diet (P=0.004) whereas expression of AKR1C4 was greater in heifers on LDM compared to HDM (P=0.01). The expression of CYP2C19 and SRD5A3 was not influenced by genetic group (CYP2C19: P=0.76, SRD5A3: P=0.18) or diet (CYP2C19: P=0.27, SRD5A3: P=0.94). These results indicate that gene expression of enzymes related to P4 metabolism can be partially regulated by dry matter intake level as well as genetic group.

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A107 FTAI, FTET and AI

### **Impact of injectable long-acting progesterone and/or hCG three days after TAI on the conception rate of lactating Holstein cows**

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**Keywords:** Holstein, injectable progesterone, timed AI.

The aim of the present study was to evaluate the effects of administration of injectable long-acting progesterone (P4LA), associated or not with hCG treatment after timed-AI on conception rates of dairy cows. The study was conducted in two commercial farms (Farm A: 27.4L of milk/cow/day and free stall system and Farm B: 40.9L of milk/cow/day and cross ventilation system), both in São Paulo, Brazil. On a random day of the estrous cycle, 908 Holstein cows received a synchronization of ovulation treatment [D0: P4 (Sincrogest®, Ourofino Agronegócio) + EB (2 mg, i.m., Sincrodiol®, Ourofino Agronegócio); D8: device removal + PGF (0.53 mg, im, Sincrocio®, Ourofino Agronegócio) + ECP (1.0 mg, im, SincroCP®, Ourofino Agronegócio); D10 AM: TAI]. Three days after TAI, the animals were homogeneously distributed according to the daily milk yield, days in milk, parity and body condition score and divided into four experimental groups (2x2 factorial): 1. Control, cows receiving no additional treatment (n=250); 2. P4LA-900, treatment with 900 mg i.m. of P4LA (Sincrogest injetável®, Ourofino Agronegócio; n=245); 3. hCG, treatment with 2,000 IU i.m. of hCG (Chorulon®, MSD Schering, hCG) and 4. P4LA+hCG, treatment with both treatments (n=250). Data analysis was performed with the procedure GLIMMIX of SAS 9.3®. There was no effect of hCG or an hCG-by-P4LA interaction for none of the evaluated variables. Greater conception rate 30 days after TAI (CR30) was observed in cows treated with P4LA-900 (34.3 vs. 26.6%; P=0.002), and these results were independent from hCG treatment. Both the conception rate at 60 days (CR60) and the rate of pregnancy loss between 30 and 60 days after TAI (PL) were not affected by P4LA-900 or hCG, P>0.05. There was an interaction between P4LA-900 treatment and farm for CR60 (P=0.09), being observed a positive effect of P4LA on farm A (27.9 [362] vs 37.2% [363]), but not on farm B (18.7 [123] vs 15.2% [132], without and with P4LA, respectively). Interestingly, there was an interaction between P4LA treatment and season (warm vs. not warm) on CR30 (P=0.04) and CR60 (P=0.07) after TAI. Greater CR was verified in cows treated with P4LA during the warm season of the year (CR30: 16.3 vs 30.2% and CR60: 15.2 vs 26.9%). However, this increase was not observed during cooler seasons of the year (CR30: 32.9 vs 36.7% and CR60: 31.9 vs 33.9% without and with P4LA, respectively). In conclusion, treatment with 900 mg of P4LA three days after TAI in lactating Holstein cows increased conception rate at 30 days, mainly during the hot season of the year. There was no effect of hCG on conception rate.

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A108 FTAI, FTET and AI

### **Impact on the conception rate and expression of estrus using different GnRH or esters of estradiol as inducer of ovulation in TAI protocols in crossbred dairy cows**

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**Keywords:** estradiol, Girolando, TAI.

The objective of this study was to compare the expression of estrus and conception rate with the use of different estradiol esters [estradiol benzoate (BE) or estradiol cypionate (EC)] or gonadotropin-releasing hormone (GnRH) as ovulation inducers in artificial insemination protocols for fixed time AI (IATF) in Girolando cows (*Bos taurus* x *Bos indicus*). At random stages of the estrous cycle (Day 0), a total of 618 cows with an average BCS of  $3.04 \pm 0.02$ ; DPP of  $147.6 \pm 4.39$ , and average production of  $22.4 \pm 0.4$  L/day, received an intravaginal progesterone release device (P4) and 2.0 mg EB (IM, Gonadiol®, MSD Animal Health, São Paulo Brazil). On the removal of the P4 device (Day 8), cows received 530 mg of sodium cloprostenol (IM, Ciosin®, MSD Animal Health, São Paulo, Brazil), 400 IU equine chorionic gonadotropin (IM, eCG; Novormon®, MSD Animal Health, São Paulo, Brazil) and marked with tail-chalk to verify later manifestation of estrus. Then, all cows were allocated to one of the following experimental groups: 1) EC [n=204; 0.5 mg CE, IM (ECP®, Zoetis, Sao Paulo, Brazil), on the 8th and artificial insemination (AI) after 48h]; 2) EB (n=205; 2.0 mg BE, IM on days 9 and 24 h after IA) or 3) GnRH [n=209; 10mg Burserelein (Prorelin®), IM at TAI on day 10]. Cows that had the tail-chalk removed were considered to have showed estrus. Thirty days after AI the cows were submitted to ultrasonography for pregnancy diagnosis (transrectal linear transducer 5.0 MHz, CHISON D600VET, USProducts, Eletromedicina, Brazil), the pregnancy was characterized by observing the embryo's heartbeat. Statistical analyzes were performed using SAS PROC GLIMMIX software (SAS® 9.3). Estrus expression was greater in BE group when compared to the EC and GnRH groups, respectively (79.5 vs 60.6 vs 46.9%,  $P=0.0001$ ), and similar between the EC and GnRH groups. However, there was no statistical difference among groups in terms of conception rate [BE=32.7% (64/196); EC=26.6% (52/198); GnRH and=26.7% (55/206),  $P=0.83$ ]. Therefore it is concluded that the expression of estrus was greater following BE than CE and GnRH. In conclusion, the different ovulation inducers produced similar conception rates after TAI, although estrus expression was enhanced when estradiol esters were used as ovulation inducers.

**Acknowledgments:** Marajoara Farm, São Luiz Farm, MSD Animal Health.



A109 FTAI, FTET and AI

## **First use progesterone implants on reproductive performance of Nelore heifers in a TAI protocol**

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**Keywords:** corpus luteum, follicle, Zebu heifers.

Some studies indicate that high concentrations of plasma progesterone (P4) decrease LH pulsatility and, consequently, the follicular growth. As a result, high circulating P4 can decrease the efficiency of protocols for fixed time artificial insemination (TAI) that rely on utilization of intravaginal devices loaded with P4. This study aims to verify if first use P4 implants, loaded with 1.2 g of P4, will interfere with follicular dynamics in beef heifers receiving a TAI protocol. In this experiment Nelore heifers (n=33) without corpus luteum (CL) at ultrasound exam, were randomly distributed to receive one of the following treatments. At P4 device insertion day (D0) all heifers received 2 mg of estradiol benzoate (BE) and an intravaginal device with P4 0.6 g (monodose group, n=11); or a previously used for 8 days P4 device of 1.2g (used group, n=11); and finally a device with P4 1.2 g without previous utilization (Max group, n=11). Eight days later (D8), 2mL of PGF2 $\alpha$  was administered, simultaneously to 300 IU of eCG and ECP 0.5 ml to all animals; then the device was removed. Throughout D0 to D17, were performed ultrasound exams to evaluate ovaries and measure follicular diameter at 4, 8 and 10 days, and the CL on day 17. Blood samples were collected for P4 measurement on 0, 2, 4, 8 and 17 days. The follicular diameter, CL diameter, and P4 concentration variables were analyzed for homogeneity and normality tests, and later compared by ANOVA test. Variables that presented P<0.05 were compared by the DUNCAM test. There were no statistical differences among groups when we compared the follicular diameter on 4, 8 and 10 days, as well as the CL diameter on day 17 (P>0.05). The monodose device (0.6 g of P4) released greater (P<0.05) amounts of P4 than the Max device (P4 1.2 g) and also than the Max device (previously used) on days 2 and 4. In conclusion, these results suggest that implants with greater amounts of progesterone (1.2g) do not appear to change follicular growth profile in Nelore heifers, and can be indicated to be used without previous utilization in Nelore heifers.





A110 FTAI, FTET and AI

## **Ciclicity induction and reproductive efficiency of 14 months old Nelore heifers submitted to fixed time artificial insemination**

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**Keywords:** AI, precocity, synchronization.

Protocols to induce cyclicity in zebu heifers with 24 months age increases cyclicity and conception rates after timed artificial insemination (TAI), however there is little information addressing that for Nelore heifers with 14 months age. The objective of the present study is to evaluate the influence of the protocol to induce cyclicity on reproductive efficiency of Nelore heifers. A total of 626 heifers with  $14.0 \pm 0.04$  months age,  $261.3 \pm 0.94$  kg of live weight and  $BCS = 3.05 \pm 0.01$  (1-5), from two commercial beef farms (Camapuã and Três Lagoas, MS, Brazil) and distributed into 5 different cohorts were enrolled in the trial. Females were randomly allocated, within each cohort, into two experimental groups: Induction (n=307) and Control (no additional treatment; n=319). The Induction group received (Day -22) a progesterone (P4; CIDR®, Zoetis) device previously used for 24 days, which remained for 10 days. Immediately after P4 removal (Day -12), it was administered 0.6 mg IM of estradiol cypionate (EC; E.C.P®, Zoetis). Twelve days after (Day 0) all heifers were submitted to the same TAI protocol, receiving a auricular ear implant containing 3 mg of Norgestomet (CRESTAR®, MSD) and 2 mg IM estradiol benzoate (EB; Gonadiol®, MSD). At Day 8, the ear implants were removed and it was administered 0.6 mg IM de EC, 0.265 mg IM of sodium cloprostenol (Ciosin®, MSD) and 300 IU of equine chorionic gonadotrophin (eCG, Novormon®, MSD). Heifers were inseminated 48 h after (Day 10; 1st TAI). At Day 32 (22 days after 1st TAI), all females were resynchronized to receive the 2nd TAI with the same protocol described above, except by the EB dose at beginning of treatment (1 mg). At Day 40, the ear implants were removed and the pregnancy was diagnosed by transrectal ultrasonography examination (Chison 8200VET, Kylumax). Non pregnant heifers received the same protocol used for 1st TAI. The second pregnancy diagnosis was performed at Day 72 to evaluate P/AI after 2nd TAI. Moreover, ultrasonography examinations were performed on Days -22, -12 and 0 to evaluate the presence of a corpus luteum (CL). Statistical analysis was performed using the PROC GLIMMIX procedure of SAS (SAS® 9.3). The CL rate at Day -12 was similar between groups (Induction=1.3 vs. Control=1.3%;  $P=0.95$ ) however it was greater at Day 0 in heifers from Induction group (75.3%) in comparison to Control group (7.8%;  $P<0.0001$ ). Regarding the P/AI, no differences were observed among groups after 1st TAI (Induction=43.0 vs. Control=42.9%;  $P=0.99$ ), after 2nd TAI (Induction=35.6 vs. Control=32.6%;  $P=0.67$ ) and accumulated (1st TAI + 2nd TAI; Induction=63.5 vs. Control=61.8%;  $P=0.75$ ). Therefore, it is concluded that the protocol to induce cyclicity increases the percentage of cyclic Nelore heifers at 14 months of age; however, does not change P/AI after 1st and 2nd TAI.

