



A052 FTAI, FTET and AI

Fertility of crossbred dairy cows submitted to FTAI with eCG

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Keywords: artificial insemination, cattle, equine chorionic gonadotropin.

The aim of this study was to evaluate the effect of eCG on fertility of crossbred (*Bos taurus* x *Bos indicus*) lactating cows submitted to a protocol of fixed time AI (FTAI) between October and November 2013. A total of 265 cows was used, 165 multiparous and 100 primiparous, with an average milk production of 24.3 ± 0.47 kg/d and average of 127.7 ± 5.38 DIM. 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 0.5 mg D-Cloprostenol im (Sincrocio®, Ouro Fino, Brazil) and were randomly assigned into two groups: eCG (n = 132) – received 400 IU eCG im (Novormon®, MSD, Brazil); and Control (n = 133) – did not receive eCG. At the afternoon of D8, devices were removed and all cows received 0.5 mg 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 PROC GLIMMIX of SAS and the results are presented as least squares means \pm SE following eCG and Control groups, respectively. Pregnancy per AI (P/AI) at 30 ($37.1 \pm 5.4\%$ vs. $22.3 \pm 4.5\%$; P = 0.02) and 60 ($27.8 \pm 5.1\%$ vs. $16.1 \pm 4.0\%$; P = 0.03) days was higher in eCG group. However, pregnancy loss did not differ between groups ($19.6 \pm 7.6\%$ vs. $21.5 \pm 10.4\%$; P = 0.80). Furthermore, there was a tendency (P = 0.11) for interaction between the use of eCG and the number of previous postpartum services. Cows treated with eCG and inseminated at 1st AI postpartum had higher P/AI at 30 ($42.6 \pm 8.2\%$ vs. $18.1 \pm 6.0\%$; P = 0.02) and 60 ($32.3 \pm 7.9\%$ vs. $12.0 \pm 4.9\%$; P = 0.08) days. When cows were divided according to milk production: higher (30.0 kg/d; n = 134) or lower (18.5 kg/d; n = 131), there was also a tendency for interaction (P = 0.11). Higher producers that received eCG had higher P/AI at 30 ($36.0 \pm 7.3\%$ vs. $15.0 \pm 4.4\%$; P < 0.01) and 60 ($26.5 \pm 6.7\%$ vs. $10.2 \pm 3.5\%$; P < 0.01) days. However, in lower producers this did not happen at 30 ($30.8 \pm 15.8\%$ vs. $25.2 \pm 16.6\%$, P = 0.48) nor at 60 ($29.2 \pm 6.1\%$ vs. $24.7 \pm 6.5\%$, P = 0.48) days. It is concluded that the use of eCG on D7 of the protocol increases fertility of crossbred dairy cows submitted to FTAI.

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A053 FTAI, FTET and AI

Intensive reproductive management in beef cows - Quick resynch

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Keywords: color Doppler, early diagnosis of pregnancy, re-synchronization.

The study aimed to verify whether the hormonal protocol for re-synchronization, beginning 13 days after artificial insemination (AI), affects pregnancy rates of beef cows, and the efficiency of a new timed AI (TAI) performed 23 days after the first AI. For this purpose, 224 females (cows and heifers) of Gyr and Nelore breeds were allocated in Control or Resynch group, according to the postpartum period (151.2 ± 115.2 d), body condition score (3.1 ± 0.6), body weight (412.4 ± 78.6 kg) and ovarian condition (corpus luteum and/or follicle ≥ 8 mm). All animals were first inseminated after the same hormonal protocol. On D0 (day 0): insertion of intravaginal device containing progesterone (P4 - 750mg, Prociclar[®], Hertape Calier) and 2mg of estradiol benzoate injection (EB - 1.0mg/ml, Benzoate[®] HC, Hertape Calier). On D8: device withdraw, 0.150mg of cloprostenol injection (PGF2 α - 0.075mg/ml, Veteglan[®], Hertape Calier) and 0.5ml of follicle stimulating hormone (25UI - LH and 25UI - FSH, Pluset[®], Hertape Calier); and D9: 1mg of EB. Timed AI was performed between 48-56h after P4 device withdraw and the diagnosis of pregnancy occurred 30d after AI (control group, n=75). In Resynch group, re-synchronization protocol started 13d after AI (new P4 device and 1mg of EB). On D21 after AI, the P4 implant was removed and the diagnosis of pregnancy – based on functionality of the CL (Siqueira et al., 2013. J Dairy Sci., 96(10):6461-72) – was performed with Color Doppler sonography (M5, DPS medical equipment, Sao Paulo). Afterwards, the animals with negative diagnose received 0.150mg of PGF2 α , 1mg of estradiol cypionate (EC - 1.0 mg/ml, cypionate HC[®], Hertape Calier), and were inseminated 48-56h later. Pregnancy rates between groups were compared by Chi-square test at 5% of probability. The hormone treatment and management associated with the re-synchronization protocol did not affect ($P > 0.05$) pregnancy rates (18.7 vs. 18.1%, respectively for Control and Resynch). In Resynch group, the pregnancy rate of the second AI was higher ($P < 0.01$) than first (33.3 vs. 18.1%, respectively). Altogether, the pregnancy rate after the end of the trial was greater ($P < 0.002$) in Resynch (42.3%) than in Control group (18.7%). These results confirm our hypothesis that re-synchronization protocol beginning 13d after AI does not affect pregnancy rate and the first hormonal protocol improves the result of second AI. The low overall pregnancy rate (34.4%) was probably due to the end of breeding season. We conclude that Resynch protocol is a useful tool to accelerate the number of pregnancies produced during the breeding season.

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A054 FTAI, FTET and AI

Retrospective study of factors affecting the fertility of lactating Girolanda cows

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Keywords: cattle, multifactorial, reproduction.

The aim of the study was to identify important factors that may influence fertility of lactating Girolanda cows. We utilized 7134 reproductive and productive records of cows in a dairy farm located in the state of Minas Gerais during 2009-2012. Statistical analysis was performed using the Statistical Analysis System for Windows (SAS, 2001) by multivariate logistic regression using the LOGISTIC procedure of SAS. Variables were removed by backward procedure, according to the statistical criteria of Wald with $P > 0.20$. The final statistical model included the effects of season [winter (May to August) and summer (November to March)], days in milk (DIM; 50 days, between 50 and 90 days, 90-150 days and above 150 days), number of inseminations (> 3 AI and ≤ 3 AI), milk production in 305 days of lactation (up to 4000 Kg, between 4000 and 6500 Kg and above 6500 Kg) and animal category (primiparous or pluriparous). After this initial analysis, variables were analyzed assumed a binomial distribution using the GLIMMIX procedure of SAS. There was no interaction between these variables ($P > 0.05$). In the study, the pregnancy rate was influenced by season [summer (34.6%, 797/2301) and winter (45.0%, 1510/3347); $P = 0.001$], number of inseminations [> 3 AI (34.7%, 543/1563) and ≤ 3 AI (41.7%, 2354/5642), $P = 0.001$], days in milk [DIM < 50 (38.7%, 313/809)^{bc}, 50 $<$ DIM < 90 (42.3%, 977/2311)^{ab}, 90 $<$ DIM < 150 (44.0%, 896/2035)^a and DIM > 150 (35.5%, 702/1979)^c; $P = 0.003$], milk production [milk < 4000 kg (43.8%, 295/673)^a, 4000kg $<$ milk < 6500 kg (39.9%, 1738/4360)^b and milk > 6500 kg (39.7%; 857/2159)^b; $P = 0.04$]. However, there was no difference in pregnancy rate between animal categories [primiparous (41.6%, 981/2359) and pluriparous (39.5%, 1916/4846); $P = 0.18$]. We conclude that the fertility of crossbred cows (Girolanda) of average/high milk production is influenced by multiple factors, in which it was found lower pregnancy rate in the summer, in cows with more than three breedings, at early and later DIM, and cows producing above 4000 Kg in 305 days of lactation.



A055 FTAI, FTET and AI

Time of last GnRH treatment (GnRH56h vs GnRH72h) on follicular dynamics of Holstein cows synchronized with the 5-day protocol

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Keywords: dairy cows, TAI, GnRH.

This study was designed to evaluate the effect of time of induction of ovulation with GnRH (GnRH56h vs GnRH72h) in short (5d) synchronization protocols in high producing lactating cows. The hypothesis was that anticipating the last GnRH to 56h would improve synchronization of ovulations, allowing a greater proportion of cows to have the AI-to-ovulation interval within 12-24h. Holstein cows (n = 39; producing 54.3 ± 1.9 Kg/day and body condition of 2.8 ± 0.1) received in a random stage of the estrous cycle (D0), GnRH (100 µg, Factrel, Zoetis, USA) and a progesterone intravaginal device (CIDR®, Zoetis, USA). On Day 5, the progesterone device was removed and all animals were treated with 25mg of dinoprost tromethamine (PGF2α, Lutalyse®, Zoetis, USA). All animals also received a second PGF2α 24h after CIDR removal. Then, cows were randomized in two treatments: 1) GnRH56h: GnRH given at 56 h after CIDR removal and; 2) GnRH72h: GnRH given at 72 h after CIDR removal. For both treatments, timed AI occurred at 72h after CIDR removal. Ultrasound exams were performed with an ultra-portable ultrasound equipment (8-5 MHz, linear probe, Ibex – E.I. Medical Imaging, USA) and done by the same technician every 12h starting at CIDR removal until ovulation. Continuous variables were analyzed with proc MIXED of SAS. Binomial distributed variables were analyzed with the proc GLIMMIX of SAS (Version 9.3 for Windows; SAS Inst., Cary, NC); and the experimental unit “cow” was included in all models as a random variable. The time of GnRH treatment affected the interval from CIDR removal to ovulation (GnRH56h = 87.8 h vs GnRH72h = 97.9 h; $P < 0.05$), without altering ovulation rate (GnRH56h = 95% vs GnRH72h = 100%; $P = 0.93$) or the diameter of the ovulatory follicle (GnRH56h = 17.7 mm vs GnRH72h = 18.3 mm; $P = 0.67$). Because time of ovulation was anticipated in GnRH56h group, the average AI-to-ovulation interval was less in cows receiving GnRH at 56h (GnRH56h = $15.8 \pm h$ vs GnRH72h = 25.9 h; $P < 0.05$). As a result, a greater proportion of cows in GnRH56h had AI-to-ovulation intervals of 12-24h (GnRH56h = 84% vs GnRH72h = 26%; $P < 0.05$). In conclusion, these results suggest that GnRH given at 56h after progesterone device removal can improve the synchrony of ovulations and allow more cows to have ideal AI-to-ovulation intervals following 5d-short progesterone-based protocols.



A056 FTAI, FTET and AI

Effect of GnRH administration at embryo transfer following a E2/P4 based protocol for FTET in Girolando heifers and cows

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Keywords: FTET, GnRH, lactating cows.

The aim of this study was to evaluate the effect of administering GnRH at the time of embryo transfer in a fixed time embryo transfer program (FTET) on the fertility of Girolando heifers, dry cows and lactating cows. At random days of the estrous cycle (D-11), 2mg estradiol benzoate (Gonadiol, MSD Animal Health, SP, Brazil) was administered IM and an intravaginal device with 1.9 g progesterone (P4, CIDR, Zoetis, SP, Brazil) was inserted. On D-4, 25 mg dinoprost (Lutalyse, Zoetis, SP, Brazil) was given IM. On D-2, 1.0 mg estradiol cypionate (ECP, Zoetis, SP, Brazil) was administered IM and the device was removed. On D18, the animals were examined by ultrasonography and only those with a CL received an embryo. On D-11, the animals were also classified according with their body condition score (BCS) following a 0 (very thin) to 5 (obese) point scale. At FTET, the animals were homogenously allocated to one of two groups: Control (no further treatment) or GnRH [100µg gonadorelin (Cystorelin, Merial, SP, Brazil)] IM. Two pregnancy diagnosis were conducted, the first on D31 and the second on D66. The analysis of binomial and continuous data were done using PROC GLIMMIX and MIXED of SAS, respectively. Significance was considered when $P \leq 0.05$ and tendency when $0.05 < P \leq 0.10$. No interaction of treatment and animal category was observed on conception rate at 31 days ($P = 0.33$), 66 days ($P = 0.32$) and on pregnancy loss ($P = 0.92$). No effect of treatment was observed on 31 days [Control = 52.8% (477/903) vs. GnRH = 52.3% (473/904); $P = 0.83$] and 66 days conception rate [Control = 42.3% (382/903) vs. GnRH = 44.03% (398/904); $P = 0.46$]. However, treatment with GnRH tended to reduce pregnancy loss between 31 and 66 days [Control = 19.9% (95/477) vs. GnRH = 15.9% (75/473); $P = 0.10$]. Heifers had greater 66 days conception rate [49.5% (412/832)] than dry cows [37.0% (44/119); $P < 0.01$] and lactating cows [39.6% (610/1539); $P < 0.01$]. Lactating cows had greater pregnancy loss [21.4% (166/776); $P < 0.01$] compared to heifers [14.0% (67/479); $P = 0.10$] and tended to have greater pregnancy loss compared to dry cows [12.0% (6/50); $P = 0.10$]. Effect of BCS on 66 days conception rate was observed in lactating cows from the Control group [Thin (BCS ≤ 2.5) = 26.8% (22/84) vs. Good (BCS ≥ 2.75) = 42.3% (203/481); $P < 0.01$]. Cows treated with GnRH at FTET had no effect of BCS on 66 days conception rate [Thin = 36.8% (33/89) vs. Good = 39.6% (184/468); $P = 0.62$]. In conclusion, the administration of GnRH at FTET may reduce pregnancy loss between 31 and 66 days of gestation and increase conception rate in lactating cows with low BCS.



A057 FTAI, FTET and AI

Effects of estradiol cypionate (ECP) and equine chorionic gonadotropin (eCG) on pregnancy rate in Nelore heifers.

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Keywords: *Bos indicus* heifers, P4 + ECP + eCG, TAI.

The study aimed to verify the effects of timed AI protocols using ECP and eCG on the pregnancy rate (PR) in Nelore heifers. Animals were treated with a P4 intravaginal device + estradiol benzoate (EB; IM) + prostaglandin F2 alpha (PGF; IM) on day 0 (d0 = beginning of the protocol) for TAI. Two hundred twenty-four cycling heifers (CL presence or follicles > 8 mm diameter) or in anestrus (no CL and follicles < 8 mm), received on d0 a P4 device intravaginal (single dose Cronipres, Biogenesis Bagó) + 2 mg EB + 0.075 mg PG; on d8 (P4 removal + PGF + eCG (300 IU) + ECP (2 mg) and TAI in d10. Animals returning to visible estrus within 25 days after TAI were inseminated (AI) 12 hours after estrus detection. Heifers returning to estrus after AI remained with clean up bulls for 45 days. The pregnancy diagnosis was performed with ultrasound 45 days after clean up bulls left the herd. The data were analyzed by ANOVA and Fisher's exact test at a level of significance of $P < 0.05$. Overall, 58,0 % (n=130) of the heifers were in anestrus and 42,0 % (n=94) were cycling. All animals though received the timed AI protocol. The PR of anestrus and cycling heifers following the TAI + IA were 38.5 and 58.5 %, respectively. In addition, PR following clean up bull breedings were 22.3 and 14.8 %. In this study, heifers bred through timed AI had improved reproductive performance as compared to AI following visually detected estruses + AI ($P = 0.0001$). Similarly, heifer that received TAI also performed better than heifers that remained for 45 days with cleaup bulls ($P < 0.0005$). Thus, adding up results from TAI + visual estrus and AI, PR was 46.9 %. Pregnancy rates were within normal ranges for beef nelore heifers, even with 58.0 % been in anestrus at beginning of the study. In conclusion, combining ECP and eCG on d8 of the progesterone-based TAI protocol in Nelore heifers that were initially treated with EB and PGF resulted in satisfactory PR; and it appears to be a good alternative to improve fertility results in comercial bovine herds.