**Acknowledgments:** São Mateus Farm, Engano Farm, Bovifértil and CNPq.



A111 FTAI, FTET and AI

### **Influence of follicle diameter at the time of TAI on the occurrence of estrus and conception rate in *Bos taurus* beef cows in anestrus**

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**Keywords:** *Bos taurus*, follicular diameter, TAI.

Follicular diameter at the time of artificial insemination at fixed time (TAI) is associated with the response of treatments for ovulation synchronization. Thus, the aim of the present study was to evaluate the diameter of the largest follicle (LF) present at the IATF time on the occurrence of estrus rate and the pregnancy rate per AI (P/AI) in anestrus lactating *Bos taurus* beef cows. Non-cyclic cows (n=435) from five commercial farms located in three different regions of Rio Grande do Sul state and kept on native pasture-grazing conditions were enrolled in this experiment. At the beginning of the synchronization protocol (D0), cows had an average of body condition score of  $2.77 \pm 0.02$  (scale of 1 to 5) and received a progesterone device (P4; CIDR®, Zoetis) and 2 mg IM of estradiol benzoate (EB; Gonadiol®, Zoetis). At D9, the device was removed and was administered IM 12.5 mg of dinoprost tromethamine (Lutalyse®, Zoetis) and 1 mg estradiol cypionate (ECP®, Zoetis). At this time, the females had the tail head painted with a paint stick (Raidl-Maxi, Raidex GmbH, Dettingen / Erms, Germany) to identify females that displayed estrus between device removal and TAI. On D11, cows were artificially inseminated and had the LF identified and measured by transrectal ultrasonography. Only females having no corpus luteum at the time of insertion and removal of the P4 device were used. Statistical analysis was performed using the SAS program. The average diameter of the LF was  $14.2 \pm 0.2$  mm. Analysis of the ROC curve (receiver operating characteristic), demonstrated that the critical LF diameter for the gestation establishment was 15 mm and the area under the curve was 66.1 [confidence interval (95% CI) was 0.661 to 0.780,  $P < 0.001$ ]. Cows that had LF  $> 15$  mm (n=257;  $17.0 \pm 0.1$ ) had higher occurrence of estrus (84.8% vs. 63.0%;  $P < 0.0001$ ) and higher P/IA (53.9% vs. 22, 6%  $P < 0.0001$ ) than those with LF  $\leq 15$  mm (n=178;  $12.5 \pm 0.1$ ). Also, a linear relationship was observed between the LF diameter and the probability of estrus occurrence [ $\exp(1.5503 + 0.1802 * LF / 1 + \exp(-1.5503 + 0.1802 * LF))$ ,  $P < 0.0001$ ] and P/IA [ $\exp(-3.4719 + 0.1985 * LF / 1 + \exp(-3.4719 + 0.1985 * LF))$ ,  $P < 0.0001$ ]. There was no quadratic relationship between the LF diameter and the occurrence of estrus ( $P = 0.19$ ) or P/AI ( $P = 0.30$ ). Thus, as the LF diameter at TAI increases, so it does the occurrence of estrus and P/AI, being 15 mm the threshold size for pregnancy establishment in suckled non-cyclic taurine beef cows enrolled in synchronization of ovulation protocols for TAI.

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A112 FTAI, FTET and AI

### **Influence of uterine environment (lactating dam or nulliparous) and dam's fertility on future reproductive performance of their offspring in Holstein cows**

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**Keywords:** fertility, Holstein, uterine environment.

The objective of this retrospective study was to evaluate whether reproductive efficiency of the dam and/or its uterine environment (lactating dam or nulliparous heifer) would affect future reproductive efficiency of their offspring. Fertility records (conception to 1st postpartum AI or TC1; and calving to conception interval or IPC) milk production standardized for 305 days of lactation (average production of 12.335 kg/lactation) in a total of 56.132 primiparous Holstein cows (offspring), from 226 commercial dairy herds located in the Central California Valley (USA), were used in this analysis. Information regarding IPC of the dam as well as the dam's parity (dam being a nulliparous heifer N=25750 or a lactating cow N=30384) were paired with the reproductive performance of their offspring, which were cows at their first lactation. Data were analyzed with the proc HPMIXED of the SAS software (version 9.3). When we divided the dam's IPC in classes (<50 d, 51-100 d, 101-150 d, 151-200 d, 201-300 d, and >300d), we observed that both TC1 and IPC of the offspring during their first lactation was affected ( $P < 0.01$ ), respectively: Dam's with IPC < 50 d=38.4% and 113d; 51-100 d=36.9% and 120d; 101-150 d=35.1% and 121d; 151-200 d=35.8% and 123d; 201-300 d=34.7% and 124d; > 300 d=31.5% and 127d. Unexpectedly, the uterine environment of the dam did not ( $P=0.15$ ) affect future fertility of their offspring and daughters from heifers had TC1=37.3% and IPC=119 d and daughters from lactating cows had TC1=36.0% and IPC=121 d. These results suggest that dams with poor reproductive efficiency will tend to produce daughters with compromised fertility; however, lactation status of the dam did not seem to affect fertility in their offspring.



A113 FTAI, FTET and AI

### **Artificial insemination and FTAI in dairy herds in Acre state: a case report**

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EMBRAPA.

**Keywords:** artificial insemination, dairy cattle, FTAI.

The state of Acre has one of the lowest daily average milk production per cow in Brazil reflecting a poorly technified production. This fact has produced a great requirement for genetic improvement of the herd so that producers can increase their productive efficiency. Artificial insemination (AI) is a widespread technique and aims the genetic improvement using semen of genetically superior bulls. Recently, to overcome the main difficulty of this technique, which is the estrus detection, AI has been used in fixed time (FTAI). However, both techniques must be implemented appropriately to avoid reduction in reproductive efficiency. The objective of this study was to report results of pregnancy rate obtained after the introduction of AI, with estrus detection, associated with FTAI in dairy herds. The AI program was started from a theoretical-practical course for family dairy farmers. Two farms located in Feijó city, in the state of AC, were enrolled. Pregnancy diagnosis was performed using ultrasonography (Mindray DP 2200) and herd data was collected. Pregnancy rates were evaluated before (natural mating) and after implementation of AI + FTAI. Pregnancy diagnosis was performed in all animals before the program implementation [first assessment (A1)] and 12 months later [second assessment (A2)]. At A1, a pregnancy rate of 58% (n= 48/83) was observed in the farm 01 for animals in reproductive age, using FTAI + AI, and natural mating during a specified period of 2 months. After A1, 9 animals were culled due to low production and advanced age. In the farm 02, a pregnancy rate of 50% (n= 13/26) was observed for animals in reproductive age, using FTAI and natural mating during a specified period of 2 months. There was no culling in farm 02. At A2, in the farm 01, a pregnancy rate of 77% (n=57/74) was observed, in which 82.5% of the pregnancies resulted from AI (conception rate of 48%) or FTAI (conception rate of 38%). In the farm 02, a pregnancy rate of 73% (n=19/26) was observed, in which 93% of the pregnancies resulted from FTAI (conception rate of 50%). In conclusion, the use of AI associated to FTAI increased the pregnancy rates in dairy herds, with satisfactory numbers of pregnancies.



A114 FTAI, FTET and AI

## **Intensification of reproductive management in a dairy herd**

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**Keywords:** dairy cattle, FTAI, reproductive management.

In order to evaluate the impact of the intensive use of fixed time artificial insemination (FTAI) on reproductive efficiency in a dairy herd in São Paulo State, Brazil, an analysis of 4512 artificial inseminations (AI; 1688 in primiparous and 2824 in multiparous cows) was performed between 2009 and 2014. These data were from 320 lactating dairy cows, managed in a free stall system with average production of 35.4±9.4 kg of milk/d. For analysis, two groups were established based on the reproductive management strategy: Non-Intensive (N-INT; 2009-2011), where cows were treated with two applications of PGF2 $\alpha$  at 40±7 and 54±7 days in milk (DIM), and on 73±7 DIM cows were subjected to FTAI; and Intensive (INT, 2012-2013), in which cows received PGF2 $\alpha$  (at 40±3 DIM), and after 14 d (54±3 DIM) were subjected to the FTAI. In both groups, after the voluntary waiting period (VWP; 40 DIM), cows observed in estrus were inseminated. For the N-INT group pregnancy diagnosis was conducted every 14 d, and for the INT, it was done every 7 d. Data were analyzed using the Freq, GLIMMIX and Lifetest procedures of SAS. The percentage of inseminations performed by FTAI was higher (P<0.001) in the intensive management period (INT: 56.9% [1474/2592] vs N-INT: 29.1% [559/1920]). In the survival analysis, N-INT group received the first AI (P<0.01) and became pregnant (P<0.01) later than the INT group. At 70 DIM, 65.4% (394/572) of N-INT cows had not been inseminated yet, while in the INT group only 35.2% (314/892). Furthermore, it was seen that 34.2% (184/538) and 45.4% (408/899) of cows became pregnant at 103 DIM (three 21-d cycles after the VWP) for N-INT and INT groups, respectively. The N-INT group had lower P/AI at 31 d post insemination (27.9% [539/1920] vs. 37.1% [903/2592]; P<0.01) and at 59 d (23.8% [463/1920] vs. 32.4% [777/2592]; P<0.01). However, there was no difference in pregnancy loss (13.6% [76/539] vs. 13.6% [126/903]; P>0.10). When we analyzed the interaction between groups and AI number, there was no effect (P=0.17) on P/AI at 31 d (23.6% [160/577], 28.4% [379/1343], 34.2% [341/898], and 34.8% [562/1694] for N-INT 1stAI, N-INT ReAI, INT 1stAI, INT ReAI, respectively), however on 59 d there was effect (22.6% [133/577], 25.0% [330/1343], 34.3% [302/898], and 30.5% [475/1694] for N-INT 1stAI, N-INT ReAI, INT 1stAI and INT ReAI, respectively; P=0.04). Therefore, the intensification of the reproductive management increased service rate to first AI, and consequently decreased the calving to conception interval. Furthermore, it improved fertility of the first postpartum AI.

**Acknowledgments:** FAPESP, CAPES, CNPq, São Jorge Farm and GEA Farm Technologies.



A115 FTAI, FTET and AI

### **Improvement of pregnancy rate by using the 6-days J-synch protocol in recipient cows transferred with *in vitro* produced embryos**

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**Keywords:** corpus luteum, embryo, ovary.

Proestrus length affects the ability of the follicle to growth and achieve ovulation, so pregnancy rate could be improved if proestrus is prolonged. Taking this into account, a new protocol for FTAI using estradiol and progesterone has been proposed named as J-Synch. In this new treatment the removal of the device is advanced to Day 6 instead Day 7 or 8, and GnRH is given 72 h later to induce ovulation instead of ECP at device removal. The objective of this study was to determine pregnancy rate in recipient females obtained with the conventional 7 d vs. the new 6 d progesterone treatment. The experiment was performed in five replicates using 945 cycling Hereford crossbreed recipients with body condition score >2.5 (1 to 5 scale). All the females received an intravaginal device (0.5 g progesterone, DIB, Syntex, Argentina) plus 2 mg EB i.m. (Syntex) (Day 0). In the 7-day progesterone + ECP treatment group (n=481) the DIB was removed on Day 7 in the morning and 500 µg cloprostenol (Ciclase DL, Syntex), 400 IU eCG (Novormon, Syntex), and 0.5 mg ECP (Cipiosyn, Syntex) was administrated i.m.. In the experimental J-Synch group (n=464), DIB was removed on Day 6 at time of cloprostenol and eCG was given i.m.. For this group the females received one dose of GnRH analogue (100 µg gonadorelin acetate, Gonasyn Gdr, Syntex) given 72 h after device removal. Fixed time embryo transfer (FTET) was performed on Day 16 and 17 with blastocysts produced by *in vitro* fertilization using sexed semen. Pregnancy rate was determined by ultrasonography 40-45 days after embryo transfer. Data were analyzed using logistic regression. Pregnancy rate was higher for the 6-days treatment (49.3%, 229/464) than conventional treatment (40.9%, 197/481; P<0.05). No effect for the day of the embryo transfer was found (FTET on Day 16: 45.9%, 249/543; FTET on Day 17: 44.3%, 178/402; P=NS). In conclusion, the extension of the period between progesterone fall and the treatment with the inducer of ovulation reached by the advancement of DIB removal on Day 6 and GnRH administration on Day 9 (i.e. J-Synch protocol) could improve pregnancy rate in recipient cows.



A116 FTAI, FTET and AI

### **Simultaneous thawing of ten semen straws reduces conception rates of beef cows submitted to TAI**

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**Keywords:** Nelore, semen thawing, TAI.

The aim of this study was to evaluate the effects of different methods of semen thawing on conception rate of beef cows undergoing artificial insemination at fixed time (TAI). A total of 1174 cows (1130 lactating and 44 non-lactating) with an average body condition score (BCS)  $3.00 \pm 0.01$ , from seven commercial farms, received at the beginning of treatment (Day 0), a progesterone release device (P4; DIB®; MSD Animal Health) combined with 2.0 mg estradiol benzoate IM (BE; Gonadiol®, MSD Animal Health). Eight days after (Day 8), the device was removed and cows received simultaneously 1 mg IM of estradiol cypionate (ECP Zoetis Animal Health), 530 mg IM sodium cloprostenol (Ciosin®, MSD Animal Health) and 300 IU IM of equine chorionic gonadotropin (Folligon®, MSD Animal Health). On Day 10, all cows were inseminated with semen straws (0.25 ml) thawed by two different methods, as follows: 1) Conventional (n=575), in which 10 semen straws were thawed simultaneously at 36°C using automatic defroster (CITO Warm Water Thaw; DSP-0063/AC)); or 2) Paused (n=599) in which only three straws were thawed simultaneously using the same temperature and automatic defroster to defrosting. After 30 seconds of thawing, straws were used sequentially (approximately one TAI per minute). Pregnancy diagnosis was done by transrectal ultrasonography (5.0 MHz, Aloka and Mindrey) 30 days after TAI. Statistical analysis was performed using SAS PROC GLIMMIX (9.3 SAS® Institute, Inc., Cary, NC, USA, 2003). The Paused method resulted in a greater conception rate (50.7%) compared to the conventional method (42.3%;  $P=0.01$ ). In conclusion, simultaneous thawing of three straws promotes better results than the simultaneous defrosting of 10 straws in lactating beef cows inseminated at fixed time.

**Acknowledgments:** Firmasa Tecnologia para Pecuária, Grupo Piveta, Camilha and Carolina Agulhon (Romaria Farm).



A117 FTAI, FTET and AI

### **Does artificial insemination procedure influences the cortisol plasma concentrations in cattle?**

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USP.

**Keywords:** artificial insemination, cortisol, radioimmunoassay.

The goal of this study was to determine whether the artificial insemination procedure (AI) is able to influence cortisol plasma concentration, due to stress that animals are subjected, as well as to correlate cortisol concentration and uterine hemodynamics data assessed by ultrasound Doppler in postpartum Nelore cows. Eighteen females were divided into two groups: inseminated (AIG, n=9) and non-inseminated (CG, n=9). All animals underwent estrus and ovulation synchronization; however, only AIG animals had semen deposited in the uterine body. All animals were evaluated for uterine hemodynamics using a Doppler ultrasound in the color mode, to assess vascularization score of the uterine horns (VS) and in the spectral mode, to evaluate uterine artery resistance index (RI) in five moments: 30 hours before AI, 4, 24, 48 and 168 hours after AI. Simultaneously, blood samples were collected from the jugular vein from all animals. Plasma was evaluated with a specific radioimmunoassay kit for determination of cortisol concentration. Cortisol levels are shown in nmol/L. Data were analyzed using PROC MIXED to detect differences between the groups and the PROC CORR of SAS was used to verify the correlation between the variables. Statistical significance was considered when  $P < 0.05$ . There was no difference in cortisol concentrations between groups (AIG=78.70±8.93, CG=68.71±5.30,  $P=0.33$ ). Regarding uterine hemodynamics, AIG had lower RI values (AIG=0.67±0.01; CG=0.73±0.02;  $P < 0.05$ ), indicating increased vascularization, however, no difference was observed between the groups regarding VS (AIG=1.81±0.08; CG=1.75±0.05;  $P > 0.05$ ). There was also no correlation between cortisol concentration and VS ( $R = -0.00527$ ,  $P = 0.96$ ) nor between cortisol concentration and RI ( $R = -0.00577$ ,  $P = 0.95$ ). In these circumstances, and considering the limited amount of animals (n=18), we could not observe differences in plasma cortisol concentration between animals that were inseminated or not, and there was no correlation between plasma cortisol concentration and uterine hemodynamics assessed by Doppler ultrasound.

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A118 FTAI, FTET and AI

## **Protocols for synchronization of follicular wave emergence and ovulation and the use of eCG can accelerate postpartum establishment of pregnancy in primiparous Nelore (*Bos indicus*) cows subjected to natural mating**

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**Keywords:** anestrous, bull, cyclicity induction.

We studied the effect of using protocols for synchronization of follicular wave emergence and ovulation associated or not to eCG on time to conception during the breeding season (BS) of primiparous Nelore cows kept on pasture with bulls. The study took place at Vera Cruz IV Farm, Barra do Garças – MT. At D0, 350 primiparous cows (30-50 DPP) were evaluated by ultrasonography (Mindray DP2200VET). Only those with complete uterine regression were randomized (taking into account their BCS) to one of three groups: without treatment (control), treated (protocol without eCG), or treated+eCG (protocol with eCG). Control cows received no previous treatment before bull mating, while treated cows received similar protocol except for the use of eCG (Ecegon®; Biogénesis Bagó, Argentina). The protocol consisted on the insertion of a 1g P4 device (Cronipres® Mono Dose, Biogénesis Bagó, Brazil) and 2mg estradiol benzoate (Bioestrogen®, Biogénesis Bagó, Brazil) IM on D0. On D9, device was removed and 1mg estradiol cypionate (Croni-Cip®, Biogénesis Bagó, Argentina) was given IM. Additional 300 IU of eCG was given only to treated+eCG cows. All cows were kept at the same pasture and exposed to bull mating on D9. The proportion bull:cow was 1:10 within the first 10d and 1:20 within the other 110d of the BS. Ultrasound exams were done at every 40d, registering gestational age to predict the quantity of new gestations every 21d cycle (C1, C2, C3, C4 and C5), starting at bull exposure (C1). Data was analyzed by PROC GLIMMIX, SAS. Control cows had lower conception at C1 (5.7%<sup>c</sup>, 7/123) than treated (30.4%<sup>b</sup>, 35/115) and treated+eCG (51.8%<sup>a</sup>, 58/112; P=0.001). One cycle later (C2) there was an increase on conception of all groups, but the difference between them was kept: 17.1%<sup>c</sup> (21/123), 42.6%<sup>b</sup> (49/115), 58.9%<sup>a</sup> (66/112); P=0.001. Similar effect occurred at C3 [27.6% (34/123)<sup>c</sup>, 52.2% (60/115)<sup>b</sup> and 70.4% (79/112)<sup>a</sup>; P=0.001] and C4 [42.3% (52/123)<sup>c</sup>, 58.3% (67/115)<sup>b</sup> and 74.1% (83/112)<sup>a</sup>; P=0.001]. Only at C5 control cows got closer to treated, but treated+eCG kept higher conception: 65.0% (80/123)<sup>b</sup>, 68.7% (79/115)<sup>ab</sup> and 82.1% (92/112)<sup>a</sup>; P=0.01. Thus, it was shown that (1) PP acyclicity is an important villain of reproductive efficiency of primiparous cows kept on pasture and that (2) treatment with TAI-like protocols is crucial to improve and accelerate PP conception. Also, the use of eCG potentially increased PP conception by allowing more than half of cows to conceive at the first PP service, which is higher than 5.7% and 30.4% found for control and treated cows without eCG. The velocity of PP conception is crucial to achieve 12m interval between parturitions and maximum herd reproductive efficiency. Thus, the use of TAI-like protocols associated with eCG can anticipate PP cyclicity and is a potential tool to improve conception of primiparous cows exposed to bulls, with impact until the end of the BS.

**Credits:** Farm Vera Cruz IV, FAPESP 2012/07510-1, CNPq 486089/2013-4.



A119 FTAI, FTET and AI

### **Loss of active ingredient in the process of reuse of intravaginal devices of progesterone**

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UNIFENAS.