A058 FTAI, FTET and AI

Use of D-Cloprostenol at different intervals of administration for estrous synchronization in cyclic Saanen goats

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Keywords: artificial insemination, goat, prostaglandin.

This study evaluated the efficiency of two doses of 37.5 µg of d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) latero-vulvar at intervals of 7 (G7; n=19), 10 (G10; n=18) or 11.5 days (G11.5; n=17) for estrus synchronization in cyclic Saanen goats. Blood samples were collected from 23 randomly chosen goats divided into G7 (n = 8); G10 (n = 8) and G11, 5 (n = 7) for subsequent determination of plasma P4. Estrus was monitored after the 1st and 2nd administration of d-cloprostenol, in 23 goats, which had blood samples collected, as well as in 54 goats enrolled in the trial. To determine the beginning and end of estrus, each goat was observed twice daily (09:00 and 16:00h) for about 15 minutes in the presence of a buck. Onset of estrus was considered when females allowed to be mounted by the male. Monitoring continued until the end of estrus. The goats were inseminated from 18 to 24 hours after the onset of second estrus. Parametric variables were analyzed through one-way analysis of variance and compared by tukey test (5% significance). Non parametric variables were analyzed using the chi-square test (SAEG®, Funarbe, Viçosa, Brasil). All goats had P4>1 ng/mL at the time of the 1st and 2nd administration of d-cloprostenol. The percentage of goats that showed estrus after 1st and 2nd administration of cloprostenol was 69.6% (16/23) and 90.7% (49/54), respectively. The percentage of animals in estrus was 94.7% - G7, 88.9% - G10 and 88.2% - G11.5. The interval to estrus and duration of estrus after first (G7 = 53.8 ± 15.4h and 24.5 ± 9.8h; G10 = 54.3 ± 9.3h and 29.2 ± 16.0h; G11.5 = 47.3 ± 5.8h and 23.7 ± 11.8h) and second (G7 = 43.8 ± 10.7h and 27.0 ± 12.1h; G10 = 50.6 ± 13.7h and 22.3 ± 10.4h; G11.5 = 45.5 ± 27.6h and 33.5 ± 19.4h) administration of d-cloprostenol did not differ (P>0.05) between treatments. The conception rate of the G7 [55.6% (10/18)] was greater (P=0.06) than G10 [18.8% (3/16)] and G11.5 [26.7% (4/15)]. It can be concluded that, although the three protocols have been effective in synchronizing estrous in Saanen goats during the breeding season, the protocol with an interval of seven days achieved better conception results.



A059 FTAI, FTET and AI

Factors affecting expression of estrus of lactating dairy cows using activity monitors

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Keywords: duration, follicle, heat.

The objective of this study was to determine parameters associated with increased physical activity as a result of estrus expression. A total of 1163 estrus episodes from 346 lactating Holstein cows were recorded. Cows were monitored continuously by an activity monitoring system (AMS; Heatime®, SCR engineers) attached to the cow's necklace. Data was recorded in real time every two hours. Immediately after the initiation of the increase in activity detected by the monitoring system (i.e. estrus episode) cows had BCS measured and their ovaries scanned by ultrasound (Aloka SSD-500, Aloka Co. Ltd, Tokyo, Japan). Pregnancy per AI (P/AI), disease episodes, and milk production data were collected and recorded for the entire experimental period. Data was analyzed using ANOVA and logistic regression using proc MIXED and GLIMMIX. Mean duration of estrus was 11.3±5.3 h with mean index activity peak of 72.8±20.2 (index created based on standard deviations of the mean activity). The follicle diameter of the animals in high activity was 18.8±0.3 mm. There was a positive correlation between duration and peak ($r^2=0.70$), with estrus episodes with high peak of activity (index of peak of activity between 51 and 100) being longer (14.3±0.2 vs. 6.2±0.2 h, $P < 0.0001$) than those with small peak of activity (index between 35 and 50). Estrus duration was affected by parity as multiparous cows expressed it for a shorter period than primiparous (10.8±0.3 vs. 12.2±0.3 h, $P = 0.0013$). Cows with moderate BCS (BCS > 3.00) had longer duration of estrus episodes than cows with low BCS (BCS < 2.75); 13.0±0.4 vs 11.5±0.3 h, respectively ($P = 0.0022$). Pregnancy per AI was not influenced by parity or follicle diameter. Peak of activity and BCS influenced P/AI. Pregnancy/AI was greater when animals had an increased activity peak (intensity > 400%; $P = 0.02$) and BCS higher than 2.75 ($P = 0.02$). The AMS measurements of intensity were not correlated with follicle diameter at AI or milk production around the time of estrus. Furthermore, estrus expression patterns as measured by the AMS were not affected by disease episodes during the transition period, but increased ($P = 0.02$) when secondary signs of estrus behavior were observed. In conclusion, duration and intensity of estrus episodes were quite variable and mainly influenced by parity and BCS. Increased estrus intensity improved P/AI, but was not correlated with follicle diameter at AI or milk production. Further studies are needed to determine management or selection tools to improve estrus expression and fertility.



A060 FTAI, FTET and AI

Progesterone re-utilization in eight and a half days on the pregnancy rate in Nellore cows in the state of Pará

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Keywords: fixed-AI, multiparous, primiparous.

The aim of this study was to evaluate pregnancy rates in Nellore cows submitted to a protocol for fixed-time AI using a device impregnated with P4 during its 1st, 2nd and 3rd use in a protocol lasting 8.5 days. Another objective was to assess the influence of the animal category in pregnancy rates. The study was conducted in the São Felix do Xingu city, southern Pará state-Brazil. It was used 3.615 females (635 heifers, 582 lactating primiparous, 1.924 lactating multiparous and 474 cows without calves). The cows had calved at least 30 days before enrolment in the protocol. On day 0 (D0) animals were randomly inserted with a intravaginal progesterone device (DIB®, Coopers Animal Health, São Paulo, Brasil) and these devices were at their 1st, 2nd and 3rd use. Simultaneously, animals received 2.0 ml intra-muscle (IM) of estradiol benzoate (Gonadiol®, MSD animal health, São Paulo, Brasil). On day eight and a half (D8.5) in the afternoon, intravaginal devices were removed, followed by the application of 1.0 ml IM of estradiol benzoate (Gonadiol®, MSD animal health, São Paulo, Brasil), 1.5 ml of Sodium D-cloprostenol (Ciosin®, MSD animal health, São Paulo, Brasil) and 1.5 ml of equine chorionic gonadotropin (Novormon®, Syntex S. A. Argentina). Fixed-time AI was performed on D10 in the morning. Pregnancy diagnosis occurred 35 days after insemination by ultrasonographic evaluation (CHISON D600Vet, USP Brasil Eletromedicina LTDA, São Paulo, Brasil). Data were analyzed by the Chi-square (χ^2) method in SAS version 8.0 considering the level of significance of 5%. The total pregnancy rate was 52% (1.878/3.615). The pregnancy rate according to the number of uses of the P4 device was 54.5%, 53.1% and 48.3% for 1st, 2nd and 3rd use ($P < 0.05$), respectively. Among these results, it is important to highlight the great performance of primiparous cows with pregnancy rates of 49.7% (289/582). In addition, pregnancy rate was 45.8% (291/635) for heifers, and 53.8% (1.038/1.924) for lactating multiparous and 54.9% (260/474) for cows without calves, which were similar to results reported by Cabral. R. Bras. Ci. Vet. v.20, n.1, p.49-53, 2013. Therefore, by reusing progesterone devices for 8.5 days it is possible to achieve satisfactory pregnancy rates across different animal categories.



A061 FTAI, FTET and AI

Resynchronization at 22 days after first TAI in suckling beef cows: preliminary results

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Keywords: anestrus, pregnancy, TAI.

Bos taurus beef cows (n = 92) maintained in native pasture in Rio Grande do Sul, South of Brazil with 45 ± 15 days postpartum and body condition score (1-5) of 2.8 were distributed into two experimental groups (TAI+Bull and TAI+Ressinc22) during 90 days of the breeding season (BS). Ten days before BS (D10) all females were synchronized for TAI (Day 0). Protocol consisted in the insertion of an Intravaginal Progesterone Releasing Device (IPRD, 0.75g of P4; PROCICLAR®, Hertape Calier Animal Health, Brazil) and 2mg IM of estradiol benzoate (EB) (HC Benzoate®, Hertape Calier) on Day 10 (D10). On D2, IPRD was withdrawn and 150µg IM D (+) cloprostenol (Veteglan Luteolytic®, Hertape Calier), 25IU IM FSH/LH (Pluset®, Hertape Calier) and 1mg IM of estradiol cypionate (EC) (cypionate HC®, Hertape Calier) were administered. TAI was performed on D0 (early BS). Bulls were introduced in the TAI + bull (n = 45) group 10 days after TAI. Females of the TAI + Ressinc22 (n = 47) group were resynchronized on Day 22 (D22) using the same protocol described above, except for the dose of EB at the beginning of treatment which was reduced to 1mg IM. On D30 the pregnancy diagnosis was performed in all females with ultrasound. Females in TAI group + Ressinc22, besides pregnancy diagnosis had their IPRD removed, and the non-pregnant females were treated with 1 mg of EC and 150µg im D (+) Cloprostenol. These females received the second TAI on Day 32 (D32). TAI+Ressinc22 Group was again regrouped with the TAI + bull group until the end BS. Statistical analysis was conducted with the PROC GLIMMIX and LIFETEST of SAS. Conception rate after 1st TAI was similar between treatments (TAI + bull = 42.0%, 19/45 and TAI + Ressinc22 = 48.0%, 23/47, P = 0.41). Conception rate after resynchronization was 66.6% (16/24), since the pregnancy rate after return of the first heat was 26.9% (7/26) in TAI + bull group. Cumulative pregnancy rate in the first 60 days of BS was higher (P = 0.01) in the TAI + Ressinc22 group (80.8%, 38/47) than TAI + bull group (55.6%, 25/45). Pregnancy rate at the end of breeding season was similar (P = 0.24) in both groups (TAI + bull = 86.6%, 39/45 and TAI + Ressinc22 = 91.5%, 43/47). Additionally, the cyclic cows at the start of the breeding season had greater conception rate to TAI (62.5% vs. 27.7%) as well as greater pregnancy rate during BS (28.5d vs 17.3d, P = 0.003). These preliminary results showed that resynchronization 22 days after the first TAI may represent a good strategy to increase number of pregnancies from AI and improve reproductive performance of suckling beef cows managed with reproductive seasons lasting 90 days.



A062 FTAI, FTET and AI

Conception rate in heifers and lactating Girolando cows inseminated with sexed and conventional semen

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Keywords: artificial insemination, conventional semen, sexed semen.

Milk production of small farms of Brazilian southeastern has been improved. To improve the herd milk production and maintain profitability in these properties are require the use of reproductive technologies. For this purpose the use of artificial insemination with conventional or sexed semen using high genetic value bulls is an interesting stratigie. In order to evaluate the efficiency of the use of sexed semen in cows producing 16 liters of milk per day under pasture and receiving 1 kg of concentrate for each 3 kg of milk produced, and crossbred cyclic heifers (25 animals of each category for each type of semen [sexed or conventional]) were used. For this 3/4 and 7/8 Girolando females had their estrus synchronized to receive sexed or conventional semen from the same Gyr bull. At the beggning of the synchronization (D0), the females received an intravaginal device containing 1.0 g of progesterone (Sincrogest, Ouro Fino Saúde Animal, Cravinhos-SP) plus i.m. administration of 2.0 mg of Estradiol Benzoate (Sincrodiol, Ouro Fino Saúde Animal, Cravinhos-SP). On D8, 500 mg of sodium cloprostenol (Sincrocio, Ouro Fino Saúde Animal, Cravinhos-SP) was administrated and the intravaginal device was removed. At this time, females had their estrous behavior observed and those females detected in estrus were inseminated 18 to 24 hours. For the insemination, females were blocked by category (cow or heifer) and randomly assigned to receive sexed or convencional semen. The pregnancy per AI (P/AI) was evaluated at 35-42 days after AI and the analysis performed using chi-square test (χ^2) of SAS program. Cows and heifers inseminated using conventional semen presented greater P/AI 72 % (18/25) and 68 % (17/25), than those females inseminated using sexed semen 44 % (11/25) and 52 % (13/25), respectively. Thus, Girolando cows and heifers presented similar P/AI according the type of semen used. The use of sexed semen determined lower P/AI than the use of convencional semen.



A063 FTAI, FTET and AI

Evaluation of different nutritional and physical development parameters on reproductive efficiency of Nelore heifers at 13 months of age submitted to FTAI

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Keywords: FTAI, precocity, pregnancy rate.

The present study aimed to evaluate the relationship between nutritional and physical development parameters and pregnancy rate results in Nelore beef heifers. The study was conducted in three commercial beef herds located in Camapua, Tres Lagoas and Agua Clara-MS. A total of 650 heifers with 13.0 ± 0.9 months of age was submitted to the same synchronization protocol for fixed time artificial insemination (FTAI). On a random day of the estrus cycle (Day -10) the females were evaluated for live weight, body condition score (BCS, 1-5 scale), subcutaneous fat thickness at the rump area (SFTR; ultrasonographic exams done with Aloka SSD500, Aloka, Japan) and the relation height/rib depth (RIBD). Ten days later (Day 0), all animals received an auricular ear implant containing 3.0 mg of Norgestomet (CRESTAR®, MSD Animal Health) and 2.0 mg of estradiol benzoate (EB; Gonadiol®, MSD Animal Health) intramuscularly (IM). At Day 8, the auricular implants were removed and they received 0.6 mg IM of estradiol cypionate (EC; ECP®, Zoetis), 0.265 mg IM of cloprostenol (Ciosin®, MSD Animal Health) and 300 IU of equine chorionic gonadotropin (eCG; Novormon®, MSD Animal Health). All females were then inseminated 48 h after implant removal. The pregnancy diagnosis was performed by transrectal ultrasonography (Aloka SSD500, Aloka, Japan) 30 days after FTAI. All variables were analyzed with the GLIMMIX procedure of SAS 9.3. The females were classified in categories according to the cutoff values provided by the Receiver Operating Characteristic curve (ROC): 13.0 months ($\leq 13m$; average = 12.8 months) and >13 months of age ($>13m$; average = 14.5 months); low BCS (LBCS, ≤ 3.0) and high BCS (HBCS, > 3.0); low SFTR (LSFTR, ≤ 2.47 mm) and high SFTR (HSFTR, > 2.47 mm); higher (HRIBD, $>44.0\%$) and lower RIBD (LRIBD, $\leq 44.0\%$). Interestingly, pregnancy rate to the FTAI protocol had a positive relationship with age [$\leq 13m = 27.8\%$ (72/259) vs. $>13m = 40.9\%$ (160/391); $P=0.01$], BCS [LBCS = 29.7% (119/400) vs. HBCS = 45.4% (113/249); $P<0.001$], SFTR [LSFTR = 23.4% (62/265) vs. HSFTR = 44.4% (168/378); $P=0.0003$] and RIBD [LRIBD = 27.0% (77/285) vs. HRIBD = 41.9% (150/358); $P<0.001$]. In conclusion, parameters related to nutritional and physical development had a positive relationship with fertility of Nelore heifers at 13 months of age that were submitted to FTAI protocol. This information could be used to increase efficiency of FTAI programs used in young animals and develop a reproductive program in order to reduce the age at first calving and the interval between generations in Nelore breed.

Acknowledgment: CRV Lagoa and MSD Animal Health.



A064 FTAI, FTET and AI

Reproductive performance of dairy buffaloes submitted to TAI protocols based on P4/E2 plus eCG during breeding and nonbreeding seasons

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Keywords: artificial insemination, corpus luteum, follicle.