**Keywords:** concentration, devices, progesterone.

The process of reutilization of intravaginal devices (VIP), although not indicated by manufacturers, is commonly used in Brazil. However, more information on cleaning these devices for reutilization is much needed (Neri et al., Arq. Bras. Med. Vet. Zootec., v.67, n.2, p.405-410, 2015) to elucidate possible problems during the cleaning process and disinfection of VIP aiming its reutilization. Usually, there is a great concern in regards to the loss of progesterone in this cleansing process. Thus, the current study is justified due to lack of information on this subject matter. This study then aimed to quantify the amount of P4 lost during cleaning of VIP when left resting in solution with or without detergent. We used ten new silicon-based VIP, loaded with 1g of P4 (Primer® - AgenerUniao – Brazil). IVP devices were placed in a clean bucket filled with 5 liters of filtered water with or without addition of 1% detergent solution. The implants were fully submerged in water. Immediately before each collection, the pre-set times samples of 0,1ml were collected with automatic pipette at times 0 (before put the VIP), 30, 60 and 120 minutes after putting the VIP. Before collect the samples containers were homogenized. The samples collected were diluted 100x to adjust the range of measurement Kit RIA (0.5 to 40ng/ml). In each container with 0.1ml of original sample were added 9,9ml of other solution. About the final total sample, 1ml was collected and placed on an eppendorf and frozen at -20°C for later evaluation of P4 by RIA (RIA IM 1188, Immunotech Inc., Prague, CzechRepublic). Each implant had originally 1g P4. The amount of P4 lost was determined by the concentration/ml x 5.000 (solution volume). As the dosage of P4 was expressed in ng/ml the loss percentage was calculated by the following formula:  $(\text{TotP4Sol}/109) * 100 = \text{TotP4Sol}/107$ . Average of P4 was accessed during periods and compared by ANOVA and TUKEY test. In containers without detergent the P4 averages differed among themselves on evaluated periods (26.6+8.7<sup>a</sup>; 35.0+9.2<sup>b</sup> and 40.6+10.4<sup>b</sup> ng/mL for measured times of 30, 60 and 120 minutes for P>0.05). Similarly, detergent containers had an increase of [P4] overtime (42.2+11.2<sup>a</sup>; 57.0+13.5<sup>b</sup> and 69.2+15.1cng/mL for measured times of 30, 60 and 120 minutes respectively). For the same time of sample collections the inclusion of detergent led to an increase in P4 in the solution (P<0.05). Despite the P4 loss, the percentage of P4 losses were negligible if we consider the total amount of P4 in the device. For the solution having no detergent the average of P4 loss was (1.33%, 1.75% and 2.03%, at 30, 60 and 120 minutes). The addition of detergent in the solution resulted in average P4 percentage loss of (2.36%, 2.85% and 3.46% for 30, 60 and 120 minutes in solution). In conclusion, although there were losses, these P4 losses are not significant when the implants are left in solution with or without detergent for up to 120 minutes.

**Thanks:** Fapemig, Capes and CNPq.



A120 FTAI, FTET and AI

### **Embryo production of Santa Inês ewes superovulated with FSH**

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**Keywords:** embryo, sheep, superovulation.

In this study we evaluated the effect of administration of decreasing doses of FSH 12/12 or 24/24 on superovulatory response and embryo production of Santa Inês ewes. We used 34 ewes divided into two treatments. For SOV protocol synchronization of estrus was obtained by placing vaginal CIDR with 0.3 g of P4 for 14 days, with device exchange on the seventh day, this was also applied 37.5 ug of D - cloprostenol. Ovarian stimulation was performed with total dose of 133mg of FSH. In treatment 1 (T1; n=17) donors received eight applications in decreasing doses every 12 hours and treatment 2 (T2; n=17) consisted of 4 applications of FSH in decreasing doses every 24 hours, started 48 hours before removal CIDR in both treatments. Twelve hours after CIDR removal ewes received 25 g of lecorelin as ovulation inducer; AI with frozen semen by laparoscopy was performed 12 and 22 hours after lecorelin and embryo collection through hemilaparoscopy occurred five days (d5) after AI. Ultrasonographic evaluation was performed in 50% of donors. The statistical analysis was performed with the program SAEG 9.1 applying the parametric and non-parametric tests. The average number of collected structures (T1=3.47, T2=2.55) were similar between treatments (P> 0.05). Viable embryos (T1=2.40, T2=0.82), freezable embryos (T1=2.07, T2=0.82) and the average number of LC (T1=7.47, T2=5.73) in the group of ewes superovulated with decreasing doses of FSH distributed in eight applications 12/12 was higher than the group of ewes who received four applications of FSH at 24/24 (P<0.05). In addition, the superovulatory response was similar (P>0.05) between donors T1 (88.24 %) and T2 (64.70%). The results suggest that the FSH regimen of eight-doses at 12-hour intervals is a better option for in Santa Inês ewes.



A121 FTAI, FTET and AI

### **Reducing beef cows handling to perform timed artificial insemination with sex-sorted sperm**

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<sup>1</sup>FMVZ/USP; <sup>2</sup>Sexing Technologies; <sup>3</sup>Aliança Assessoria.

**Keywords:** estradiol esters, sexed semen, TAI.

This study aimed to compare different ovulation inductors (estradiol benzoate or cypionate) during timed artificial insemination (TAI) protocols with sex-sorted sperm to reduce cattle handling (3 vs. 4 managements) and to evaluate the reproductive efficiency of suckled beef cows. At a random stage of the estrous cycle (Day 0), a total of 591 Nelore (*Bos indicus*) multiparous cows with BCS  $2.45 \pm 0.02$  received a new insert of progesterone (P4 device; CIDR®, Zoetis) plus 2 mg IM estradiol benzoate (EB; Gonadiol®, Zoetis). At P4 device removal (Day 8), cows received 25 mg IM of Dinoprost (Lutalyse®, Zoetis) and 300 IU of eCG (Novormon®, Zoetis) and were randomly allocated into one of four experimental groups in a factorial 2 x 2 design: 1) 1 mg of estradiol cypionate (EC; E.C.P®, Zoetis) at P4 device removal and TAI with non sex-sorted sperm (EC-0h-Conv; n=148); 2) the same treatment of EC-0h-Conv, inseminated with sex-sorted sperm (EC-0h-Sex; n=149); 3) 2 mg of EB 24 hours after P4 device removal and TAI with non sex-sorted sperm (EB-24h-Conv; n=145); and 4) the same treatment of EB-24h-Conv, inseminated with sex-sorted sperm (EB-24h-Sex; n=149). All TAIs were performed by three experienced AI technicians, 60 hours after P4 device removal using the same batch of three different sires (A, B and C). Cows were examined for pregnancy by transrectal ultrasonography (5.0 MHz linear transrectal transducer, Chison D600VET, Eletromedicina, Brazil) 30 d after TAI. Statistical analysis was performed using the PROC GLIMMIX procedure of SAS (SAS® 9.3). The BCS was considered as a covariate and AI technicians included in the model as random effect. There was no interaction ( $P=0.07$ ) between different ovulation inductors and semen type on pregnancy per AI (P/AI) which was, as follows: EC-0h-Conv=68.2% (101/148); EC-0h-Sex=51.0% (76/149); EB-24h-Conv=52.4% (76/145); and EB-24h-Sex=49.0% (73/149). However, EC promoted greater P/AI in comparison to EB [EC=59.6% (177/297) vs. EB=50.7% (149/294);  $P=0.03$ ] and non sex-sorted sperm resulted in greater P/AI compared to sex-sorted sperm [Non sex-sorted=60.4% (177/293) vs. Sex-sorted=50.0% (149/298);  $P=0.007$ ]. Furthermore, there was a difference according to sires on P/AI, regardless the ovulation inductors or type of semen used [sex-sorted sperm: Sire A=39.8% (39/98); Sire B=50.0% (51/102) and Sire C=60.2% (n=59/98). Non sex-sorted sperm: Sire A=54.5% (55/101); Sire B=62.1% (64/103) and Sire C=65.2% (58/89);  $P=0.01$ ]. Thus, non sex-sorted sperm resulted in greater P/AI and different sires resulted in different P/AI. Also, EC resulted in greater P/AI in comparison to EB, allowing reduced handling of suckled beef cows to be timed inseminated with sex-sorted sperm without compromising P/AI.

**Acknowledgments:** Sexing Technologies, Aliança Assessoria Agropecuária and Fazenda Santa Cristina.



A122 FTAI, FTET and AI

### **Relationship between population of antral follicles and uterine diameter in Nelore heifers**

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J.C. Arevalo Junior, W. Blaschi, T.R.R. Barreiros**

UENP.

**Keywords:** *Bos indicus*, follicular population, puberty.

The relationship between the number of follicles and hormone levels were shown to possible parameters to represent the reproductive potential *Bos taurus* females (Ireland et al., 2011, *Reprod. Fertil. Dev.* 23: 1-14). The aim of this study was to compare the relationship between the population of antral follicles and uterine diameter. Three hundred and thirty six Nelore heifers, 25.0±3.2 months old (BCS: 3.0 to 3.5, range 1-5) managed in *Brachiaria brizantha* and mineral supplementation containing 9% phosphorus were used during the month of October 2014. The animals were submitted to two transrectal ultrasonography exams (7.5 MHz, Mindray, China) with 10 days interval, for evaluation of cyclicity, viewing CL, counting all the visualized antral follicles (AFC) and measurements of uterine diameter, after obtaining three transversal evaluation and the average dimensions. The results were submitted to analysis of variance and correlation with significance level of 5% and heifers were classified as low (12.5±2.7; n=73), intermediate (21.7±2.4; n=86) AFC and high (35.0±7.7; n=177) (P=0.001). The uterine diameter was similar (P>0.05) for high (11.0±1.9 mm), intermediate (10.8±2.0 mm) and low AFC (10.3±2.7 mm). Prepubertal (n=280), pubertal heifers (n=56) had difference (P=0.03) in values for AFC, resulting in 27.3±11.1 vs. 23.7±8.0 follicles, respectively. The uterine diameter was greater (P=0.001) in pubertal (12.2±2.6 mm) than in prepubertal heifers (10.6±1.9 mm). The AFC was positively correlated with the size of the uterus (CR=0.11; P=0.03), however there was a positive correlation only in prepubertal heifers (CR=0.17; P=0.003) (pubertal: CR=- 0.05; P=0.6). The results suggest that follicular population has been possible to influence the uterine development prepubertal Nelore heifers.



A123 FTAI, FTET and AI

## Resynchronization protocols improve reproductive efficiency of suckled beef cows subjected to a breeding season during autumn-winter

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**Keywords:** bovine, breeding season, TAI.

The present study evaluated the impact of resynchronization (RE) after the first timed artificial insemination (TAI) in suckled beef cows (n=960; Angus and Brangus) submitted to breeding season (BS) during the autumn-winter. Females were subjected to one of four reproductive managements: 1) only natural mating (MN) during 90 days of the BS (n=266); 2) TAI at the first day of the BS (Day 0) followed MN until the end of the BS (TAI+MN; n=200); 3) TAI followed RE 30 days after the 1st TAI and MN until the end BS (TAI+RE30+MN; n=245) or; 4) TAI followed RE 22 days after the 1st TAI and MN until the end EM (TAI+RE22+MN; n=249). Cows were randomly assigned according to the calving date into the breeding groups and allocated in winter pastures composed of *Avena sativa*, *Lolium multiflorum* and *Trifolium repens*. The TAI protocols were similarly for TAI and RE. The treatment consisted of the insertion of a progesterone (P4) device (DIB®, MSD Animal Health) plus 2 mg of estradiol benzoate (EB, GONADIOL®, MSD Animal Health). Eight days later, the P4 devices were removed and were administered 500 mg of sodium cloprostenol (Ciosin ® MSD Animal Health), 1 mg of estradiol cypionate (ECP®, Zoetis) and 400 IU of equine chorionic gonadotropin (Folligon®, MSD Animal Health). The TAI was performed 48 hours after P4 device removal. Cows from MN group were exposed to bull during the entire 90 days BS, while the other groups, the bulls were introduced 10 days after the last TAI, using the proportion of one bull for each 20 cows. The pregnancy diagnosis were performed 30 (diagnosis of MN or pregnancy after the 1st TAI), 70 (diagnosis of MN or pregnancy after the 2nd TAI) and 120 (final diagnosis, 30 days after the end of BS) days after the onset of BS. Statistical analysis was performed by logistic regression using SAS PROC GLIMMIX. The effect of cow was considered as a random effect. At 30 days of BS, females undergoing a MN (3.0%) had lower pregnancy rate (P<0.001) compared to other groups (TAI+MN=40.0%; TAI+RE30+MN= 40.0%; TAI+RE22+MN=39.8%). At 70 days of BS, the groups receiving RE [TAI+RE30+MN (69.4%) and TAI+RE22+MN (66.3%)] obtained greater pregnancy rate (P<0.001) than the other groups [MN (16.9%) and TAI + MN (48.0%)]. However, the TAI+MN group had greater pregnancy rate than the MN group (P<0.001). The pregnancy rate at the end of BS was greater in cow receiving RE [TAI+RE30+MN (83.7%a) or TAI+RE22+MN (81.5%a)] than the MN (45.1%c) or TAI+MN (71.0%b). Still, females subjected to TAI+MN obtained greater pregnancy rate at end of the BS than those submitted to only MN (P <0.001). Thus, the use of resynchronization programs, RE30 or RE22, increase the proportion of pregnancy from IA and the reproductive efficiency of *Bos taurus* cows submitted to BS during the autumn-winter.

**Acknowledgments:** MSD Animal Health, CAPES.



A124 FTAI, FTET and AI

## **Supplementation with melengestrol acetate (MGA) post TAI improves fertility in suckled Nelore cows**

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**Keywords:** MGA, progestogen, TAI.

The objective of this study was to evaluate if oral supplementation of Melengestrol Acetate (MGA®, Zoetis, SP, Brazil) after TAI and the supplementation period increases pregnancy rate in Nelore cows submitted to TAI protocols with different gonadotropin stimulation (Calf removal (CR) or eCG). A total of 2301 multiparous suckled Nelore cows was enrolled to the experiment, distributed in 24 lots with similarly days post partum, and access to pasture of *Braquiaria* and water *ad libitum*. All cows were synchronized with the protocol: 2.0 mg of Estradiol Benzoate im (2.0 ml of Gonadiol®, Zoetis, SP, Brazil) + intravaginal progesterone device (CIDR®, Zoetis, SP, Brazil) in d-11; 12.5 mg of PGF2α im (2.5 ml of Lutalyse®, Zoetis, SP, Brazil) in d-4; Removal of CIDR® + 0.5 mg of Estradiol Cypionate im (0.3 ml of ECP®, Zoetis, SP, Brazil) in d-2; AI in d0. In d-2 15 lots (Farm 01) received 300 IU of eCG im (1.5 ml of Novormon®, Zoetis, SP, Brazil) and 9 lots (Farm 02) received CR. After insemination each lot of animals were randomly distributed in three treatments: Control- Animals did not receive MGA; MGA 6- MGA supplementation during 6 days, from day 13 to day 18 after TAI; MGA 14- MGA supplementation during 14 days, from day 5 to day 18 after TAI. Melengestrol Acetate dose utilized was 0.5 mg/day (2.28 g of MGA®/day) supplied with mineral salt every day. Mineral salt intake was evaluated for 3 days before the start of supplementation. Pregnancy diagnosis was performed by ultrasound 30 days after insemination. Pregnancy per AI (P/AI) was determined by dividing the number of pregnant cows by the number of inseminated cows. Was used the PROC GLIMMIX of SAS program for data analysis, random variables were included in the model and withdrawals (criterion of Wald ) when  $P > 0.2$ . Two contrasts were used to determine the effect of MGA supplementation [Control vs. MGA (MGA 6 + MGA 14)] and to determine the effect of MGA supplementation duration (MGA 6 vs. MGA 14). Significant differences were considered when  $P \leq 0.05$ . There was no interaction between BCS or stimulus (CR and eCG) on pregnancy rate. Cows supplemented with MGA had higher P/AI [62.7% (983/1568)] compared to control group [56.2% (412/733)] independent of gonadotropin stimulation. The duration of supplementation, independent of gonadotropin stimulation, did not change pregnancy rates [MGA 6: 61.9% (520/840) vs. MGA 14: 63.6% (463/728)]. We concluded that MGA supplementation post TAI increased pregnancy rates in suckled Nelore cows, independent of gonadotropin stimulation and duration of supplementation.



A125 FTAI, FTET and AI

## Supplementation with sunflower seed alters the endometrial lipid composition in beef cows

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**Keywords:** linoleic acid, lipids, PGF2 $\alpha$ .

Compounds that are rich in linoleic acid, such as sunflower seeds, provide lipid changes in the endometrium, and may be involved in the ability of PGF2 $\alpha$  biosynthesis. Previous studies (Cordeiro et al. 2015, *Theriogenology*, 83, 9, p. 1461-1468) observed that the conception rate increased in Nelore cows supplemented with sunflower seed for 22 days from the TAI (66.7 % vs 47.8 %) and in crossbred heifers submitted to TET (55.7 % vs. 36.9 %). We aimed to test the hypothesis that supplementation with sunflower seed promotes endometrial changes in lipid composition. Thus, we compared the composition of fatty acids in endometrial tissue in cows supplemented or not with sunflower seed. Nelore (n=30) cows received an intravaginal device containing progesterone (1g; DIB, Syntex Biochemistry & Pharmaceutical, Buenos Aires, Argentina) associated with an im injection of estradiol benzoate (2mg; Benzoate HC, Hertape Calier Animal Health, Juatuba, Brazil). The devices were removed after eight days, when cows were treated im with cloprostenol sodium (2mg; Sincrocio®, Ourofino Animal Health, Ribeirão Preto, Brazil), estradiol cypionate (0.5 mg; ECP®, Zoetis, São Paulo, Brazil) and eCG (300IU; Folligon®, MSD Animal Health, Madison, USA). Two days after removal of the device females were assigned into six groups to receive 1.7 kg/cow/day of 40% soybean meal, 44% crude protein (CP) + 60% sunflower seed for 6 (n=4), 14 (n=5) and 22 days (n=6), or 53% soybean meal, 44% CP + 47% corn for 6 (n=4), 14 (n=5) and 22 days (n=6). Both diets were formulated with 72% TDN and 24% CP. Females were slaughtered 24 hours after the end of supplementation and endometrial tissue was isolated and stored at -196°C. The fatty acids in endometrial tissue were assessed by gas chromatography. Data were analyzed by SAS Proc GLIMMIX. The fatty acid profile (54 compounds) was analyzed and 43 fatty acids were present in the endometrial tissue. The lacking fatty acids in endometrial tissue were C4:0, C11:0, C12:1, C: 13:0, C13:0 iso, C13:0 anteiso, C14:0 iso, C15:1, C18:1 T16, C18: 2 C12 T10 and C21:0. The fatty acids that showed a higher percentage compared to Control group were C18:1 T10-T11-T12 and C10:1. The fatty acids that showed low percentage compared to the Control group were C15:0 iso, C20:5, C20:3N3, C23:0, C24:0 and C22:5. In conclusion, the supplementation with sunflower seed promotes changes in the endometrial lipids. More studies are needed to examine the possibility of reducing embryonic mortality with such supplementation.

**Acknowledgments:** FAPESP, FUNDUNESP and Santa Encarnação Farm.





A126 FTAI, FTET and AI

### Conception rate to a TAI protocol in Nelore (*Bos indicus*) cows submitted to three or four managements using Sincrogest® or CIDR®

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**Keywords:** fertility, management, progesterone.

The aim of this study was to evaluate the conception rate in multiparous Nelore (*Bos indicus*) cows using two intravaginal progesterone (P4) devices (Sincrogest®; 1.0 g of P4 or CIDR®; 1.9g P4) during the TAI protocol 3 (Day 0, Day 8 and Day 10) or 4 (Day 0, Day 7, Day 9 and Day 11) managements. A total of 372 cows Nelore multiparous, from a herd located in Aquidauana/MS, was homogeneously distributed according to body condition score (mean of 2.90±0.02) and days postpartum (mean 38.8±0.3 days) at the beginning of the TAI protocol. At initiation of the TAI protocol (D0) the animals were treated with 2.0 mg EB IM (Sincrodiol®, Ouro Fino, Brazil) and insertion of the intravaginal device release of P4 (SINCROGEST® or CIDR®). To avoid additional managements from this point onwards, the cows were managed separately according to the type of protocol (3 vs. 4 managements). In cows receiving the protocol with 3 managements, withdrawal of P4 device was performed on D8 (Sincrogest® or CIDR®) associated to the treatment with 0.530 mg of sodium cloprostenol IM (Sincrocio®, OuroFino, Brazil), 300 IU IM of equine chorionic gonadotropin (eCG; SincroCG®, Ouro Fino, Brazil) and 1.0 mg IM of estradiol cypionate (ECP®, Pfizer, Sao Paulo, Brazil). The TAI was done on Day 10 (a.m.). For cows enrolled in the protocol with 4 managements, 0.530 mg of sodium cloprostenol (Sincrocio®, Ouro Fino, Brazil) was administered on D7. The, on Day 9, P4 devices (Sincrogest or CIDR) were removed associated with 300IU of equine chorionic gonadotropin (eCG; SincroCG®, Ouro Fino, Brazil) and 1.0 mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil). The TAI was performed on Day 11 (a.m.). After TAI, the groups of 3 and 4 managements were again regrouped until the time of pregnancy diagnosis performed by ultrasonography (Mindray, DP2200vet, São Paulo, Brazil) 30 days after TAI. Data were analyzed using the SAS procedure GLIMMIX 9.3. No interaction was observed between P4 device and the number of managements on conception rate [Sincrogest®\*3managements = 50.56% (45/89); CIDR®\*3managements = 54.90% (56/102); Sincrogest®\*4managements=52.63% (50/95); CIDR®\*4managements = 44.2% (38/86); P=0.41]. Likewise, there were no differences in conception rate between the groups treated with different P4 devices [Sincrogest® = 51.63% (95/184) vs. CIDR® = 50.00% (94/188); P=0.46], as well as number of managements, [3 managements = 52.88% (101/191); 4 managements = 48.62% (88/101); P=0.43]. Thus, it is possible to obtain similar conception rates to TAI in lactating zebu cows treated with Sincrogest® or CIDR®, regardless of number of managements employed (3 or 4 managements) during the TAI protocol.