This study aimed to compare the reproductive performance of dairy buffaloes submitted to TAI protocols based on P4/E2 during breeding (BS; May, June and July) and nonbreeding season (NBS; November, December and January). Thus, 351 dairy buffaloes were used in this study. Animals were raised in five herds in Ribeira Valley area, São Paulo State-Brazil. To compare the effect of the seasons, each herd had similar numbers of animals submitted to TAI in both seasons, totaling 168 animals in BS and 183 in NBS. On random days of the estrous cycle (D0; 4:00 PM), all buffaloes received an intravaginal progesterone releasing device (1 g of P4; Sincrogest®, Ourofino Agribusiness) plus 2.0 mg of estradiol benzoate im (Sincrodiol®, Ourofino Agribusiness). On day 9 (D9; 4:00 PM), females received 0.53 mg of PGF2 α im (Cloprostenol, Sincrocio®, Ourofino Agribusiness) and 400 IU of eCG im (Novormon®, MSD Animal Health), followed by removal of progesterone device. On day 11 (D11; 4:00 PM), 10 mg of buserelin acetate (GnRH Sincroforte®, Ourofino Agribusiness) were administered im. The TAI was performed 16 hours after application of GnRH (D12; 8:00 AM). At the beginning of each protocol (D0), it was recorded the number of days in milk (DIM) and body condition score of females (BCS, 1-5). Ultrasonography (Chison D600Vet, China) was conducted to determine: the cyclicity rate (presence of corpus luteum on D0 and/or D9); diameter of the dominant follicle in D9 and D12 (ϕ DF); daily growth rate of the DF (between D9 and D12; DLgrDF); ovulation rate (Ov) and CL diameter 10 days after TAI (ϕ CL); pregnancy diagnosis at 30 (P30) and 45 days (P45) after TAI, and the rate of embryonic mortality (EM) in this period. Continuous variables were presented as mean and standard error of the mean (mean \pm SEM). The comparison between variables was performed through analysis of variance (ANOVA) using the Glimmix procedure of SAS®. Difference was considered when P<0.05. It was observed that despite of the similarity about DIM (111.1 \pm 8.8 vs. 144.3 \pm 8.6 days; P=0.27) and BCS (3.3 \pm 0.1 vs. 3.4 \pm 0.1, P=0.41) during BS and NBS, the females showed different cyclicity rate [76.2 (128/168) vs. 42.6% (78/183); P=0.03]. None of other variables differed between BS and NBS, respectively: ϕ DF D9 (9.6 \pm 0.2 vs. 9.8 \pm 0.2 mm, P=0.35.); ϕ DF D12 (13.1 \pm 0.2 vs. 13.2 \pm 0.2 mm, P=0.47); DLGRDF (1.9 \pm 0.1 vs. 1.9 \pm 0.1 mm/day; P=0.81); Ov [86.9 (146/168) vs. 82.9% (150/180); P=0.17]; ϕ CL (19.0 \pm 0.3 vs. 18.4 \pm 0.3 mm, P=0.20); P30 [66.7 (112/168) vs. 62.7% (111/177); P=0.31]; P45 [64.9 (107/165) vs. 60.2% (106/176); P=0.37]; EM [1.8 (2/111) vs. 3.6% (4/110); P=0.96]. In conclusion, dairy buffaloes have similar reproductive efficiency in BS or NBS when subjected to TAI programs based on P4/E2 and eCG.

Acknowledgments: Ourofino Agribusiness.



A065 FTAI, FTET and AI

Resynchronization protocol for FTAI in Zebu cows

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Keywords: artificial insemination, gestation rate, reproductive performance.

Resynchronization programs in which a new FTAI protocol is initiated in all animals even before pregnancy diagnosis are already used in some breeding systems. In beef cattle in which the reproductive season is used, the conception of a large number of females at the beginning of season is important not only for the current breeding season but also for the following one. The purpose of this study was to verify the efficacy of the use of a resynchronization protocol after the first FTAI using progesterone devices and estradiol benzoate to synchronize the follicular growth at the beginning of the protocol. Literature is controversial on the safety of estradiol esters in females at the beginning of pregnancy. A total of 183 multiparous beef cows (Nelore breed – *Bos indicus*) was used in a reproductive season program, divided into three treatment groups. G1: Conventional artificial insemination with the use of a teaser bull, G2: FTAI followed by estrus observation and conventional artificial insemination; G3: FTAI followed by resynchronization. The same FTAI protocol was used to all groups: Day 0, insertion of an intravaginal progesterone device and administration of 2 mg of estradiol benzoate (EB); Day 8, removal of device and administration of 500 mg of cloprostenol sodium and 1 mg of estradiol cypionate; Day 10, FTAI. Twenty days after FTAI, all cows from group G3 had an implant inserted and 1 mg of EB administrated. Upon pregnancy diagnosis, at day 28 post-AI, non-pregnant animals received 500 mg of cloprostenol sodium, 1 mg of estradiol cypionate, and the device was removed. The 2nd FTAI was performed on Day 30. The percentage of pregnant cows at the 1st FTAI and the total number of pregnant cows in the first 30 days of the breeding season were compared among the groups. The interval from the beginning of the breeding season to conception was submitted to the Kaplan-Meier distribution and a survival plot was prepared during the first 30 days of the reproductive season. Probability lower than 5% were considered significant. There were no differences in pregnancy rate after the first FTAI between groups 2 and 3 (42.6 and 52.5%, respectively - $P > 0.05$). Such lack of difference indicates that the start of a new FTAI protocol in pregnant cows, as occurred in group 3, does not impair pregnancy in those animals. In G1, pregnancy rate following the 1st artificial insemination was 55.7%. The resynchronization protocol increased the number of pregnant cows at the beginning of season. The total number of pregnant cows by the end of 30 days of season was 55.7^a, 57.4^a and 82.0^b for G1, G2 and G3, respectively ($P < 0.05$). It was concluded that resynchronization of females, using the above mentioned protocol, before pregnancy diagnosis is safe and does not impair pregnancy maintenance in the initial stages of pregnancy. Resynchronization protocols are effective to increase the number of pregnant cows at the beginning of the breeding season.

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A066 FTAI, FTET and AI

Effect of supplementation with sunflower seed in the expression of transcripts involved in the uterine biosynthesis of eicosanoids

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Keywords: endometrium, linoleic acid, PGF2 α .

Embryo mortality between 15 and 19 days of pregnancy in cattle is caused by the increase in the release of endometrial PGF2 α , resulting in luteolysis. Synthesis of PGF2 α can be inhibited in animals supplemented with linoleic acid-rich compounds, among them a sunflower seed. In previous studies, increased conception rate was observed in Nelore cows supplemented with sunflower seed for 22 days starting at the time of TAI (66.7% vs 46.3%; P = 0.02; Peres et al., 2008, *Acta Scientiae Veterinariae*, 36, p.639) and crossbred heifers submitted to TET (55.7 % vs. 36.9 %; P < 0.01; Membrive et al., 2013, *Acta Scientiae Veterinariae*, 36, p.603). It was hypothesized that sunflower seed alters the expression of genes involved in biosynthesis of eicosanoids uterus. This study aimed to examine the effects of sunflower seed supplementation on the the abundance of transcripts for genes involved in the eicosanoid biosynthesis as prostaglandin E synthase 1 (PTGES1) and 2 (PTGES2), aldo-keto reductase B1 (AKR1B1) and C4 (AKR1C4), cyclooxygenase-2 (COX-2), phospholipase A2 (PLA2), prostaglandin E receptor type 2 (PTGER2) and 4 (PTGER4). Nelore cows (n = 33) received an intravaginal device containing progesterone (1g; DIB , Syntex Biochemistry & Pharmaceutical Industries SA) associated with an intramuscular (IM) injection of estradiol benzoate (2mg; Benzoate HC, Hertape Calier Animal Health SA). The devices were removed 8 days later, when heifers were treated IM with cloprostenol sodium (2mg; Sincrocio®, Ourofino Animal Health Ltda), estradiol cypionate (0.5 mg; ECP®, Zoetis Animal Health Ltd.) and eCG (300IU; Folligon®, Intervet Veterinary Ltda of Brazil). Two days after removal of the device, females were divided into four groups to receive 1.7 kg / animal / day of 40% soybean meal, 44 % crude protein (CP) + 60 % sunflower seed for 6 (n = 8) and 14 days (n = 7) or 53% soybean meal 44% CP + 47 % corn for 6 (n = 9) and 14 days (n = 9). Both diets were formulated with 72 % TDN and 24 % CP. On the last day of supplementation, endometrial samples were collected from the the posterior part of the uterine horn ipsilateral to the corpus luteum via transcervical biopsy. Samples were stored at -196°C and evaluated for the expression of genes by real-time PCR. Data were analyzed using Proc Mixed of SAS. Cows supplemented with sunflower seed had higher (P < 0.05) expression on D14 than on D6 for PTGER4 (0.06 \pm 0.02 vs. 0.02 \pm 0.01, respectively), AKR1C4 (0.65 \pm 0.40 vs. 0.05 \pm 0.03, respectively), COX -2 (0.14 \pm 0.03 vs. 0.02 \pm 0.00, respectively). There was no difference in the expression of transcripts in treated or not with sunflower seed in same-day examined animals. It is concluded that the transcription of genes evaluated was not modified by supplementation with sunflower seed.

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A067 FTAI, FTET and AI

Effect of association of FTET and TAI in reproductive programs of Nelore females

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Keywords: *Bos indicus*, FTET, TAI.

The fixed time embryo transfer (FTET) technique in beef herds can be an efficient alternative for multiplication of superior animals, presenting reproductive indices that justify their application. The aim of this study was to evaluate the pregnancy rate of Nelore (*Bos indicus*) cows submitted to synchronization protocols that allow for fixed-time AI (TAI) and/or FTET. Thus, 634 lactating Nelore cows were randomly assigned to one of four experimental groups according to the technique performed in two consecutive steps: 2TAI (n=160), 2FTET (n=152), TAI followed by FTET (TAI/FTET; n=160) and FTET followed by TAI (FTET/TAI; n=158). All animals received an intravaginal progesterone device (P4; Primer, Tecnopec) plus administration of 2 mg of estradiol benzoate (EB; RIC-BE, Tecnopec) at random stage of the estrous cycle (D0). On D8, P4 devices were removed and the females received D-cloprostenol (0.530 mg; Prolise, Tecnopec), 1 mg of estradiol cypionate (ECP, Zoetis Animal Health) plus 300 IU of eCG (Folligon, MSD Animal Health). The females undergoing TAI were bred 48 hours after device removal. Females sent to FTET were evaluated for the presence of CL 9 days after removal of the devices and only cows with CL were transferred. Cows in both groups were resynchronized 30 days after the first TAI or 23 days after FTET. The same protocol was once again used as previously described, except for the dose of EB at the beginning of the protocol (1 mg). Females previously submitted to TAI at the first service were randomly divided into two experimental groups: 2TAI (second TAI service) and TAI/FTET (second FTET service) and females previously submitted to FTET at the first service were randomly divided into two experimental groups: 2FTET (second FTET service) and FTET/TAI (TAI second service). The post-partum period at first service was 41.4 days for TAI and 47.4 days for FTET. At the second service it was 82.8 days for TAI and 89.8 days for FTET. The pregnancy diagnosis was performed by ultrasonography at 60 days of gestation. The pregnancy rate after the first service differed ($P < 0.0001$) among reproductive managements: 2TAI (59.4%^a; 95/160), TAI/FTET (59.4%^a; 95/160), FTET/TAI (31.7%^b; 50/158) and 2FTET (32.7%^b; 51/156). Similarly, pregnancy rates after the second service also differed ($P = 0.06$) among managements: 2TAI (50.8%; 33/65)^a, TAI/FTET (40.6%; 26/64)^{ab}, FTET/TAI (51.9%; 55/106)^a e 2FTET (35.0%; 36/103)^b. Furthermore, the cumulative pregnancy rate (first + second service) also differed ($P < 0.0001$) across reproductive managements: 2TAI (80.0%; 128/160)^a, TAI/FTET (75.6%; 121/160)^{ab}, FTET/TAI (66.5%; 105/158)^{bc} and 2FTET (55.8%; 87/156)^c. In conclusion, the use of two consecutive FTET produced lowered cumulative pregnancy rate in lactating Nelore cows. However, the association between TAI and FTET programs can be considered as a good strategy to increase number of offspring from ET, without lessening reproductive efficiency.

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A068 FTAI, FTET and AI

Effect of different doses of FSH on the superovulatory response in Santa Inês ewes

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Keywords: embryo, Santa Inês, superovulation.

The embryo production in sheep is influenced by several factors, including superovulation protocol (SOV). The aim of this study was to evaluate the effect of different doses of FSH on superovulatory response and embryo production of Santa Inês ewes. Fifty seven animals were used and divided into three treatment with 19 animals per treatment. Santa Inês ewes were submitted to the protocol "Day 0", where the pre-estrus synchronization was achieved with placement of vaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (MAP) for 6 days associated with 300IU eCG and 0.5 mL D-cloprostenol at removal of MAP, with busserelin (18µg) given 36 hours later. The SOV was performed with different doses of FSH (T1 - 200mg, T2 - 133mg, T3 - 80mg) in 8 applications starting 84 hours after removal of the MAP. At the seventh and eighth days of the application of FSH, animals received 0.25 ml of D-cloprostenol. Busserelin (18µg) was administered to induce ovulation at 12 hours after the eighth dose of FSH. Artificial inseminations (AI) were performed 12 and 22 hours after application of busserelin, using frozen semen by laparoscopy technique. The embryo collection was performed by laparotomy, five days after AI. Ultrasound evaluation of follicular dynamics was performed in the donor in the first and eighth dose of FSH. The collected embryos were assessed for quality according to IETS (1998). The number of follicles larger than 4mm in the eighth dose of FSH of SOV and the total collected structures were evaluated by ANOVA. The number of viable and freezable embryos were compared using the Kruskal-Wallis test. In addition, response rate was evaluated with the Fisher's exact test. It was observed that the average number of collected structures (T1 = 3.9, T2 = 4.3, T3 = 2.2), viable embryos (T1 = 1.5, T2 = 3.9, T3 = 0.3), and freezable embryos (T1 = 1.4, T2 = 3.3, T3 = 0.3) were similar among treatments ($P > 0.05$). However, the response rate of the SOV donors was greater ($P < 0.01$) in T1 (68.4%) and T2 (38.9%) than in T3 (16.7%). There was no difference ($P > 0.05$) across treatments with respect to the average of follicles > 4 mm at the eighth dose of SOV (T1 = 8.4, T2 = 6.5, T3 = 4.1). These results suggest that the dose of 133mg of FSH for SOV in Santa Inês ewes shows similar results to 200mg, and both seem better than 80mg.



A069 FTAI, FTET and AI

Reuse of progesterone intravaginal devices maintains satisfactory reproductive performance of dairy buffaloes submitted to TAI during breeding season

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Keywords: artificial insemination, follicle, progesterone.

This study aimed to evaluate the reuse of progesterone intravaginal devices (P4) on reproductive performance of dairy buffaloes submitted to timed artificial insemination protocol during breeding season (autumn and winter). Thus, 142 lactating buffaloes kept on three farms in the Ribeira Valley, São Paulo State-Brazil were used. At random days of estrous cycle (D0; 4:00 PM) the buffaloes received an intravaginal progesterone releasing device (1 g of P4; Sincrogest®, Ourofino Agribusiness, Sertãozinho, Brazil) plus 2.0 mg of estradiol benzoate im (Sincrodiol®, Ourofino Agribusiness, Sertãozinho, Brazil). The trial design included 3 experimental groups, in which the P4 devices were randomly distributed according to the number of previous uses: New (1x, n=48), previously used for 9 days (2x, n=47) or used for 18 days (3x, n=47). On D9 (4:00 PM), females received 0.53 mg of PGF2 α IM (Cloprostenol, Sincrocio™, Ourofino Agribusiness, Sertãozinho, Brazil) and 400 IU of eCG IM (Novormon®, Merck Animal Health, São Paulo, Brazil), followed by removal of progesterone device. In D11 (4:00 PM), 10 mg of buserelin acetate (GnRH, Sincroforte®, Ourofino Agribusiness, Sertãozinho, Brazil) were administered IM. The TAI was performed 16 hours after GnRH (D12; 8:00 AM). On D0, number of days in milk (DIM) and body condition score (BCS; 1-5) were recorded for all buffaloes. Ultrasound assessments (Chison D600Vet, Shenzhen, China) were performed to determine: cyclicity rate (CLrt, presence of corpus luteum on D0 or D9); diameter of the dominant follicle (ϕ DF) in D9 and D12; ovulation rate (Ov) and CL diameter (ϕ CL) 10 days after TAI. Pregnancy diagnosis occurred at 30 (P30) and 45 days (P45) after TAI, despite the occurrence of embryonic mortality (EM). Continuous response variables were presented as mean \pm standard error mean. The comparison between variables was performed through analysis of variance using the GLIMMIX procedure of SAS (Institute Inc., Cary, NC). Difference was considered when $P < 0.05$. All responses were similar between groups 1x, 2x or 3x, respectively: DIM (114.9 \pm 17.5 vs. 98.0 \pm 10.2 vs. 109.9 \pm 14.2 days, $P=0.96$); BCS (3.1 \pm 0.1 vs. 3.3 \pm 0.1 vs. 3.2 \pm 0.1, $P=0.31$); CLrt (81.2 vs. 78.7 vs. 80.8%, $P=0.65$); ϕ DF D9 (9.0 \pm 0.4 vs. 9.0 \pm 0.3 vs. 9.6 \pm 0.4 mm, $P=0.40$); ϕ DF D12 (12.2 \pm 0.5 vs. 12.3 \pm 0.3 vs. 12.6 \pm 0.4 mm, $P=0.58$); Ov between D9 and D12 (6.3 vs. 12.8 vs. 19.2%; $P=0.17$), Ov (77.1 vs. 87.2 vs. 82.9% $P=0.69$); ϕ CL (18.8 \pm 0.6 vs. 18.7 \pm 0.6 vs. 19.1 \pm 0.5 mm $P=0.71$); P30 (64.0 vs. 70.0 vs. 61.0%; $P=0.70$), P45 (62.0 vs. 65.0 vs. 61.0%; $P=0.93$) and EM (3.0 vs. 6.0 vs. 0.0%, $P=0.30$). In conclusion, it is possible to reuse P4 intravaginal devices in TAI programs performed in dairy buffaloes during breeding season without compromising reproductive performance.

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A070 FTAI, FTET and AI

Prevalence of inflammatory process in the reproductive tract affecting the reproductive performance in crossbred dairy cows

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Keywords: cervicitis, cytology, endometritis.

Endocervical inflammation and uterine inflammation during early postpartum have affect the conception. However, the presence of cervicitis was not indicative of endometritis (Deguillaume et al., J. Dairy Sci. 95, 1776-1783, 2012). The objective was to evaluate the prevalence of endometrial and endocervical inflammation in crossbred cows at greater than 60 days in milk (DIM). Endometrial and endocervical cytological samples were collected from 149 crossbred cows at slatherhouse (Uberlândia-MG). Only uterine tratcts having completed uterine involution at macroscopic evaluation were used. The endometrial sample was collected using cytobrush. The thresholds of 5% and 6% neutrophils was considered to classify the inflammation in the cervix and in the uterus, respectively (Ahmadi et al., Veterinarski Arhiv 76(4), 323-332, 2006; Deguillaume et al., J. Dairy Sci. 95, 1776-1783, 2012). An additional histological analysis from the cervix and corpus of the uterus were performed. As results, 4.0% (6/149) of the cows presented inflammation on the cervix, 5.4% (8/149) presented endometritis and only 2.0% (3/149) presented both of inflamations. Interestingly, the degrees of cervix inflammation were: 66.6% (6/9) of the cows presented no inflammatory infiltration on cervical mucosa, 22.2% (2/9) had mild inflammatory infiltration and 11.1% (1/9) demonstrated moderate inflammatory infiltration. Furthermore, two of these three cows presented also inflammation in the endometrium. In conclusion, the present study demonstrated that 11.4 % of crossbred dairy cows with 60 DIM have some inflammation on endometrial and/or cervix.



A071 FTAI, FTET and AI

Evaluation of different eCG doses on pregnancy rate in lactating Nelore cows resynchronized with FTAI

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Keywords: eCG, FTAI, resynchronization.

The economic efficiency of livestock beef cattle is linked to the production of calves, which are destined for meat production or herd replacement. In order to provide a second chance for cows that were diagnosed as not pregnant following the previous AI, the use of resynchronization has become widely used in beef cattle. The objective of this study was to evaluate the efficiency of the use of eCG in resynchronization programs in lactating pluriparous Nelore cows with interval of 35-75 days postpartum. Objectives were to understand the interaction between dose, presence or absence of estrus, presence or absence of the corpus luteum (CL) and pregnancy rate. Seven hundred and seventy five cows were used in the first fixed-time artificial insemination (FTAI) and cows that were found not pregnant (449) were used after 30 days. Cows were divided into three homogeneous groups (G) in a balanced way. The ultrasonographic examination to observe the presence or absence of CL structures was performed before the first FTAI and previously to resynchronization. All the experimental animals received the same protocol, however with different doses of eCG (0, 200 or 300 IU). On day 0, cows received a new intravaginal device containing 1 g of progesterone (Sincrogest®, Ouro Fino, Brazil) and 2 mg of estradiol benzoate intramuscularly (Sincrodiol®, Ouro Fino, Brazil). On day 8, the devices were removed and cows received IM 1 mg of estradiol cypionate (ECP®, Pfizer Saúde Animal, Brazil), 0.530 mg cloprostenol sodium (Sincrocio®, Ouro Fino, Brazil), G-0 did not receive eCG, G-200 received 200 IU of eCG (® Folligon, Intervet / Schering-Plough, Brazil), and G-300 received 300 IU. Behavioral estrus was recorded and all the cows were inseminated 48 hours after the removal of the progesterone device. Pregnancy diagnosis was performed 30 days after AI. The likelihood ratio test was applied to determine the importance of each variable and meaningful interactions were kept in the model. The McNemar test was used to compare the proportion of cows with CL. In animals showing signs of estrus after progesterone device withdrawal (0IU 65.6%, 200IU 71.6%, 300IU 82.0%) the different treatments did not alter pregnancy results (0IU 75.2%, 200 IU 62.1%, 300 IU 74.8%). In contrast, for animals that did not show estrus, there were no differences in incidence of pregnancy among the G-200 and G-300, but G0 cows had lower pregnancy results ($P < 0.05$) (0IU 19.6%, 200IU 41.9%, 300IU 44.4%). The percentage of cows that had CL in the first AI (16.3%) was significantly lower than those with CL at the time of resynchronization (67.3%). The cumulative pregnancy rate after 40 days of breeding season was 77.5 %. According to this study, doses of 200 IU and 300 IU eCG when used in resynchronization programs in the first 30 days after FTAI, did not seem to differ in terms of pregnancy results. The cumulative pregnancy rate at 40 days of breeding season indicates that this tool can be used as a way to optimize reproductive management in beef cattle herds.



A072 FTAI, FTET and AI

Follicular dynamics in Nelore females (*Bos indicus*) with high or low numbers of antral follicles

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Keywords: antral follicle, *Bos indicus*, synchronization.

The aim of this study was to evaluate the influence of the high or low antral follicular count (AFC) on some parameters of follicular dynamics and ovarian diameter in *Bos indicus* cattle. A total of 250 suckling multiparous (40-60 days postpartum) Nelore cows was evaluated using an intravaginal microconvex array probe (Aquila PRO, Pie medical, Maastricht, The Netherlands) for counting follicles ≥ 3 mm. After assessment of AFC, 58 cows with BCS 2.8 ± 0.1 (1-5 scale) were randomly assigned in one of two experimental groups: G-high (AFC ≥ 40 follicles, n = 34) or G-low (AFC ≤ 10 follicles, n = 24). During the estrous cycle (D0), cows were randomly submitted to a synchronization of ovulation protocol using an ear implant containing 3 mg of norgestomet (Crestar®, MSD Animal Health, São Paulo, Brazil). Animals also received intramuscular (i.m.) injection of 2 mg of estradiol benzoate (Gonadiol®, MSD Animal Health, São Paulo, Brazil). At the ear implant removal (D8), they were injected with 0.150 mg of d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil), 300 IU of equine chorionic gonadotropin (Novormon®, Syntex SA, Buenos Aires, Argentina) and 1 mg of estradiol cypionate (ECP®, Zoetis, São Paulo, Brazil), i.m. Fixed-time artificial insemination (FTAI) was performed 48h after ear implant removal. Ultrasound exams were performed at D0, from D4 to D10 at every 24 h, from D10 to D12 at every 12 h (ovulation control) and D18. Parameters were determined on D5 (AFC, diameters of the largest follicle and ovary), D10 (diameter of the dominant follicle at FTAI) and D18 (diameter of the CL). We also evaluated time to ovulation after ear implant removal and the rate of follicular growth. Data were analyzed using ANOVA and comparisons were performed by the Tukey test ($P \leq 0.05$). Results are presented as mean \pm standard deviation. The mean number of follicles was 49.2 ± 5.5 (G-high) and 11.4 ± 3.9 (G-low; $P = 0.001$). Cows of G-high had greater ovarian diameter on D5 (27.7 ± 4.0 mm) than G-low (19.5 ± 3.3 mm; $P = 0.001$). In the same day, the diameter of the largest follicle was similar between G-high (8.6 ± 1.5 mm) and G-low (7.7 ± 2.1 mm; $P = 0.18$). There was no difference in the rate of follicular growth (G-high 2.4 ± 1.4 vs. G-low 2.2 ± 1.2 mm/day; $P = 0.59$) and for diameter of the dominant follicle at FTAI (G-high 11.8 ± 2.0 vs. G-low 12.6 ± 2.0 mm; $P = 0.26$) between groups. Cows of G-high ovulated at 68.6 ± 8 hours and cows of G-low at 70 ± 7 hours ($P = 0.64$) after ear implant removal. The diameter of the CL was similar between G-high (18.4 ± 3.0 mm) and G-low (18 ± 3.2 mm; $P = 0.67$). We observed similarities in the parameters of follicular dynamics in Nelore cows with high or low numbers of antral follicles submitted to synchronization of ovulation. However, cows with high AFC had greater ovarian diameter.



A073 FTAI, FTET and AI

Association between FTAI and FTET programs using lecirelin acetate

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Keywords: conception, lecirelin, re-synchronization.

The aim of this study was to evaluate the efficiency of lecirelin acetate (Gestran Plus®, ARSA, Argentina) in the re-synchronization between FTAI and FTET programs. Two experiments were conducted. In experiment 1, 99 multiparous Nelore cows, BCS 2.75 and 35 to 70 days postpartum were submitted to a FTAI program, in which they received an intravaginal device with 1 g progesterone (P4; PRIMER®, Tecnopec, Brazil) and 2 mg estradiol benzoate (EB; Ric BE®, Tecnopec, Brazil) im on D0. On D8.5 the device was removed and 500 µg cloprostenol (Estron®, Tecnopec, Brazil), 10 mg FSHp (Folltropin®, Bioniche, Canada) and 1 mg EB were given im. FTAI was performed 44 h later. On the 20th day after FTAI, all cows received 25 µg Gestran Plus®. At 28 days after FTAI, pregnancy diagnosis of the FTAI cows was done by ultrasound evaluation, and nonpregnant cows with a corpus luteum (CL) received FTET. The *in vitro* produced embryos were produced simultaneously to the resynchronization protocol, utilizing oocytes retrieved from ovaries of donors sent to slaughterhouse. The conception rate to FTAI was 44.4% (44/99). For nonpregnant cows, FTET rate was 76.3% (42/55), conception rate was 57.1% (24/42), and pregnancy rate was 43.6% (24/55). In experiment 2, 183 Nelore females (nulliparous = 107, multiparous postpartum = 76) underwent the same FTAI program described above. On the 20th day after FTAI, ultrasonography was performed and the animals without CL (n = 102) were divided into two groups. The Gestran group with 44 animals received 25 µg lecirelin acetate and the Control group with 58 animals received no further treatment. On the same day, other cows were subjected to OPU to perform IVF. All other procedures were similar to Experiment 1. Statistical analysis was performed using Chi-square. The conception rate to FTAI was 39.3% (72/183). The Gestran group had 84.1% (37/44) rate of FTET, against 74.1% (43/58) for the Control group (P = 0.23). Forty days after FTET, animals underwent ultrasound examination for pregnancy diagnosis. The conception rate of Gestran group was 62.2% (23/37) vs 34.9% of the Control group (15/43, P = 0.02). The pregnancy rate of Gestran group was 52.3% (23/44) vs 25.9% (15/58) of the Control group (P = 0.01). The cumulative pregnancy rate in experiment 1 (FTAI + FTET) was 68.6% (68/99) and in experiment 2, was 60.1% (110/183). The association of FTAI and FTET programs with the use of lecirelin acetate used at 20 days after FTAI caused a significant increase in reproductive efficiency, mainly because it allowed to equivalent of two services within 21 days of the breeding season with satisfactory rates of pregnancy in Nelore females.



A074 FTAI, FTET and AI

Effects of different doses of estradiol cypionate in E2/P4 based protocols at TAI and FTET in lactating cows

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Keywords: lactating cows, estradiol cypionate, TAI/FTET.

This study aimed to evaluate the use of different dosages of estradiol cypionate (ECP) to synchronize ovulation in dairy cows subjected to a TAI and FTET protocols. In Exp.1 (FTAI), 643 Holstein cows received the following protocol: d0- 2mg of estradiol benzoate im (2.0mL Estrogin®, Farmavet, SP, Brazil) + CIDR (1.9g of P4, Zoetis Animal Health, SP, Brazil) + GnRH im (2.0mL Cystorelin®, Merial Animal Health, SP, Brazil); d7- PGF im (5.0mL Lutalyse®, Zoetis Animal Health, SP, Brazil); d9- CIDR withdrawal + PGF; d11- FTAI. Animals were randomly assigned to receive at d9 0.5mL (1.0mg) or 1.0mL (2.0mg) im ECP (Zoetis Animal Health, SP, Brazil). In Exp.2 (FTET), 593 lactating Girolando cows received the following protocol: d0- im 2mg of EB+CIDR; d7-im PGF; d9- withdrawal of CIDR; d18- GnRH im + FTET. Animals were randomly assigned to receive on d9 0.5mL (1.0mg) or 1.0mL (2.0mg) im ECP (Zoetis Animal Health, SP, Brazil). Binomial distributed variables were analyzed with PROC GLIMMIX. In Exp.1 ovulation rate was similar ($P=0.59$) between treatments. The treatment with 1.0mL of ECP showed higher ($P<0.01$) expression of estrus [93.2%(n=269/287)] compared with 0.5mL treatment [82.8%(n=245/296)]. Treatment did not affect ($P=0.66$) the rate of P/AI (pregnant AI) at 32d [0.5mL-24.6%(n=80/326) vs 1.0mL-23.1%(n=73/317)] and 60d [0.5mL-22.8% (n=67/326) vs 1.0mL-21.4%(n=61/317); $P=0.66$] and pregnancy loss [0.5mL-17.9%(n=13/80) vs 1.0mL-17.4%(n=12/73); $P=0.93$]. There was a P/AI interaction ($P=0.05$) between treatments and body condition score (BCS) at the 60d pregnancy diagnosis, in which both thin ($BCS\leq 2.5$) and fat ($BCS\geq 3.5$) animals showed greater P/AI in the 1mL treatment, compared to the 0.5mL treatment [thin= 0.5mL-15.4%(n=12/104) vs 1.0mL-22.0%(n=21/107); fat= 0.5 mL-26% (n=4/18) vs 1.0mL-38.6% (n=10/28)] in animals with good BCS ($BCS\geq 2.75\leq 3.25$), 0.5mL treatment showed higher P/AI at 60d compared to 1.0mL treatment [0.5mL-26.2%(n=51/204) vs 1.0mL-18.1%(n=30/182)]. In Exp.2 ovulation rate was similar ($P=0.23$) between treatments, an increased expression of estrus rate was observed in the 1mL treatment [0.5mL-68.5%(n=148/216) vs 1.0mL-81.8%(n=184/225); $P<0.01$]. There was no effect of treatment on rate of P/ET at 30d [0.5mL-51.6%(n=147/285) vs 1.0mL-46.8%(n=144/308); $P=0.24$] and 60d [0.5mL-40.4%(n=115/285) vs 1.0mL-35.1%(n=108/308); $P=0.18$] and pregnancy loss [0.5mL-21.8%(n=32/147) vs 1.0mL-25%(n=36/144); $P=0.52$]. In the cows without CL at d0 there was a tendency ($P=0.06$) in the 1.0 mL treatment to reduce the P/ET at 60d [0.5mL-40.3%(n=50/124) vs 1.0mL-29.3%(n=39/133)]. In thin and fat cows there was no effect (thin $P=0.97$; fat $P=0.94$) between treatments in P/ET at 60d, in animals with good BCS, the 1.0mL reduced the P/TE at 60d [0.5mL-40.3%(n=60/149) vs 1.0mL-29%(45/155); $P=0.04$]. In conclusion, increasing dosage of estradiol cypionate increased the percentage of animals with expression of estrus, did not affect ovulation rate and affected the P/AI or P/ET at 60 d by BCS.