**Acknowledgments:** Alliance Advisory, Santa Cristina Farm and Ouro Fino Agribusiness.



A127 FTAI, FTET and AI

## **Pregnancy rate in Nellore females supplemented with oral progestogen after TAI**

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**Keywords:** cyclicity, fertility, progesterone.

This study aimed to determine the impact of supplementation with oral progestogen after TAI on the pregnancy rate of cyclic and anovular Nellore cows. Two hundred and eighty three cows with 35 to 68 days postpartum and average body condition score of 3.35 (from 1 to 5) were subjected to two clinical and gynecological examinations (ten-day interval) with the aid of transrectal ultrasonography using linear transducer frequency 6.0MHz (Pie-Medical, Falco 100, São Paulo, Brazil) to estimate the reproductive status, being considered in anovular the cows without the presence of corpus luteum (CL) at both evaluations. Subsequently, all cows were subjected to the following synchronization protocol: at a random day of the estrous cycle (Day 0) they received an intravaginal progesterone device (P4, CIDR®, Zoetis, São Paulo, Brazil) associated with 2 mg of estradiol benzoate by intramuscular (IM) (GONADIOL®, Zoetis, São Paulo, Brazil). On day 8 (D8) the intravaginal progesterone device was removed and 25 mg of Dinoprost tromethamine was administered IM (Lutalyse®, Zoetis, São Paulo, Brazil), 1 mg of estradiol cypionate IM (ECP®, Zoetis, São Paulo, Brazil) and 300IU of eCG IM (NOVORMON®, Zoetis, São Paulo, Brazil). In Day 10 (D10), all cows were inseminated. On the 13th day after TAI, the cows were evenly distributed according to the prior cyclicity at the beginning of the protocol into two groups: MGA (n=154) cows supplemented with melengestrol acetate (MGA PREMIX®, Zoetis, São Paulo, Brazil) at a dose of 2.28 g per cow/day given in the trough with the mineral salt for a period of 6 days (between the 13th and 18th day after TAI) and NoMGA (n=129) cows received in the trough just the mineral salt without supplementation with MGA. Pregnancy diagnosis was done by transrectal ultrasonography 30 days after insemination. For the Statistical analysis of the variables, we applied the Chi-square test, using the 5% significance level (SPSS - version 19). There was no difference in pregnancy rates between MGA and NoMGA groups [52.6% (81/154) vs. 48.1% (62/129); P=0.414]. When analyzed separately, according to the reproductive status prior to the protocol, there was no difference between groups: cyclic: MGA and NoMGA [58.7% (27/46) vs. 52.3% (23/44); P=0.540] and anovular: MGA and NoMGA [50.9% (55/108) vs. 40.0% (34/85); P=0.131]. However, there was a numerical increase in pregnancy rates of anovular cows supplemented with progestogen, when compared with the control. Thus, supplementation with MGA between the 13th and 18th day after TAI did not interfere on pregnancy rates of anovular or cyclic Nellore cows. However, it is necessary to carry out further studies with a greater number of cows per experimental group.



A128 FTAI, FTET and AI

### **Pregnancy rate and litter size of English Bulldog bitches inseminated with fresh semen**

**G.H.M. Araujo<sup>1</sup>, B.M.S. Hernandez<sup>2</sup>, F.C. Leme<sup>2</sup>, A.C. Camplesi<sup>3</sup>, C.F. Moya-Araujo<sup>4</sup>**

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**Keywords:** canine, reproductive biotechniques, vaginal cytology.

The present study aimed to evaluate the estrous cycle of Bulldog bitches using vaginal cytology, and to inseminate these with fresh semen, leading to the determination of pregnancy rate and the size of the litter. Sixteen (16) English Bulldog breed bitches were used, all with good reproductive history and proved fertility, age ranging from 1.5 to 6 years. Associated with the proestrus detection (external signs: vulva edema and a blood-stained discharge) was performed the vaginal cytology in order to determine the oestrus moment, as well was observed the female behavior of male acceptance. The artificial inseminations (AI) started when the vaginal cytology showed 90% of superficial cells, then repeated every 48h until the vaginal cytology change to metaestrous (increasing the nucleated cell counting) and/or the bitch stop to accept the dog mating. Digital manipulation was used to perform the semen collection, all male breeders underwent a previous seminal examination being selected for use only those ejaculates with more than 70% of total motility, vigor 3 and, at least,  $200 \times 10^6$  of spermatozoa. The AI was performed using the intravaginal technique with all the collected ejaculate. The gestation confirmation were made by transabdominal ultrasonography between 25 and 30 days after the last AI, the ultrasonographic evaluation after that was carried out periodically in order to determine the best moment to perform the c-section. The vaginal cytology evaluation, associated with the comportamental changes in the bitch was efficient to detect the estrous onset in 100% of the bitches. Fourteen out of the 16 bitches inseminated got pregnant (87.5%). The litters had a mean number of 5.6 puppies, of those 53.1% (42/79) were male and 46.8% (37/79) were female. In conclusion, AI with fresh semen and whole ejaculate lead to good results in terms of fertility and a large litter size in English Bulldog bitches under these experimental conditions.



A129 FTAI, FTET and AI

### **Pregnancy rates in Nelore (*Bos taurus indicus*) heifers treated with PGF2 $\alpha$**

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**Keywords:** artificial insemination, estrus, prostaglandin.

The aim of this study was to evaluate the pregnancy rate of Nelore heifers treated with PGF2 $\alpha$ . The experiment was conducted in a farm near Paragominas, Para, in July and September of 2011. We used 694 heifers with average and SEM of 24.1 $\pm$ 2.0 months and body condition score (ECC) average 3.5 (range 1 to 5). All heifers underwent transrectal ultrasonography, CHISON D600VET model for ovary evaluation. Heifers that had corpus luteum (n=268) received a dose of 1.5 mL (375 mg) prostaglandin (Ciosin®) on day zero (D0) in the morning and were moved to a pasture for estrus observation in the morning (6 to 7 am) and afternoon (17 to 18h). After nine days (D9) the heifers that did not show estrus behavior (n=110) received a second dose of 1.5 mL (375mg) of prostaglandin (Ciosin®) done intramuscularly (IM). Animals identified in estrus were inseminated on average 12 hours later, being AI done during mornings (n=124) or afternoons (n=144). Inseminations were performed from D2 to D9 and from D10 to D16 with the use of semen from two different Nelore sires and 6 AI technician. Pregnancy diagnosis was carried out 35 days after the last insemination using ultrasonography, Model Ultrasonic Transducer - Mindray / DP-2200vet. The data were analyzed with the statistical Chi-square test with 5% significance level, using SAS (2010). Overall, pregnancy rates according to the number of PGF2 $\alpha$  and insemination period was evaluated (morning/afternoon). Pregnancy rate was 51.49% (n=138/268) in 16 AI working days. There was difference (P<0.05) in pregnancy rates according to the number of PGF2 $\alpha$  applications, where only one application of PGF2 $\alpha$  was enough to cause estrus induction in 58.96% (n=158/268) of all animals and a subsequent gestation in 56.96% (n=90/158) of the animals receiving just one PGF2 $\alpha$  and 43.64% (n=48/110) for two PGF2 $\alpha$  treatments. The pregnancy rate for the period of insemination was 45.16% (n=56/138) on the inseminations carried out in the morning and 56.94% (n=82/138) during the afternoon, being observed statistical difference between periods (P<0.05). The results show a satisfactory pregnancy rate following conventional artificial insemination program in Nelore heifers treated only with PGF2 $\alpha$ . In conclusion, a single application of PGF2 $\alpha$  is effective to obtain good pregnancy rates, with afternoon breedings having greater pregnancy results.



A130 FTAI, FTET and AI

### **Tolerana® improves the conception rate of inseminated cows**

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E.S.C. Morani<sup>1</sup>, M. Roncoletta<sup>1</sup>**

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**Keywords:** fertility, materno-fetal tolerance, TAI.

Fertility or reproductive efficiency is the most important component of production efficiency on bovine production systems. The economic value of cows reproductive efficacy were 3.24 bigger than other consume characteristics, indicating the importance of reproductive on bovine maternal herds system productivity (MELTON, 1995). Tolerana® is an innovated product that can help on this context, it acts mediating physiologic events of the maternal pregnancy recognition and maternal immune tolerance (communication between embryos and endometrium), that results in reduction of loss pregnancy or improve the embryo mortality prevent in mammals, resulting in great improvement of conception rate in cows. The goal of this project was to compare the conception rate of inseminated cows distributed in 02 groups (Treated and Control), considering that comparison was always done within each herd. In that way, breed, nutritional score and TFAI protocols are not influence factors on comparative conception rate between groups. Beef cows were used in test (n=7000 Nelore breed cows, average score 3 and with 75 days post-partum in average), distributes in 16 different herds and 250 Holstein cows (1 herd, average score 2.5 and 28kg/day of average milk production). The treatment was always randomly distributed and sequential within each pen of synchronized cows (1 was treated, another not, and successively until finish with that group). Statistical analyses were based on T-Test, using a linear model (pregnancy/non pregnancy) within each category (beef/dairy), using SAS System 6.9. Factors like breed, herd and category were not considered in this statistical model. The recommended dose of Tolerana® was 200µg/cow in one administration. The product was filled into 0.25mL French semen straw. The administration of Tolerana® was performed just after AI gun removal, in which another gun was passed through the cervix and the product was deposited on the uterine lumen. Preliminary results showed a gain of 9.4 percent points conception rate of beef cows, and 11.94 percent points in dairy cows. The average conception rate was 46.2% for Control Group and 55.6% for Treated Group. In dairy cows the average conception rate was 22% (Control Group) and 33.94% (treated Group). There are other ongoing experiments, looking for maximal optimization of the product, that is still at experimental stage and registration (MAPA); and thus not commercially available yet. However, with these results it appears that Tolerana® has an enormous potential – with a great impact in bovine reproduction; minimizing costs related to AI implementation and contributing to the genetic progress in bovine herds.