A075 FTAI, FTET and AI

Reproductive efficiency of Nelore females submitted to three consecutive FTAI programs with 32 days of interval between inseminations

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Keywords: beef cows, resynchronization, FTAI.

This study aimed to evaluate the reproductive efficiency of Nelore females submitted to three consecutive FTAI programs with 32 days of interval between inseminations. The programs were conducted in a commercial farm located at Camapua-MS. A total of 2,200 females of the three following categories: heifers (HEIF; n = 584), primiparous (PRIM; n = 507) and multiparous cows (MULT; n = 1109) was submitted to the same fixed time artificial insemination (FTAI) protocol (Day -10). At this day, the females received a progesterone (P4) releasing device (Cronipress Monodose M-24®) and 2.0 mg intramuscular (IM) estradiol benzoate (EB; Bioestrogen®). On Day -2, the P4 device was removed and it was administrated 300IU IM of eCG (Novormon), 1.0 mg IM of estradiol cypionate (EC; ECP®) and 0.75 mg IM of cloprostenol (PGF; Croniben®). All females were inseminated 48 h after the P4 device removal, first day of the breeding season (Day 0). On Day 22, all females were resynchronized with the insertion of the P4 device and the 1.0 mg IM injection of EB. On Day 30, the females were submitted to ultrasonography examinations (Chison 9300VET, Kylumax, Brasil) for pregnancy diagnosis. The non-pregnant females, in addition to the P4 device removal, received 300IU of eCG, 1.0 mg IM of EC and 0.75 mg IM of PGF and were timed inseminated 48 h after later (Day 32). On the pregnant females it was only removed the P4 device. On Day 54, all females that received the second FTAI were resynchronized using the same described resynchronization protocol. The pregnancy diagnosis of the second FTAI was performed on Day 62, and the third FTAI occurred on Day 64. The statistical analysis was performed by the GLIMMIX procedure of SAS 9.3. The overall conception rates were verified among the three inseminations: first FTAI = 55.1% (1,015/1,843); second FTAI = 37.4% (303/811); and third FTAI = 41.7% (213/511). However, an interaction ($P < 0.0001$) was observed between the animal categories and the number of inseminations. In HEIF, the conception rates were greater in the first FTAI = 50.9% (297/583)^a, compared to the second FTAI = 35.8% (98/274)^b and third FTAI = 35.4% (63/178)^b. Similar results were obtained in MULT cows: first FTAI = 65.4% (494/755)^a vs. second FTAI = 37.3% (96/257)^b vs. third FTAI = 44.0% (70/159)^b. Differently, in PRIM females the conception rates did not differ between the three inseminations: first FTAI = 44.4% (224/505) vs. second FTAI = 38.9% (109/280) vs third FTAI = 46.0% (80/174). The final pregnancy rate of the breeding season was 83.1% (1,531/1,843), being 78.6% (458/583) for the HEIF, 87.4% (660/755) for MULT cows and 81.8% (413/505) for PRIM cows. In conclusion, the use of three consecutive FTAI with 32 interval between inseminations results in satisfactory efficiency, independently of the category used, being multiparous more efficient than other categories. Such programs enable the establishment of a 64 days breeding season, without the use of natural mating.

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A076 FTAI, FTET and AI

Synchronization of ovulation in suckling beef cows with hCG or estradiol cypionate for timed artificial insemination

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Keywords: beef cow, ovulation, TAI.

Synchronization of ovulation and conception rate to TAI was evaluated in suckling *Bos taurus* beef cows (n=246) with 45±15 days postpartum maintained in native pastured system in Rio Grande do Sul. Females were randomly assigned in the groups and presented at the beginning of treatment the body condition score of 2.9 (1 to 5). Females were evaluated with ultrasound on the day zero (D0) of the protocol (Day 0), day 8 (D8), immediately before TAI (D10) and 7 days after TAI (Day 17). All cows were synchronized with Intravaginal Progesterone Releasing Device (IPRD) (0.75g of progesterone, Proclinar®, Hertape Calier Animal Health, Brazil) and 2mg im of estradiol benzoate (EB; Benzoato HC®, Hertape Calier) on D0. On Day 8, the IPRD was removed and 150µg of D (+) cloprostenol (Veteglan Luteolytic®, Hertape Calier), and 25IU IM of FSH/LH (Pluset®, Hertape Calier) were administered. Females in EC (n=84) group received 1 mg IM of Estradiol Cypionate (EC; Cipionato HC®, Hertape Calier). Females on D8 in hCG (n=81) group received 500IU IM of hCG (Vetecor, Hertape Calier) at the time of TAI. The females in EC + hCG group (n = 83) received both treatments. All cows were submitted to TAI 54h after withdrawal of IPRD. At the beginning of the protocol 29.2% (72 /246) of the cows had a corpus luteum. Some of the cows (n=102) had the time of ovulation monitored at every 12 hours starting at the withdrawal of IPRD [EC (n = 34), hCG (n = 34) and hCG + EC (n = 33)]. Statistical analysis was performed using PROC GLIMMIX of SAS. The diameter (mm) of the dominant follicle (DF) on Day 8 (8.7 ± 0.2 , 8.8 ± 0.2 , 8.6 ± 0.2) did not differ between treatments EC, EC + hCG or hCG ($P = 0.79$). However, the DF on D10 was bigger ($P = 0.001$) for cows treated with hCG (12.9 ± 0.3 mm) compared to cows from EC (11.3 ± 0.2) or EC + hCG group (11.8 ± 0.2). The interval (h) between the withdrawal of IPRD and ovulation was less ($P = 0.01$) for hCG group (71.2 ± 1.7) compared to the groups treated with EC or EC + hCG (76.6 ± 2.2 and 74.2 ± 1.7 , respectively). The ovulation rate did not differ ($P = 0.61$) among EC (85.2%, 29/34), hCG (91.1%, 31/34) or EC + hCG groups (90.9%, 30/33). Corpus luteum diameter (mm) was greater ($P = 0.04$) on D17 for the hCG treated group (21.4 ± 0.3) compared to others (EC = 19.1 ± 0.8 or EC + hCG = 20.4 ± 0.8). However the plasma progesterone concentrations (ng/mL) on D17 were EC = 2.0 ± 0.1 ; hCG = 2.4 ± 0.1 and EC + hCG = 2.3 ± 0.1 ($P = 0.19$) and the conception rate on the 28th day after TAI (EC = 43.0%; hCG = 47.0% and EC + hCG = 48.8%, $P = 0.76$) was also similar. Despite some differences in follicular diameter, and shorter interval to ovulation in hCG treated cows; both hCG and/or EC produced similar pregnancy rates in TAI programs in suckling *Bos taurus* cows.

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A077 FTAI, FTET and AI

Pregnancy rate in ewes submitted to shorter or longer protocols using progesterone devices in and out of the breeding season

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Keywords: laparoscopy, Santa inês, texel.

The objective of this study was to evaluate the pregnancy rate in short (6 days) and long term protocols (12 days) with progesterone device inside and outside of the breeding season. There were 79 ewes Texel breed (TE) and Santa Inês (SI) that were between 2.0 and 4.0 years old, mean body condition score 3.0 (range 1-5). The ewes were maintained on *Panicum maximum* cv. Mombaça, receiving 250 g of concentrate with 18% crude protein (CP), with access to water and mineral salt *ad libitum*. Initially, each ewe received an intravaginal progesterone device (D0, CIDR, Zoetis, Animal Health, Brazil) which remained for 6 (G-6) or 12 days (G-12). At device removal we administered intramuscularly, 0.075 mg of cloprostenol (cloprostenol, Veteglan Hertape Calier®, Brazil) and 300 IU of equine chorionic gonadotropin (eCG, Novormon®, MSD Animal Health, Brazil). Two experiments were performed: experiment 1 outside of the breeding season, in November (n = 43) and Experiment 2 within the breeding season in March (n = 36). In experiment 1, 43 ewes were divided into two groups: G-6 (n = 24) and G-12 (n = 19). In experiment 2, 36 sheep were divided into two groups: G-6 (n=18) and G-12 (n = 18). Approximately 50 hours after CIDR removal ewes were inseminated at fixed time with laparoscopy with frozen semen containing 200×10^6 sperm cells. Pregnancy diagnosis was performed 40 days after AI by ultrasound. Statistical analysis was performed with the Chi-square test and the Yates correction for continuity was used. For all comparisons, we adopted a significance level of 5% in the Biostat 5.0 software. The pregnancy rate on experiment 1 in G-6 group was 58.33% (14/24), in the G-12 group was 52.63% (10/19) and the overall percentage, regardless of group, was 55.81% (24/43). In Experiment 2 (within the breeding season) the pregnancy rate of the G-6 group was 61.11% (11/18), and the G-12 group 66.66% (12/18), being 63.88% (23/36) overall percentage independently of group. The overall pregnancy rate of both experiments (inside and outside the breeding season) was 59.49% (47/79), being 59.52% the pregnancy rate of the G-6 (25/42) and 59.46% G-12 (22/37). There was no significant difference in pregnancy rate when comparing the time of permanence of the progesterone device nor in relation to reproductive season. We conclude that it is possible to reduce the time of the fixed-time AI protocol, both for in and out of the breeding season, without affecting pregnancy rate.



A078 FTAI, FTET and AI

Impact of TAI on productive and reproductive efficiency in Holstein cows reared in semi-arid conditions

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Keywords: Holstein, reproductive efficiency, semi-arid.

This study was conducted at the Experimental Station of São Bento do Una (ESSB/IPA) Pernambuco, Brazil, (Latitude 08 31'35" and Longitude 036 27'38.4") and temperature-humidity index of 63.5. Cyclic Holstein cows with a lactation period of 60 to 150 days; average production of 18.9 kg/milk/day; having 28 to 108 days in milk and BCS varying from 2.0 to 4.0. The herd was kept in semi-intensive system, receiving a diet composed of cactus pear, sorghum silage and protein concentrated with 24% of crude protein (CP) besides mineral supplement and water ad libitum. All cows were monitored for cyclicity and underwent gynecological examination. Reproductively sound animals were randomly distributed into two experimental groups: G1 (AI) and G2 (TAI). In G1 (n=157) cows underwent estrus observations twice a day, during 12 months, and those detected in estrus were artificially inseminated 12h later. In G2 (n=132) cows were allocated in groups of approximately 20 females receiving TAI each 60 days during a period of 12 months. All cows in G2 received a hormonal protocol with an intravaginal device containing 1.9g of P4 (CIDR, Pfizer, São Paulo, Brazil) and 2.0mg of EB (RIC-BE, Tecnopec, Sao Paulo, Brazil) intramuscularly at day (D0). At day 7 of the protocol (D7) it was administered 0.530mg of sodium cloprostenol (Ciosin, MSD, Sao Paulo, Brazil) and 400 IU of eCG (Folligon, MSD, São Paulo, Brazil); at day 8 (D8) intravaginal devices were removed. On day 9, cows received 1.0mg of EB im and were bred by fixed-time AI at 54 h after intravaginal device removal. Cows from G1 were submitted to ultrasonography at D9 to evaluate follicular diameter, and all cows from both groups (G1 and G2) were artificial inseminated with conventional semen. Some variables evaluated were number of services per conception, conception rate and embryo loss at day 30 and 60 after TAI. Milk production was assessed in each group through standard 305-day lactation period. Data were analyzed with ANOVA and T-test (5%) using the statistical program SPSS 16 for windows. Follicular diameter at D9 in cows from G2 varied from 6.8 to 23.2 mm. Number of services per conception for G1 was 1.91 and for G2 1.36; $P < 0.05$. Conception rate at day 30 was 42.0% for G1 and 65.2% for G2 and at day 60, 41.4% for G1 and 62.9% for G2; $P < 0.05$. Embryo loss for G1 was 1.50% and 3.50% for G2; $P > 0.05$. The median average milk production per cow and the average daily production obtained in G1 were 3.537Kg and 11.60 kg, and to G2 were 6.645 kg and 21.78 kg; $P < 0.05$. Therefore, it is concluded that the TAI increases productive and reproductive efficiency of Holstein cows reared in semi-arid conditions.



A079 FTAI, FTET and AI

Uterine size matters for fertility in lactating dairy cows

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Keywords: diameter, pregnancy, synchronization.

We hypothesized that greater size of uterus is related to reduced fertility in lactating dairy cows. A total of 705 Holstein dairy cows were synchronized for ovulation using Gonadotropin releasing hormone (GnRH) and prostaglandin F2 α (PGF) in the Double-Ovsynch protocol (GnRH-7d-PGF-3d-GnRH-7d-GnRH-7d-PGF-56h-GnRH-16h-timed AI). Blood samples were taken to evaluate progesterone (P4) concentrations at the time of last PGF and final GnRH. Based on P4, cows that were not synchronized ($n = 52$) were excluded from further analysis ($P4 < 2.0$ ng/mL at PGF and/or $P4 > 0.5$ ng/mL at GnRH). A total of 653 cows (307 primiparous, 346 multiparous) were included in the final analysis for fertility. Overall pregnancies per AI (P/AI) were 48.9% for primiparous and 39.3% for multiparous cows. At time of final PGF (72h before AI), uterine diameter was determined for both uterine horns by ultrasound at the greater curvature. Length of uterus (cervix to ovary) was determined by hand palpation compared to a hand ruler. Total volume of uterus was estimated by determining volume of each horn as the volume of a cylinder ($V = \pi r^2 h$; $r = \text{diameter}/2$ at greater curvature; $h = \text{length of horn}$). Differences between groups were determined with Student's t-test and logistic regressions were performed with Proc Logistic in SAS. Overall, pregnant cows ($n = 286$) were less than non-pregnant cows ($n = 367$) for uterine diameter (18.0 ± 2.3 , 18.7 ± 2.3 ; $P = 0.0001$), uterine length (25.1 ± 2.1 , 25.6 ± 2.4 ; $P = 0.01$), and total uterine volume (130.0 ± 38.3 , 143.1 ± 41.8 ; $P = 0.0001$). However, multiparous cows were greater than primiparous cows for uterine diameter (18.9 ± 2.4 , 17.9 ± 2.2 ; $P = 0.0001$), uterine length (25.8 ± 2.2 , 24.8 ± 2.2 ; $P = 0.0001$), and total uterine volume (147.3 ± 42.6 , 126.2 ± 35.6 ; $P = 0.0001$) and therefore analyses were performed by parity. For multiparous cows, pregnant cows ($n = 136$) were less than non-pregnant cows ($n = 210$) for uterine diameter ($P = 0.003$), uterine length ($P = 0.04$), and total uterine volume ($P = 0.001$). In primiparous cows, pregnant cows ($n = 150$) were not different from non-pregnant cows ($n = 157$) for uterine length ($P = 0.42$) but tended to be less for uterine diameter ($P = 0.05$) and uterine volume ($P = 0.07$). Logistic regression indicated that P/AI for all cows was related to uterine diameter ($P < 0.0001$), uterine length ($P = 0.01$), and total uterine volume ($P < 0.0001$) with cows with greater uterine size having reduced P/AI. Similarly for just multiparous cows, diameter ($P = 0.003$), length ($P = 0.04$), and volume ($P = 0.002$) were related to P/AI. However for primiparous cows, P/AI tended to be related to uterine diameter ($P = 0.06$) and volume ($P = 0.07$) but was not related to length ($P = 0.42$). Uterine diameter was also related to circulating P4 concentrations in multiparous ($r = -0.15$; $P = 0.005$) but not primiparous ($r = -0.08$; $P = 0.16$) cows. Thus, uterine size is clearly related to fertility in synchronized lactating dairy cows with greater uterine size being associated with reduced fertility. These changes in uterine size may be a causative factor for reduced fertility in dairy cows particularly multiparous dairy cows.



A080 FTAI, FTET and AI

Impact of supplementation with long-acting progesterone during early diestrus on fertility of Nelore cows submitted to TAI

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Keywords: cattle, embryo, interferon-tau.

Increased circulating progesterone (P4) concentrations during early pregnancy favor conceptus development (O'Hara L. *Reprod Fertil Dev* 26:328-36). In addition, long-acting P4 supplementation on day 3 post-ovulation increases the plasma P4 concentrations for 72 hours and does not alter the CL development (Pugliesi G. *Reprod Dom Anim* 49:85-91). We aimed to evaluate the effect of administration of a long-acting P4 formulation after timed-AI (TAI) on fertility of beef cows. On Experiment 1, suckling Nelore cows between 30 to 50 days post-partum and with a averaged body condition score of 3.7 (scale: 1 to 5) received an intravaginal P4 device (Sincrogest; Ouro Fino, Cravinhos, SP) and an im injection of estradiol benzoate (2 mg; Sincrodiol, Ouro Fino) on Day-10 (Day 0 = day of TAI). On Day-2, devices were removed and cows received im estradiol cypionate (1 mg; ECP; Zoetis, São Paulo, SP), eCG (300 IU; Novormon; Zoetis) and sodium cloprostenol (0.530 mg; Sicrocio; Ouro Fino). On Day 0 cows were TAI and diameter of preovulatory follicle was measured through transrectal ultrasonography. On Day 4 post-TAI the ovulation was confirmed by ultrasound, and animals were split to receive im 1 mL of vehicle (Control group; n= 393) or 150 mg (1mL) of long-acting P4 (Sincrogest injectable; Ouro Fino; P4-treated group; n=390). On Experiment 2, two ultrasound evaluations 10 days apart were performed in suckling Nelore cows with 35 to 65 days post-partum and averaged body condition score of 3.3. Cows without a CL at both evaluations (considered in anetrus) were synchronized and split in the Control (n=189) and P4-treated (n= 187) groups as described on Experiment 1. In both experiments the pregnancy diagnosis was done between 35 and 60 days post-TAI, and conception rates were analyzed by logistic regression using the PROC Glimmix from SAS software. On Experiment 1, the conception rate did not differ ($P>0.1$) between Control (53.2%; 209/393) and P4-treated (56.2%; 219/390) groups. Considering only ovulated cows, the conception rate also did not differ (62.6%; 209/334 and 65.6%; 219/334; $P>0.1$) for Control and P4-treated groups, respectively. On Experiment 2, the conception rate was 46.0% (86/187) for the Control group and 55.6% (105/189; $P=0.05$) for the P4-treated group considering all cows, and 49.1% (86/173) for the Control group and 59.0% (105/178; $P=0.08$) for the P4-treated group, when considering only ovulated cows. In conclusion, the administration of long-acting P4 on the fourth day after TAI improves fertility of non-cycling beef cows.

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A081 FTAI, FTET and AI

Evaluation of the resynchronization efficiency using GnRH or estradiol benzoate at the beginning of the protocol in lactating Holstein cows

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Keywords: dairy cows, follicular dynamics, resynchronization.

The current study evaluated the efficiency of the synchronization of a new follicular wave emergence after administering GnRH or estradiol benzoate (BE) at the beginning of the resynchronization protocol at 25 days (Day 25) after TAI in Holstein cows with unknown pregnancy status. A total of 41 lactating cows was distributed in two experimental groups at a random stage of the estrous cycle, and all animals underwent the same TAI protocol [2mg of EB + progesterone device (P4)-8d-device removal + 1mg estradiol cypionate + 0.150 mg of cloprostenol (PGF2 α)-TAI 48h later]. Twenty five days after TAI, all females were examined by ultrasound and blocked into two experimental groups GnRH (n=21) or EB (n=20), according to the presence (87.8%, 36/41) or absence of corpus luteum. Thus, the complete sequence of hormonal treatments were: P4 device insertion (Primer®, Agener-Tecnopec) and 2.0 mg of EB (RIC-BE®, Agener-Tecnopec; BE GROUP) or P4 device insertion and 25 μ g of Lecirelin (GnRH; Gestran, Tecnopec; GnRH GROUP). Between days 25 and 32, all females were subjected to ultrasonography exams at every 24 hours to evaluate the synchronization of the emergence of a new follicular wave. On Day 32 the P4 device was removed and both groups received another ultrasound exam for pregnancy diagnosis and, therefore, determine which cows would continue (non-pregnant females, n=26) or not (pregnant females, GnRH: 33.3%, 7/21 and EB: 40%, 8/20) in the resynchronization protocol. At this moment, only non-pregnant females (GnRH: n=14 and EB: n=12) received 0.150 mg of d-cloprostenol (Prolise®, Agener-Tecnopec), 1.0 mg of EB 24 hours later (Day 33) and were inseminated on Day 34. The ovulation was assumed following the disappearance of the ovulatory follicle detected by ultrasonographic exams occurring at every 12 hours until 96 hours after device removal. Then, pregnancies following the first TAI were confirmed on Day 70 (GnRH: 71.4%, 10/14 and EB: 75.0%, 9/12). The results were analyzed with the GLIMMIX procedure of SAS® and only non-pregnant animals were considered in the analyses. It was observed similar (P = 0.81) new follicular wave emergence rate [GnRH: 71.4% (10/14) vs. EB: 75% (9/12)], however, the time of emergence differed (P < 0.001) between the experimental groups (GnRH: 2.0 \pm 0.3 d vs. EB: 3.4 \pm 0.3 d). The ovulatory follicle diameter was greater (P = 0.008) in the GnRH group (16.0 \pm 0.8 mm) compared to the EB group (12.9 \pm 0.8 mm). In addition, the time of ovulation was similar (P = 0.94) between groups (GnRH: 78.5 \pm 1.7 h vs. EB: 78.7 \pm 2.9 h after P4 device removal), as well as ovulation rate [GnRH: 92.9% (13/14) vs. EB: 75.0% (9/12); P = 0.22]. In conclusion, the administration of either GnRH or EB at the beginning of the resynchronization protocol at unknown pregnancy status are efficient to induce synchronized emergence of new follicular waves and ovulation in lactating Holstein cows.

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A082 FTAI, FTET and AI

Reproductive efficiency in beef cattle

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Keywords: beef cattle, bovine, reproductive performance.

Infectious diseases related to reproduction are referred as important in cattle mostly in dairy cattle, due its ease dissemination between animals managed in intensive systems. However, in beef cattle such diseases can also negatively affect reproductive performance. In Brazil, this situation is of great relevance considering the size of beef cattle population. This study analyzed the effect of vaccinating against IBR, BVD, Bovine Parainfluenza type 3, Leptospirosis (PI3), Bovine respiratory syncytial virus (BRSV) and Campylobacteriosis (Fertiguard™-Vallée S.A.) over the gestation and calving rates in a beef cattle herd kept in extensive system, with four-month breeding season, in which no previous vaccination against these diseases had been used. Four hundred and thirty four *Bos indicus* females (Zebu) under two different physiological status, that is, pregnant cows (n=246 - between 230 and 270 days of gestation) and postpartum cows (n=188 - between 10 and 50 days postpartum), were randomly divided in two groups (vaccinated and control). Animals of different groups were kept grazing together. Independent of the physiological status, cows were vaccinated with two doses, applied 50 and 20 days before the beginning of the breeding season. Fertility tested bulls were used in a proportion of 1:30. Reproductive performance in breeding season following the vaccination was evaluated and the calving percentage was also recorded. The percentages were compared by Chi-square test and statistical significance was assumed at 5% probability. Vaccination improved the gestation rate in immunized pregnant cows (84.5% vs 78.7%) and in immunized calving cows (90.3% vs 82.1%). The calving rate on the subsequent season was also higher in vaccinated cows (82.8% vs 75.7%). The immunized group, regardless of reproductive status, had improved reproductive performance as compared to cows in the control group ($P < 0.05$). Immunized cows showed better pregnancy rate by the end of the subsequent season and lower rate of pregnancy loss from the end of breeding season to calving ($P < 0.05$); which resulted in a better calving rate in comparison to the control group. Therefore, it is concluded that the vaccination previously to the breeding season can be done on cows at the end of gestation and/or after calving because it improves reproductive efficiency and calving rates in the following calving season in beef cows.

Support: FAPEMIG, CNPq, and Vallée S.A.



A083 FTAI, FTET and AI

Fixed-time artificial insemination in the State of Pará

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Keywords: FTAI, pregnancy, reproduction.

The objective was to evaluate the evolution of FTAI, identifying the most used protocols and the factors that influence the efficiency of the technique. The most commonly used protocols were CIDR® [D0: 2.0mg of EB (Gonadiol®, MSD Saúde Animal, São Paulo, Brazil) + implant of P4 (CIDR®, Zoetis, São Paulo, Brazil); D9: application of 10 mg PGF2 α (Dinoprost, Lutalyse®, Zoetis, São Paulo, Brazil) + removal of the implant + 300 IU of eCG (Folligon®, MSD Saúde Animal); D10: 1.0mg of EB; D11: FTAI]; DIB® [D0: 2.0mg of EB (Gonadiol®, MSD Saúde Animal) + implant of P4 (DIB®, MSD Saúde Animal, São Paulo, Brazil); D8.5: application of 10 mg PGF2 α (Cloprostenol, MSD Saude Animal) + removal of the implant + 300 IU of eCG (Folligon®, MSD Saúde Animal) + application of 0.5mg estradiol cypionate; D10: FTAI] and Ovsynch. Denominated T1, T2 and T3, respectively. A pregnancy per AI (P/AI) following 49,426 inseminations was evaluated according to the protocol, body condition score (BCS) in a scale of 1.0 to 5.0, and animal category (heifers, primiparous and multiparous), number of reutilizations of the device (1st, 2nd, 3rd and 4th uses), month of FTAI, time (morning or afternoon), presence and absence of shading, inseminator (best and worst P/AI), semen (best and worst P/AI) and handling (with and without stress). Data were analyzed using the SAS (2000) using the Chi-square test, (P<0.05). The mean P/AI was 52.5 \pm 10.3%, similar averages in other regions of Brazil (Baruselli et al., 2002, Brazilian Journal of Animal Reproduction 26, 218-221). The most efficient protocol (P<0.05) was the T1 (55.3 \pm 9.3%) compared to T2 (51.1 \pm 7.2%) and T3 (34.4 \pm 5.1%). The BCS influenced P/AI (P<0.05), being the best rates (54.5 \pm 16.5%) in animals with BCS of 3.5 when compared to the score 2.5 (32.9 \pm 4.3%). Cow category influenced P/AI (P<0.05), being primiparous (48.8 \pm 7.3%) lower than heifers (54.8 \pm 5.7%) and multiparous (56.5 \pm 2.3%). The use of more than once of the P4 implant, did not influence P/AI (P>0.05), being 54.2%, 54.5%, 55.9% and 56.3% for 1st, 2nd, 3rd and 4th use, respectively. Inseminations performed in November resulted in the best P/AI (60.2 \pm 11.3%; P<0.05), compared to the months of September, October and December. The time of the day in which breeding was performed influenced P/AI (P<0.05), and those performed in the morning (69.0 \pm 20.2%) were higher than those of the afternoon (49.3 \pm 5.8%). Difference was also observed in P/AI with regards to shading, as follows: with shadow (70.4 \pm 18.4%), without shadow (51.9 \pm 8.6%). The inseminator with the best P/AI obtained (78.6 \pm 21.6%) and the one with lowest P/AI was (15.9 \pm 3.6%). Similarly, there was a significant variation in P/AI according to semen, with the best P/AI reaching 77.8 \pm 22.25% and the worst semen having P/AI of 24.3 \pm 4.3% (P<0.05). Handling also had an effect, with best handling with P/AI of 77.0 \pm 18.3% and the worst 41.3 \pm 12.0% (P<0.05). Therefore, the type of protocol, the BCS, animal category, month of breeding, time of insemination, shading, AI technician and handling all influenced P/AI; however, the reuse of progesterone devices did not affect P/AI in FTAI protocols.



A084 FTAI, FTET and AI

Resynchronization as a tool to improve the efficiency of use of embryo recipients

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Keywords: bovine, hormones, reproductive efficiency.

The maintenance of recipients has a major impact on the cost of a commercial ET program in cattle. Alternatives to get the recipients pregnant as soon as possible is important for technical and financial viability of the program. Resynchronization protocols can offer this possibility, but require the application of hormones in females in the initial stage of pregnancy, even before pregnancy diagnosis. The aim of this study was to evaluate the efficiency of a resynchronization protocol on pregnancy rate in crossbred heifers used as embryo recipients. The study was conducted in a farm located in the Southwest of Minas Gerais State - Brazil. Embryos (184) were produced in vitro (IVP) using Gyr donors and sexed sorted semen. A timed embryo transfer protocol (TET) was used to synchronize the time of ovulation in the recipients. DO: progesterone intravaginal device insert PID-(Prociclar™-Brazil) plus 0.5mg of estradiol benzoate – EB (Benzoato HC™-Brasil) IM; D8: PID removal plus 0.5 mg of estradiol cypionate– EC (Cipionato HC™-Brasil) IM and 0.125mg of D-Chloprostenol IM (Veteglan™- Brazil). At the embryo transfer day (D17) recipients were evaluated by ultrasound (Mindray™-M5) and those having a corpus luteum were used. Fourteen days after embryo transfer (ET) the recipients were randomly divided into two groups: G1 (n=92) start of the resynchronization protocol by inserting a PID and application of 1 mg of EB, and G2 (n=92) untreated controls. This procedure was designed to evaluate the effect of application of estradiol in early pregnancy. Eighth days later all recipients were again evaluated by ultrasound for pregnancy diagnosis. The pregnant females in G1 had their PID removed. Animals not pregnant females in this group received 0.5mg of EC and 0.125 mg of D-Chloprostenol IM and PID was removed. Ten days later these animals were again evaluated and those that were eligible were inoculated. In G2 the beginning of the second TET protocol only occurred after the non-pregnancy diagnosis. The Chi-square X2 test was used to analyze pregnancy rate after first and second embryo transfer and the number of pregnant recipients at 30 and 45 days. The pregnancy rate after the first ET was not different between groups ($P>0.05$ – 52.2 vs 48.9% for G1 and G2). This result confirm that the insertion of a PID and application of 1 mg of EB in females at the beginning of pregnancy does not seem to impair pregnancy maintenance. After the second ET, pregnancy rate was again not different ($P>0.05$ – 47.9 vs 48.8% for G1 and G2). These results indicate that the resynchronization protocol does not affect with future fertility of the recipient. Thirty days after the beginning of the study the number of recipients that were pregnant was not different between groups (48 and 45% for G1 and G2). In contrast, at forty-five days, G1 had more pregnant recipients than G2 (67 and 45% for G1 and G2). We conclude that the use of the resynchronization protocol does not affect maintenance of the early pregnancy and will anticipate time to pregnancy in recipient animals.