**Support:** FAPESP and CNPq-RHAE.



A131 FTAI, FTET and AI

### **Intrafollicular transfer of immature oocyte: an option for bovine female multiplication**

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**Keywords:** bovine, embryos, oocyte injection.

The *in vivo* embryo production by superovulation (SOV) and the *in vitro* embryo production (IVP) are the two available options to accelerate female germplasm multiplication. Despite the capacity of SOV to produce embryos of better quality, it must be respected an interval between superovulation protocols, the FSH administration may affect the physiological pattern of follicular dynamics decreasing the embryo production in the subsequent SOV procedures. On the other hand, with the IVP technique, the donor can be submitted to OPU sessions every week. However, oocytes submitted to *in vitro* production results in poorer quality embryos. The use of a technique that associates the advantages of *in vivo* and *in vitro* systems would be the best option for multiplying animals. The technique that fulfills these requirements is the immature oocytes intrafollicular transfer (IOIFT) in which immature oocytes obtained by OPU are injected into a dominant follicle from a synchronized recipient. The aim of this experiment was to evaluate the embryo production using the IOIFT technique. Heifers (n=12) were synchronized with the following protocol: On D-10 a P4 device (Primer®) was inserted together with the administration of 2 ml of estradiol benzoate (RIC-BE®); at D-2 the devices were removed simultaneously to the administration of 2 ml of prostaglandin (Veteglan®); D-1, 1 ml of EB was administered and on D0 estrus was detected. The oocytes were injected into dominant follicles 58 hours after P4 removal. The mean follicular diameter was 14±1.7 mm. The intrafollicular injections were guided by ultrasound equipped with a 7.5MHz vaginal probe (Aloka®) using a modified aspiration system. The system was filled with follicular fluid and attached to a 27G needle. Grade 1 and 2 oocytes were obtained by aspiration of slaughterhouse ovaries. After selection, 25 oocytes were placed in the needle, with 80 µL of follicular fluid. An insulin syringe at the other end of the system served to perform the injections. A single dose of semen was used for artificial insemination (AI), 6 hours after IOIFT and embryos were recovered by uterine flushing 7 days later. Before uterine flushing, CL measurement was performed to confirm if the ovulation had occurred in the ovary where IOIFT was carried out. The CL mean diameter was 20±2.3mm and CL was observed in the same ovary of IOIFT in 9 animals (75%). The average of recovered structures on D7 was 3.7±2.3 per animal, corresponding to 15% of injected oocytes. From the total structures, 58% were viable embryos (Mc, Bi, Bl or Bx), an average of 2.2±1.9 embryos per animal. Despite the low efficiency, IOIFT is a less expensive alternative for cows multiplication, and enables the production of better quality embryos. Moreover, it is an interesting *in vivo* model for research in the embryology field.



A132 FTAI, FTET and AI

### Melatonin or progesterone treatment in lactating buffaloes during seasonal anestrus

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**Keywords:** melatonin implant, progesterone, seasonal anestrus.

The effect of treatment with subcutaneous melatonin implants (MEL) or intravaginal progesterone device (P4) in the ovulation induction and pregnancy outcomes in seasonal anestrus buffaloes (spring and summer) subjected to the Ovsynch protocol was evaluated. At the beginning of the experiment (D -20), 139 anestrus buffaloes were divided according to the body condition score, parity, postpartum period, and diameter of the largest ovarian follicle (LF) into one of 3 groups: control (CG, n=46), progesterone (P4G, n=47) and melatonin (MG, n=46). On this day, each MG buffaloes received twelve MEL implants (216mg; Regulin®, Ceva Animal Health, UK). After 20 days (D0), all females received 10µg im (intramuscular) of GnRH (Buserelin acetate, Prorelinn®, Innovare Biotecnologia e Saúde Animal, Brazil), and in the buffaloes from the P4G was inserted an intravaginal P4 device (1g, Cronipres®, Biogenesis-Bagó Animal Health, Brazil), which remained for seven days. In D7, all females received 150µg im of PGF2α (D-Cloprostenol, Croniben®, Biogenesis-Bagó). Two days after (D9), buffaloes received 10µg im of GnRH (Prorelinn®), and 16h later (D10), were submitted to TAI. After ten days (D20), females were subjected to natural mating, to cover the return to estrus. All buffaloes were evaluated by ultrasonography (Mindray DP2200Vet, China) on D -20 to check the presence of corpus luteum (CL), in the D0 and D9 to measure LF diameter, on D7 to assess ovulation rate to 1st GnRH (Ov-rt), on D20 to evaluate the CL percentage and diameter, on D30 for the pregnancy diagnosis and on D60 to quantify pregnancy loss (PgL) and the pregnancy rate for natural mating (PgM). Statistical analysis was performed by GLIMMIX procedure of the SAS®. There were no differences among experimental groups (CG vs. MG vs. P4G) for the analyzed variables, respectively: LF on D0 (11.5±0.3 vs. 12.0±0.3 vs. 11.4±0.3 mm, P=0.37), Ov-rt (45.6 vs. 38.3 vs. 39.1%, P=0.67), LF on D9 (12.9±0.5 vs. 12.9±0.4 vs. 12.8±0.5 mm, P=0.88), PgL (12.9 vs. 0.0 vs. 16.7 %; P=0.40) and PgM (28.6 vs. 12.5 vs. 36.6%, P=0.37). The P4G showed higher CL diameter in D20 and pregnancy rate at TAI than the CG and the MG (19.2±0.5 vs. 16.6±0.8 vs. 16.2±0.6 mm and 66.0 vs. 8.7 vs. 13.0%; P<0.01); higher percentage of CL on D20 and cumulative pregnancy (TAI+PgM) than the CG and similar to the MG (83.0<sup>a</sup> vs. 54.4<sup>b</sup> vs. 71.4<sup>ab</sup> % and 61.7<sup>a</sup> vs. 34.8<sup>b</sup> vs. 41.3<sup>ab</sup> %, P<0.03) and less days open than the CG and the MG (18.5±1.5<sup>b</sup> vs. 36.1±1.3<sup>a</sup> vs. 34.2±1.5<sup>a</sup> days; P<0.01). In conclusion, P4 treatment associated to the Ovsynch protocol promotes better results regarding the association with MEL in terms of the CL diameter and pregnancy rates, reducing the days open in lactating buffaloes during the seasonal anestrus.



A133 FTAI, FTET and AI

### **Treatment with prostaglandin at the beginning of the protocol in beef cows with CL increases conception rate after FTAI**

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**Keywords:** corpus luteum, prostaglandin, TAI.

In beef cows the concentrations of progesterone (P4) before the proestrus is associated with the size of preovulatory follicle that is positively associated with fertility. Thus, strategies that manipulated the concentration of P4 during the fixed-time artificial insemination (TAI) protocol could be useful to improve the results of TAI programs at commercial operations. A simple way to make this is to add a dose of prostaglandin (PGF) on the beginning of TAI protocol (P4 device insertion) of cycling cows. Thus, this work aimed to compare the conception rate at 30 days (CR) of beef cattle after TAI with or without corpus luteum (CL) at the beginning of the protocol. On a random day of the estrus cycle (Day 0) a total of 3,134 beef cows (Exp. 1, 10 different farms, 1,001 cows with CL and 2,133 cows without CL) received a P4 intravaginal device (Sincrogest®, Ourofino Saúde Animal) and 2 mg i.m. of estradiol benzoate (Sincrodiol®, Ourofino Saúde Animal). On Day 8, P4 device was removed and cows received 500 µg i.m. of sodium cloprostenol (Sincrocio®, Ourofino Saúde Animal), 1 mg i.m. of estradiol cypionate (SincroCP®, Ourofino Saúde Animal) and 300 IU i.m. of eCG (SincroeCG®, Ourofino Saúde Animal). TAI was performed on Day 10. In Exp. 2, 3,802 cows (14 different farms, 1,374 cows with CL and 2,428 cows without CL) received the same protocol previously described; however, a dose of 500 µg i.m. of cloprostenol sodium (Sincrocio®, Ourofino Saúde Animal) was administered on Day 0 in cows presenting CL at the beginning of the protocol. The GLIMMIX procedure (SAS) was used for statistical analysis. In Exp.1, no difference (P=0.08) was observed on the CR between cows with (48% [N=1,001]) or without (51.3% [N=2,133]) CL. On the other hand, in Exp.2 (PGF on Day 0 in cows with CL) the group of cows with CL on Day 0 presented higher CR (55.5% [N=1,374] vs. 52.1 [N=2,428], P=0.05). Farm effect was not detected. In conclusion, similar CR was observed between cows with or without CL at the beginning of protocol when PGF was not used at D0. Also, the addition of a dose of PGF at the beginning of the protocol in cows with CL increased the CR in comparison to other experimental group.





A134 FTAI, FTET and AI

## **Different antibiotics associated or not to sodium cloprostenol for treatment of puerperal problems in dairy cows**

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UNIFENAS.