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A085 FTAI, FTET and AI

Effect of thermal comfort index on reproductive performance of Brangus cows in tropical conditions

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Keywords: Brangus, pregnancy, thermal stress.

In tropical environments, high temperature and high humidity associated with air radiation, reduces the efficiency of heat loss and thus, increases the animal's stress (Silva et al., 2007 Brazilian Journal of Zootecnia.v.36, p.1192-1198), limiting development, production and reproduction (Vasconcelos et al., 2006 Theriogenology, v.65, p.192-200). According to HAHN (Stress physiology in livestock, v.2, 1985), for domestic animals in general, a value of temperature and humidity index (THI) at or below 70 indicates normal condition; and above 71 values characterize stress condition. For Armstrong (Journal of Dairy Science, v.77, p.2044-2050, 1994) THI above 72 is considered heat stress. The aim of this study was to evaluate the effect of thermal comfort index, using THI, on the reproductive performance of Brangus cows submitted to TAI in wetland savannah. Brangus females (n = 421) were submitted to estrus synchronization and TAI protocols between September and October 2013, in two separate groups. The protocol used was as follows: on a random day of the estrous cycle, vaginal devices with 1.9 g of progesterone (CIDR®) were inserted and 2mg of estradiol benzoate IM was applied (BE; Estrogin®). On D9 device removal was performed and 300 IU of eCG (SincroeCG®), 0.8 mg of estradiol cypionate (ECP®) and 12.5 mg of dinoprost (Lutalyse®) IM was applied. The TAI was performed 48-54 hours after device withdrawal. At the same time the THI was calculated hourly during the program, using the methodology proposed by Thom (Weatherwise, v.12, p.57-60, 1959). The THI of the whole TAI program was 72 for the first group of animals and 73 for the second one. On the day of the TAI the THI was 72 and 74 respectively, classified as mild stress index, what according Hahn, is sufficient to cause reduction in reproductive performance. However, the TAI pregnancy rate was 54.4% (143/263) and 63.3% (100/158) for the first and second groups, both results considered suitable for TAI programs. It is suggested that THI up to 73 does not characterize heat stress for the Brangus breed. Further comprehensive studies should be conducted to define the range of thermal comfort for cattle breeds adapted to the tropics.



A086 FTAI, FTET and AI

High progesterone concentration has a negative effect on the expression of LH receptors in granulosa cells from Nelore heifers

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Keywords: *Bos indicus*, progesterone, synchronization.

High progesterone concentration during hormonal protocols has a negative effect in follicle growth, lowering preovulatory estradiol and post ovulatory progesterone in Nelore heifers. A recent study showed the impact of LH on expression of LH receptor (LHR) in granulosa cells (Luo et al., Biol Reprod, v.84, p.369-78, 2011). We suggest that the lower LH pulsatility, influenced by high P4 concentration, would down regulate the expression of LHR and consequently decrease follicle development after deviation. The objective was to evaluate if the progesterone concentration affects expression of LHR in granulosa cells from dominant follicle (DF) in Nelore heifers. We utilized 52 pre-synchronized heifers (D-17: 2mg EB + intravaginal progesterone device CIDR®; D-10: 12.5mg PGF2α Lutalyse®; D-8: 0.5 mg ECP® + CIDR® removal). Heifers that responded to pre-synchronization (presence of CL) were submitted to follicular ablation and blood collection on D0 and randomly distributed in six groups. High P4 D5 (CIDR1 [new] on D0 and slaughtered on D5 with blood collection); High P4 D6 (CIDR1 and slaughtered on D6); High P4 + PE (proestrus) (CIDR1 on D0, CIDR removal, blood collection and 25mg PGF2α on D5 and slaughtered on D6); Low P4 D5 (CIDR3 [previously used for 18 days] on D0 and 25mg PGF2α on D0 and D1, slaughtered on D5); Low P4 D6 (CIDR3 on D0 and 25mg PGF2α on D0 and D1, slaughtered on D6); Low P4 + PE (CIDR3 on D0 and 25mg PGF2α on D0 and D1, CIDR removal, blood collection and 25mg PGF2α on D5 and slaughtered on D6). The ovaries were taken and DF diameter measured after dissection. The comparison of the effect of P4 concentration on LHR expression was analysed by ANOVA. Granulosa cells from heifers treated with high P4 had decreased expression of LHR ($P < 0.01$); [High P4 D5 (n=6; P4 slaughter = 9.10ng/mL; average DF = 8.6mm; average LHR = 0.05 ± 0.12), High P4 D6 (n=8; P4 slaughter = 6.76ng/mL; DF = 9.35mm; LHR = 0.28 ± 0.15), High P4 + PE (n=6; P4 D5 = 7.76ng/mL; P4 slaughter = 0.79ng/mL; DF = 11mm; LHR = 0.72 ± 0.27)] vs. [Low P4 D5 (n=7; P4 slaughter = 3.23ng/mL; DF = 12.8mm; LHR = 3.28 ± 1.13), Low P4 D6 (n=6; P4 slaughter = 2.20ng/mL; DF = 11.56mm; LHR = 2.56 ± 0.91), Low P4 + PE (n=4; P4 D5 = 4.26ng/mL, P4 slaughter = 0.42ng/mL; DF = 13mm; LHR = 1.33 ± 0.47). In conclusion, high P4 concentration decreased LHR expression in granulosa cells of DF from Nelore heifers.



A087 FTAI, FTET and AI

Plasma progesterone concentration in the short and long term protocols using progestagens in ewes inseminated at fixed time

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UNOESTE.

Keywords: ovulation, Santa Inês, texel.

The objective of this study was to evaluate the plasma progesterone concentration in sheep according to time of progestagen permanence (6, 9 or 12 days) and correlate with the pregnancy rate in protocols for artificial insemination (TAI) in sheep. There were 38 ewes of Texel (Te) and Santa Inês (SI), randomly divided into three groups according to duration of treatment with progestin: short-term (6 days, G-6; n=13), average (9 days G-9; n=13) or long (12 days, G-12; n=12). The sheep were between 4.0 and 6.0 years old and mean of body condition score was 3.0 (range 1-5). They were maintained on *Panicum maximum* cv. Mombasa, receiving 250 g of concentrate with 18 % crude protein (CP), with access to water and mineral salt ad libitum. Initially, each ovine received an intravaginal sponge with progestin (D0, Acetate of Medroxiprogesterone, Progespon®, Syntex, Brazil) which remained for 6 (G-6), 9 (G-9) or 12 days (G-12). At sponge removal animals received 0.1315 mg of prostaglandin F2 α (PGF2 α , Sincrocio®, Ourofino, Brazil) and 300 IU of equine chorionic gonadotropin (eCG, Folligon®, Intervet, Brazil), intramuscularly. Blood samples were collected by jugular venipuncture in heparinized tubes of 15 mL in four moments: at the insertion of progestin (D0); removal of progestin; artificial insemination (TAI); and 10 days after TAI. After that, blood samples were centrifuged at 750 g for 15 minutes to separate the plasma, which was frozen at -20 °C. The dosage of the plasma progesterone concentration was performed using a commercial kit (Coat- A-Count, Diagnostic Products Corporation, CA, USA). The intra-assay coefficient of variation was 2.54 % for high control and 0.09 % for under control. The laparoscopic TAI was made at least 50 hours after withdrawal of progestin. One-way analysis of variance (one-way ANOVA) was used and the means were compared by Tukey method. When the data did not show homogeneity of variance, the Welch correction was performed and the evaluation of contrasts was done by Games-Howell. Chi-square test of Pearson was used for the analysis of pregnancy rate. All analyzes were conducted with SPSS software v. 13.0. At the time of progestin withdrawal, P4 in G-12 was significantly (P<0.05) lower (0.34 ng/mL) than P4 in G-6 (1.68 ng/mL) and in G-9 (1.76 ng/mL). However, there was no difference in pregnancy rate among the G-6 (76.9 %), G-9 (61.5 %) and G-12 (91.6 %) groups. In conclusion, the longer duration of progestin influences the concentration of progesterone in the removal of the implant without affecting pregnancy rate.



A088 FTAI, FTET and AI

hCG administration in Saanen goats inseminated following synchronized estrus

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Keywords: artificially inseminated, estrous induction, goat.

The objective of this study was to evaluate the effect of intravaginal hCG administration on pregnancy rate in artificially inseminated Saanen goats after induction of synchronous estrus. A total of 73 goats (35 nuliparous and 39 pluriparous) received intra-vaginal sponges (60 mg MAP; Progespon®, Syntex, Buenos Aires, Argentina) for 6 days plus 30µg d-cloprostenol (Prolise®; ARSA S.R.L., Buenos Aires, Argentina) latero-vulvar and 200 IU eCG (Novormon® 5000; Syntex, Buenos Aires, Argentina) i.m. 24 h before sponge removal. Goats were checked for estrus twice daily and only those in estrus 24 to 36 h after sponge removal were artificially inseminated in standing position (Embrapa Artificial Insemination Technique) from 18 to 24 hours after onset of estrus. The remaining goats in estrus were naturally mated. Following artificial insemination, goats were randomly assigned to receive 300 IU hCG (Vetecor® 5000; Hertape Calier, São Paulo, Brazil) (16 Nuliparous and 14 Pluriparous) or no further treatment (Control; 15 Nuliparous and 15 Pluriparous). hCG was diluted in a 0.3 mL saline solution and deposited into the vagina with the aid of a sterile insulin syringe without needle. Qualitative variables were analyzed by Chi-square and the quantitative analysis of variance to check differences between treatments was performed with T-test (5%, SAEG®). Parameters evaluated were similar for both groups ($P>0.05$). Body condition score (1 to 5 scale) was similar ($P>0.05$) for hCG treated (3.2 ± 0.3) and control animals (3.4 ± 0.3). Pregnancy rate was checked 90 days after artificial insemination by transretal ultrasonography. The estrus response was 92% (67/73) and 60 goats were artificially inseminated at the interval established. Natural breeding resulted in 57% pregnancy rate (4/7). Pregnancy rate was similar for hCG treated (57%, 17/30) and control goats (43%, 12/30). These promising results support further more comprehensive studies with greater numbers of goats to explore the use of hCG intramuscularly.

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A089 FTAI, FTET and AI

Effect of treatment with eCG, hCG or both 4 days after TAI on the development of the corpus luteum of dairy buffaloes

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Keywords: diameter, luteinization, ultrasonography.

This study aimed to evaluate the effects of eCG, hCG, or both given at 4 days after TAI in the development of the corpus luteum (CL) of dairy buffaloes. Thus, 72 dairy buffaloes housed in Sete Barras, São Paulo state were used. At random days of the estrous cycle (D-12; 4:00 PM) all buffaloes received a new intravaginal progesterone (P4) releasing device (1 g of P4; Primer®, Tecnopec, São Paulo, Brazil) plus 2.0 mg of estradiol benzoate im (Gonadiol®, MSD Animal Health, São Paulo, Brazil). In D-3 (4:00 PM), females received 0.53 mg of PGF 2α im (Cloprostenol sodium, Ciosin®, MSD Animal Health, São Paulo, Brazil) and 400 IU of eCG im (Novormon®, MSD Animal Health, São Paulo, Brazil), followed by removal of progesterone device. In D-2 (4:00 PM), 1.0 mg of estradiol benzoate (Gonadiol®, MSD Animal Health, São Paulo, Brazil) was administered im. The TAI was performed 40 hours after application of GnRH (D0; 8:00 AM). Four days after TAI (D4) all buffaloes that ovulated (n=60) after the protocol were randomly divided into four groups: buffaloes without treatment (Control, n=14); or treated with 400 IU of eCG i.m. (eCG, n=16; Novormon®, MSD Animal Health, São Paulo, Brazil); 1500 IU of hCG i.m. (hCG, n=14; Chorulon®, MSD Animal Health, São Paulo, Brazil); or 400 IU of eCG plus 1500 IU hCG (eCG+hCG; n=16). Ultrasound examinations (DP2200Vet Mindray, China) were performed to determine: dominant follicle diameter (ϕ DF) in 3-D and D0; daily growth rate of the DF (GrowthDF; between D-3 and 0); and the diameter of the CL originated after TAI protocol in D4, D8, D12 and D16 (ϕ CL), as well as the total growth of the CL (GrowthCL; between D4 and D16). The comparison between variables was performed through analysis of variance using Glimmix procedure of SAS®. The effects of treatment, day and interaction of these two effects on ϕ CL were analyzed as repeated measures using the Mixed procedure of SAS®. Difference was considered when $P < 0.05$. There was no difference among the respective control, eCG, hCG and eCG+hCG groups for: ϕ DF D-3 - 10.1 ± 0.2 mm; ϕ DF D0 - 12.6 ± 0.3 mm; and GrowthDF - 1.4 ± 0.1 mm/day. However, treatment ($P < 0.01$), day ($P < 0.01$) and day*treatment ($P < 0.01$) effects were detected on the development of the CL respectively for Control, eCG, hCG and eCG+hCG: D4 - 14.7 ± 0.6^a ; 13.8 ± 0.5^a ; 15.2 ± 0.5^a ; 14.1 ± 0.4^a mm; D8 - 16.8 ± 0.6^b ; 17.1 ± 0.6^b ; 20.7 ± 0.7^a ; 19.1 ± 0.7^{ab} mm; D12 - 17.4 ± 0.6^b ; 18.2 ± 0.5^{ab} ; 21.6 ± 0.9^a ; 21.1 ± 0.9^{ab} mm; and D16 - 18.4 ± 0.6^a ; 19.6 ± 0.6^a ; 22.2 ± 0.9^a ; and 21.1 ± 0.9^a mm. Also detected a difference ($P=0.03$) for GrowthCL: Control - 4.6 ± 0.6^c ; eCG - 5.5 ± 0.6^{bc} ; hCG - 7.0 ± 0.7^{ab} ; eCG+hCG - 6.9 ± 0.8^a mm. It was concluded that administration of hCG 4 days after TAI in dairy buffaloes increases the diameter of the CL that was originated in the protocol of synchronized ovulation. In contrast, eCG treatment given at day 4 after TAI did not affect the development of the CL.



A090 FTAI, FTET and AI

Follicular and luteal dynamics of dairy buffaloes that were treated or not with prostaglandin at the beginning of the synchronization protocol for TAI

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Keywords: corpus luteum, prostaglandin, reproductive seasonality.