**Keywords:** antibiotic treatment, cefquinome, cloprostenol.

Uterine infections are among the main causes of reduced fertility in dairy cows, both in cases of natural mating, conventional insemination, and fixed time (TAI). The objective of this study was to evaluate the efficacy of two antibiotics, associated or not to sodium cloprostenol in the treatment of puerperal infections in dairy cows. The cows were divided evenly according to parity and body condition score and all treatments were applied intramuscularly, as follow: T1 (n=25): cefquinome (Cobactan® - MSD, Brazil), 1ml / 25kg body weight (BW) once a day for 3 days, T2 (n=25): cefquinome 1ml / 25 kg BW – once a day for 3 days plus 0.5 mg of sodium cloprostenol (Ciosin® - MSD, Brazil) on the 1st and 3rd day of treatment, T3 (n=25): oxytetracycline hydrochloride (Terramycin LA®, Zoetis, Brazil) for two days at 48 hours interval, and T4 (n=25) oxytetracycline for two days at 48 hours interval plus 0.5mg cloprostenol on the 1st and 3rd day of treatment. The animals were re-assessed between the 3rd and 5th weeks postpartum by rectal palpation and ultrasound vaginoscopy to assess the presence and degree of uterine infection. We evaluated the conception rate at 1st insemination, the number of pregnant animals up to 250 days after birth, the average number of services/conception, the calving interval at the 1st artificial insemination (AI) and services period. The trial was carried in a factorial arrangement 2x2 and differences between means were declared at 5% of significance. Significant interactions between the factors studied were not observed. The percentage of infection between the 3rd and 5th weeks was 36.0<sup>a</sup>; 24.0<sup>a</sup>; 64.0<sup>b</sup> and 48.0<sup>ab</sup>. The conception rate at the 1st AI was 24.0; 32.0; 16.0 and 20.0 (P>0.05). The percentage of pregnant dairy cows up to 250 days postpartum was 76.0<sup>bc</sup>; 88.0<sup>c</sup>; 56.0<sup>a</sup> and 68.0<sup>ab</sup>. The calving interval at the 1st AI was 67.7±18.6<sup>b</sup>; 53.2±13.8<sup>a</sup>; 74.0±22.7<sup>c</sup> and 64.7±15.1<sup>b</sup> days. The number of services/conception was 3.2±1.8; 2.9±1.5; 3.5±2.0 and 3.3±1.9 (P>0.05), whereas the services period was 158.5±86.4<sup>b</sup>; 144.6±72.0<sup>a</sup>; 179.8±96.2<sup>c</sup> and 161.3±81.2<sup>b</sup> days for the treatments T1, T2, T3 and T4, respectively. Comparing the same antibiotic, the association with cloprostenol improved the service period in all cases (P>0.05). We concluded that cefquinome is more efficient than oxytetracycline hydrochloride in the treatment of postpartum uterine infections in dairy cows. The association between antibiotics with an analog of prostaglandin can improve reproductive performance of dairy cows.

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A135 FTAI, FTET and AI

## **Use of Bioabortogen H® and Bioleptogen® as a strategy to reduce gestational loss between 30 and 60 days of beef heifers**

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**Keywords:** abortion, immunization, reproductive efficiency.

The aim of the study was to evaluate the effect of using reproductive vaccines Bioabortogen H® and Bioleptogen® (protection against IBR, BVD and leptospirosis) previously (D-40 and D0) or during and after the TAI protocol (D0 and D40) of beef heifers on 30 and 60d conception rates and pregnancy loss. The study was conducted at Farm Vera Cruz IV, Barra do Garças – MT. Heifers (n=700) aging between 15 and 24mo were evaluated by ultrasonography and if considered prepubertal (immature uterus and absence of CL) they were subjected to a treatment to induce cyclicity 40d before starting the TAI protocol [D-40: insertion of one 0.558g P4 device (Cronipres Mono Dose®, Biogénesis Bagó, Brazil) previously used for 8d, and D-30: device removal and administration of 1 mg estradiol benzoate (EB; Bioestrogen®, Biogénesis Bagó, Brazil)]. On that day, both cyclic and non-cyclic heifers were homogeneously allocated (cyclicity, ECC and live weight) to receive (VacBefore) or not (VacD0 and control) the first dose of both vaccine Bioabortogen H® and Bioleptogen® (5 mL SC each, Biogénesis Bagó, Brazil). VacBefore heifers received the booster of both vaccines on D0, the beginning of TAI protocol. VacD0 heifers received the first dose of both vaccines on D0 and the booster on D40, the day of pregnancy diagnosis 30d after TAI. Control heifers did not receive vaccination. All heifers were reassessed for cyclicity on D0 and only those considered pubertal (n=619) were kept in the study. The TAI protocol consisted in the insertion of an intravaginal device with 0.558g P4 and 2 mg EB on D0; device removal and administration of 0.150 mg of D-Cloprostenol (Croniben®, Biogénesis Bagó, Brazil) and 300 IU of eCG (Novormon®, MSD, Brazil) on D8; administration of 1 mg EB 24h after (D9) and TAI 48h after device removal. Pregnancy diagnosis was performed 30 and 60d after TAI through ultrasonography (Mindray DP2200VET). Data analysis was done by logistic regression (SAS PROC GLIMMIX). Similar average BCS (P=0.97) and body weight (P=0.48) were observed for heifers from control group (3.02±0.02, and 297.6±3.1), VacBefore (3.02±0.01 and 301.4±3.3) and VacD0 (3.02±0.02 and 299.4±4.3), demonstrating the homogeneity between groups. There was no difference in pregnancy rates at 30 [(control: 37.0% (71/192), VacBefore: 40.5% (90/222) and VacD0: 35.1% (72/205); P=0.55] and 60 days post-TAI [(control: 33.3% (64/192), VacBefore: 40.1% (89/222) and VacD0: 34.2% (70/205); P=0.33]. However, pregnancy loss between 30 and 60d of gestation was considerably inferior in heifers from VacBefore [1.1%<sup>b</sup> (1/90)] compared to control [9.9% (7/71)<sup>a</sup>] and similar to VacD0 [2.8%<sup>ab</sup> (2/72); P=0.05] Therefore, despite the use of Bioabortogen H® and Bioleptogen®, vaccines did not interfere in pregnancy rates 30 and 60d after TAI. The use was effective to reduce pregnancy loss during this period, especially when used previously to the TAI.

**Credits:** Farm Vera Cruz IV, FAPESP 2012/07510-1, CNPq 486089/2013-4.



A136 FTAI, FTET and AI

### Use of two doses of cloprostenol at different intervals for estrus synchronization in Santa Inês ewes

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**Keywords:** ovine, ovulation, prostaglandin.

The objective of this study was to compare protocols for estrus synchronization, using two doses of cloprostenol in different intervals, in March, during the breeding season, in Santa Inês ewes, in the city of Cachoeiras de Macacu-RJ. A total of 30 ewes (43.9±6.4 kg, 2.9±0.27 BCS and 3.4±1.6 years old) weaned at least for three months was equally allocated into three treatments, with intervals of: 11.5 days (G11.5: n=10), 9 days (G9: n=10) or 7 days (G7: n=10). The dose used for each administration was 37.5 µg cloprostenol (Estron®, Agener União, São Paulo, Brazil) intramuscularly. Transrectal ultrasound evaluations were performed (B-mode; SonoScape®, Shenzhen, China) for monitoring the follicular and luteal dynamic, daily from 5 d before the first dose, and every 12 h after both administrations, again for 5 d or until ovulation. For estrus detection, females were teased individually every 12 h after each cloprostenol administration for 5 d and for 5 min per animal. Normal quantitative variables were subjected to ANOVA followed by Tukey test (P<0.05). Data concerning the rate of ewes in estrus (%) were evaluated by Fisher's exact test (P <0.05). The percentage of animals in estrus after the first dose did not differ (P>0.05) among groups: G11.5 – 60% (6/10); G9 – 80% (8/10) and G7: 80% (8/10) as well as the duration of estrus (G11.5: 24.0±13.1 h; G9: 37.5±7.7h and G7: 28.5±11.0 h. After the second dose, estrus presentation rates and duration of estrus also did not differ (P>0.05) among groups, respectively: G11.5 – 90% (9/10) and 29.3±12.2 h; G9 – 100% (10/10) and 36.0±10.4 h; and G7 – 80% (8/10) and 31.5±8.9 h. There was no statistical difference (P>0.05) in the intervals from the second dose to the onset of estrus, end of estrus, and from estrus to ovulation between G11.5 (48.5±8.9 h; 80.0±8.5 h; 35.0±20.1 h), G9 (50.3±13.1 h; 83.0±9.1 h; 25.5±12.2 h) and G7 (36.5±6.2 h; 68.0±14.0 h and 20.3±6.1 h). The interval from the administration of the second dose to ovulation differed (P<0.05) among groups G11.5 (78.7±9.4 h), G9 (75.5±8.3) h e G7 (56.8±6.2 h). The G7 anticipated ovulation and presented a lower standard deviation (P<0.05). In conclusion, it appears that animals in the G7 were close to follicular dominance period or were already with the dominant follicles while G11.5 and G9 might still be in the beginning of the follicular wave. The three protocols were effective for estrus synchronization in Santa Inês ewes.



A137 FTAI, FTET and AI

## **Vetscore: a new method for nutritional assessment and selection of beef cows to TAI**

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**Keywords:** body condition score, cows, TAI.

Reproductive performance of suckled beef cows is directly associated to their nutritional status. Cows with adequate body condition score (BCS) at timed artificial insemination (TAI) are more likely to become pregnant than cows with low BCS. Although the benefits that BCS evaluation may represent in selecting cows for TAI, the method still consists in visual observation and, therefore, subjective. Based on that, the objectives of this study were to: 1) evaluate the relationship between the angle formed between the sides of the rump and BCS; and, 2) develop an objective tool to select cows for TAI according to their nutritional status. In Experiment 1, 801 suckled Nelore cows of 3 to 12 years old and weighing 400 - 625 Kg. All females were evaluated according to their BCS (1 to 5 scale; 1=emaciated e 5=obese; Ferguson et al., J Dairy Sci, v.77, p.2695-2703, 1994). Moreover, the angle formed between the sides of the rump was measured in all cows with a goniometer instrument. All analysis were performed in SAS 9.0 statistical software. The relationship between BCS and the rump angles was analyzed by regression models. There is a positive relationship between BCS and angle of the rump ( $P < 0.0001$ ), whereas the linear regression equation was  $\text{Angle} = 79.78 \pm 9.53 \times \text{BCS}$ ;  $R^2 = 0.54$ . Based on the results from Experiment 1, a device that aims to evaluate the nutritional status in a simple, direct and objective way was developed for use in the Experiment 2. This device, known as Vetscore (INPI registration n. 1020140049916), evaluates the BCS according to the rump angle and it classifies cows into 3 different nutritional status, such as: Red, cows with BCS  $< 2.75$ ; Green, cows with BCS between 2.75 and 4.25; and Yellow, cows with BCS  $> 4.25$ . In this study, 354 Nelore multiparous suckled cows, 4 - 8 years old, were subjected to a TAI protocol (2 mg BE + CIDR at D0 / 2 mL PGF + 300 IU eCG + 1 mg ECP - CIDR at D8 / TAI at 48 h). On D0 all cows were evaluated with the Vetscore and were classified according to its nutritional scale. The pregnancy status was detected with ultrasound 30 d after TAI. Pregnancy rate was analyzed using the Chi-square test. Vetscore presented a precision of 80% and cows classified as Green had higher pregnancy rate than cows classified as Red and Yellow ( $P < 0.01$ ). The pregnancy rates according to Vetscore were 38.6% (51/132), 56.74% (122/215), and 28.6% (2/7), for Red, Green, and Yellow, respectively. These results demonstrate that the new methodology to evaluate nutritional status and select cows for TAI is proven efficient. Therefore, Vetscore device may easily be applied to livestock's production system.