The study aimed to evaluate the follicular and luteal development in buffaloes submitted to a TAI protocol according to the presence or absence of a functional corpus luteum during the maintenance of Progesterone intravaginal device (P4). For this, 26 multiparous Murrah buffaloes in milk, bred in a farm located in Registro, State of São Paulo were used. Before the TAI protocol, all buffaloes were subjected to a pre-synchronization protocol, which consisted two doses of 0.53 mg of PGF2 α im (Cloprostenol, Sincrocio®, Ourofino Agribusiness), on days -24 and -12, in order to produce a corpus luteum (CL) functional at the time of initiation of the TAI protocol (D0). On D0 (4:00h PM), the buffaloes received a new P4 intravaginal device (1g progesterone; Sincrogest®, Ourofino Agrobusiness) plus 2.0 mg of estradiol benzoate im (Sincrodiol®, Ourofino Agrobusiness). At this time (D0), the 26 buffaloes were randomly assigned to two treatments: 12 animals received one dose of PGF2 α (PGF group) to keep the intravaginal device as their only source of P4; while the other 14 females remained without the PGF2 α administration (Control group), which maintained a CL during treatment in addition to P4 intravaginal device. On D9 (04:00h PM), females received 0.53 mg of PGF2 α im (Cloprostenol, Sincrocio®, Ourofino Agrobusiness), followed by removal of the progesterone device. On D10 (04:00h PM) 1.0 mg of estradiol benzoate (Sincrodiol®, Ourofino Agrobusiness) was administered im. Ultrasound assessments (Chison D600Vet, China) were performed to determine: the presence of CL on D0, D3, D6 and D9; and diameter of the dominant follicle in D3, D6, D9 and D12 (ϕ DF); Ovulation rate (Ov. rate); The daily growth rate of the dominant follicle was calculated by the difference between the observed measures, and then dividing by the number of days apart (mm/day). The comparison between the response variables was performed through analysis of variance (ANOVA) using the procedure Glimmix of SAS®. Difference was considered when $P < 0.05$. We observed the presence of CL in 100% of animals at the beginning of the TAI protocol (D0), with CL regression all animals in the PGF2 α group. The results observed for the Control and PGF2 α groups were, respectively: ϕ DF D3, 3.1 \pm 0.2 vs. 3.2 \pm 0.4 mm ($P=0.83$); ϕ DF D6, 5.6 \pm 0.4 vs. 6.5 \pm 0.8 mm ($P=0.34$); ϕ DF D9, 6.9 \pm 0.5 vs. 9.3 \pm 0.8 ($P=0.01$); ϕ DF D12, 8.3 \pm 0.9 vs. 10.6 \pm 1.0 ($P=0.10$); Ov. rate 42.9% vs. 66.7% ($P=0.24$) daily growth rate between D3 and D9 DF - 0.6 \pm 0.1 vs. 1.1 \pm 0.1 mm/day ($P < 0.01$); and daily growth rate of the DF between D9 and D12 - 0.7 \pm 0.3 vs. 0.9 \pm 0.3 mm/day ($P=0.65$). It was concluded that the presence of CL during the treatment with P4 intravaginal device reduce the development of the dominant follicle. However, after removing the progesterone intravaginal device no difference was observed between the groups for daily growth rate, diameter of the dominant follicle at the end of the treatment and ovulation rate.

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A091 FTAI, FTET and AI

Effect of different chorionic gonadotropins on final growth rate of dominant follicle in Nelore cows

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Keywords: bovine, eCG, hCG.

Studies using beef cows showed that equine chorionic gonadotropin (eCG) at a dose of 400 IU at the time of intravaginal P4 device withdrawal was able to stimulate final follicular growth and increase size and steroidogenic capacity of the CL (Baruselli et al., *Anim. Reprod. Sci.*, v.82-83, p.479-86, 2004; Sá Filho et al., *Acta. Sci. Vet.*, v.32, p.235, 2004). Other studies have demonstrated that human chorionic gonadotropin (hCG) has an LH-like action (Bertan et al., *Bras. J. Vet. Res. An. Sci.*, v.43, p.379-86, 2006). However, there are no reports of the use of hCG as a stimulator of the final growth of the dominant follicle at FTAI protocols. The aim of this study was to evaluate the effect of hCG or eCG on the final growth of dominant follicle in Nelore cows submitted to FTAI. Ninety seven lactating cows with body condition score (BCS) of 2.9 (range 1-5) were used. At a random day of the estrous cycle (D0) cows received 2 mg estradiol benzoate (Sincrodiol®, Ourofino, Brazil) and a reused intravaginal progesterone device (CIDR®, Zoetis, Brazil). At D8, when the device was removed, cows received 0.5 mg cloprostenol (Estron®, Tecnopec, Brazil) and 1 mg estradiol cypionate (ECP®, Zoetis, Brazil). Subsequently, the cows were divided into the following treatments: Control (n=19), eCG 300IU IM (n=19), hCG 300IU IM (n=19), hCG 200IU IM (n=19) and hCG 200IU SC (n=21). At the same day and 2 days later, cows were subjected to ovarian ultrasonography to evaluate the diameter of the biggest follicle and to calculate follicular growth rate (D8 to D10). Statistical analysis was performed using PROC GLM of SAS® (9.2) and data are presented as least squares means \pm SE. The diameter of the dominant follicle on D8 only differed between hCG 200IU SC and hCG 200IU IM (11.6 \pm 0.7 vs 9.1 \pm 0.7 mm, P=0.03). This difference was also present at D10 (14.2 \pm 0.7 vs. 11.5 \pm 0.7 mm, P=0.03). In other treatments, the diameter of the dominant follicle at D10 was for Control: 12.9 \pm 0.6; eCG 300IU IM: 13.2 \pm 0.7 and hCG 300IU IM: 12.3 \pm 0.6 mm. There was no difference in follicle growth rate from D8 to D10 among groups (Control: 1.3 \pm 0.2; eCG 300IU IM: 1.7 \pm 0.2; hCG 300IU IM: 1.7 \pm 0.2; hCG 200IU IM: 1.2 \pm 0.2, and hCG 200IU SC: 1.3 \pm 0.1 mm/d, P>0.05). At the day of AI, the absence of the ovulatory follicle that was present at D8 was considered premature ovulation. The hCG 300IU IM group had a higher incidence of premature ovulation than most other treatments (Control: 5.2%^a [1/19]; eCG 300IU IM: 0.0%^a [0/19]; hCG 300IU IM: 42.1%^b [8/19]; hCG 200IU IM: 10.5%^a [2/19], and hCG 200IU SC: 14.2%^{ab} [3/21]; ^{a,b}P \leq 0.05). This study identified the threshold dose and route of administration of hCG that apparently does not cause ovulation in Nelore cows. This finding will allow evaluating the potential use of hCG for stimulation of the final follicle growth, as alternative to eCG treatment.

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A092 FTAI, FTET and AI

Evaluation of two types of prostaglandin (Dinoprost vs Cloprostenol) during an E2 and P4 based estrus synchronization protocol

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Keywords: progesterone, prostaglandin, TAI.

Lactating Holstein cows with progesterone (P4) concentrations $\leq 0,09$ ng/mL at artificial insemination (AI) have higher pregnancy rates per AI (P/AI) when submitted to a timed artificial insemination protocol (TAI) based on E2 and P4 (Pereira et al., Journal of Dairy Science. 96:2837–2846, 2013). The aim of this study was to evaluate if the type of prostaglandin (Dinoprost x Cloprostenol) affects P/AI in an E2/P4 based TAI protocol with two prostaglandins injections. Eight hundred and seventeen lactating Holstein cows producing 44.6 ± 10.8 kg milk per day were randomly assigned to the following TAI protocol: d-11 2mg im EB (Gonadiol®, MSD Animal Health, SP, Brazil) injection + 100µg im GnRH (Cystorelin®, Merial, SP, Brazil) injection + intravaginal device of P4 (CIDR®, 1.9g of P4, Zoetis, SP, Brazil), d-2 CIDR® removal + 1mg im ECP injection (ECP®, Zoetis, SP, Brazil), d0 TAI. At d-4 and d-2 animals were randomly assigned to receive 25mg im of Dinoprost (Lutalyse, Zoetis, SP, Brazil) or 0.5mg im of Cloprostenol (Ciosin, MSD Animal Health, SP, Brazil). At d0 and d10 blood samples were taken from cows for P4 concentration measurements. Pregnancy per AI (P/AI) was determined by ultrasound at d32 and d60. 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 $P < 0.1$. Progesterone concentrations at d0 (Cloprostenol = 0.14 ± 0.01 ng/mL; Dinoprost = 0.14 ± 0.02 ng/mL) and d10 (Cloprostenol = 2.90 ± 0.07 ng/mL; Dinoprost = 2.90 ± 0.08 ng/mL) did not differ between treatments. P/AI tended ($P = 0.08$) to be greater at d32 for Dinoprost treated cows (36.2% [409]) compared to Cloprostenol cows (31.6% [408]). Using only synchronized animals ($P4 < 0.2$ ng/mL at d0 and $P4 > 1$ ng/mL at d10), P/AI tended to be greater for Dinoprost treated compared to Cloprostenol treated cows ($P = 0.10$; 43.8% [288] vs 38.1% [291]). Treatment effects were not detected for P/AI at d60 using all animals (Dinoprost 30.3% [406]; Cloprostenol 27.9% [405]) or only synchronized animals (Dinoprost 35.7% [286]; Cloprostenol 33.7% [288]). In lactating Holstein cows submitted to a TAI protocol based on E2 and P4 with two prostaglandin injections at d-4 and d-2 of the protocol, the use of Dinoprost tended to increase the P/AI at d32 but not at d60.



A093 FTAI, FTET and AI

The effect of low vs. high serum concentrations of progesterone during early and late stages of follicular development on follicle size and double ovulations of lactating dairy cows

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Keywords: double ovulation, ovulatory follicle, progesterone.

The objective of this study was to determine the effect of high vs. low circulating concentrations of progesterone (P4) during early and late stages of ovulatory follicular wave development on ovulatory follicle size and double ovulation rate in lactating dairy cows. Lactating dairy cows were synchronized with prostaglandin F2 α (PG; 25 mg of dinoprost tromethamine, i.m.) then gonadotropin releasing hormone (GnRH; 100 μ g of gonadorelin; i.m.) 2 d apart to initiate a new cycle. Only cows that started a new cycle were used in this study. On d 7 of the estrous cycle, all cows received GnRH (200 μ g; i.m.), were blocked by parity and AI service number and then randomly assigned to four groups. Only animals that ovulated to this 2nd GnRH injection were used in the study. The day of this GnRH injection was considered d0 of the ovulatory follicular wave and treatment. PG and/or CIDRs were utilized to induce high (H) or low (L) circulating concentrations of P4 during either 0 to 4 d (early) and/or 5 to 7 d (late) of the ovulatory wave in a 2 x 2 randomized design, H/H, L/L, L/H and H/L. All cows (n=471) received PG 7 d after initiation of treatment. One d later, CIDRs were removed and a second PG was administered to ensure complete CL regression. GnRH was administered to all cows 56 h after the first of these two PGs to induce ovulation. Ovaries were evaluated by transrectal ultrasonography at time of GnRH administration and 2d later to determine size and number of ovulatory follicles. Variables were analyzed using the GLIMMIX procedure of SAS. There was an effect of treatment on mean diameter of single ovulating follicles ($P < 0.001$) and percentage of cows with double ovulations ($P < 0.001$). Mean diameter of single ovulating follicles was smaller for HH (16.8 \pm 0.2 mm) compared to HL, LH and LL treatments (18.2 \pm 0.3; 17.8 \pm 0.2; 19.6 \pm 0.3 mm, respectively; $P < 0.02$). HL and LH had smaller single ovulating follicles compared to LL ($P < 0.001$). Single ovulating follicles tended to be smaller in LH compared to HL ($P = 0.09$). Percentage of cows with double ovulations was greater in LL (51%) compared to HH, HL and LH (13, 34, and 32 %, respectively; $P < 0.01$). Cows in HH had lower rates of double ovulation compared to HL and LH ($P < 0.001$). An interaction between parity and service number for double ovulation rate was significant ($P < 0.02$) and determined that primiparous first service cows had lower double ovulation rates compared to primiparous later service cows and multiparous first and later service cows (14 vs. 36, 35, and 40 %, respectively; $P < 0.001$). In summary, these data indicate that serum concentrations of P4 during specific stages of the follicular wave directly affect size and number of ovulating follicles.



A094 FTAI, FTET and AI

Pregnancy rates of embryo recipient cows synchronized with progesterone, estrogen, equine chorionic gonadotropin and prostaglandin F2 α

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Keywords: bovine, IVF, embryo transfer.

The present study had the objective to evaluate the pregnancy rates of recipient cows transferred with in vitro produced embryos according to the corpus luteum quality and embryo development stage. Girolando (n = 15) and Nelore (n = 7) embryo donors were enrolled in a OPU process. Cyclic crossed embryo recipient heifers (n = 630), BCS ≥ 3 (1 – 5 scale) were synchronized with an intravaginal device of progesterone (Sincrogest®, Ourofino, Brazil) and 2 mg of estradiol benzoate i.m. (Sincrodiol®, Ourofino, Brazil) on D0. On D5, 400 μ g of cloprostenol sodium (Sincrocio®, Ourofino, Brasil) and 400 IU of eCG (Folligon®, Intervet, Brazil) were i.m. administered. On D8, intravaginal devices were removed and on D9, 1 mg of estradiol benzoate was i.m. administered, with the transfer of the embryos occurring on D16 (Girolando and Nelore in similar conditions). Recipient corpus luteum (CL) quality was evaluated by rectal palpation at the time of embryo transfer, scoring the diameter as 1, 2 or 3 (1 – small; 3 – large). Embryos were classified as initial blastocyst (Bi), blastocyst (B1), expanded blastocyst (Bx), hatching blastocyst (Bn) and hatched blastocyst (Be). Statistical analysis considered the nonparametric binomial test of two proportions, with 5% of significance in order to determine the occurrence of differences in pregnancy rates. To determine differences of pregnancy rates among different stages of embryonic development and among the different qualities of CL, the multiple comparison test for proportions was used, with 5% significance. Pregnancy rate at 30 days after ET was similar when using Nelore (59.63%, 161/270) or Girolando (51.94%, 187/360) embryos (P>0.05). In contrast, pregnancy rate at 60 days was greater for Nelore (57.04%, P<0.05) than for Girolando (48.06, 173/360) embryos. Pregnancy loss at 60 days was 7.49% (14/187) with Girolando embryos and 4.35% (7/161) with Nelore. Pregnancy rate was not different across embryo developmental stage and were Bx (55.67%, 108/194) vs. Bi (51.92%, 27/52) vs. B1 (51.09%, 47/92) following transfer of Girolando embryos and B1 (64.29%, 45/70) vs. Bx (63.64%, 84/132) vs. Bi (55.17%, 32/58) for Nelore embryos. The pregnancy rate according the CL classification was not different (P>0.05) for CL1, CL2 and CL3, as follows: CL1 = 50.9% (82/161), CL2 = 52.4% (44/84) and CL3 = 53.0% (61/115) for Girolando embryos and CL1 = 60.0% (45/75), CL2 = 56.3% (49/87) and CL3 = 62.0 (67/108) for Nelore embryos. In conclusion, pregnancy results at 60 days were influenced by embryo donor breed. However, pregnancy was independent from embryo developmental stage and CL quality based on its classification by rectal palpation.



A095 FTAI, FTET and AI

The effect of sire on circulating concentrations of bovine pregnancy associated glycoproteins (bPAGs) in beef cows

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Keywords: FTAI, pregnancy loss, pregnancy rate.

Bovine PAGs are placental proteins that are secreted by binucleated trophoblast cells starting around days 22-24 of gestation and have been used to diagnose pregnancy in cattle. Recently, circulating concentrations of bPAGs on day 28 of gestation have been reported to be a placental marker for predicting embryonic mortality in beef cows (Pohler et al., J Anim Sci. 91:4158-67, 2013). More specifically, cows that establish and maintain a pregnancy until day 72 of gestation resulted in higher circulating concentrations of bPAGs on day 28 compared to cows that had a viable embryo on day 28, but subsequently experienced embryonic mortality by day 72. The biological function of bPAGs and the factors affecting bPAG secretion are not known. Therefore, the objective of this study was to examine the effect of sire of the embryo on circulating concentrations of bPAGs during early pregnancy. In this experiment, 720 Nelore beef cows were inseminated to one of 8 sires at FTAI. All cows were synchronized with the following protocol: 2.0 mg i.m. of estradiol benzoate and insertion of CIDR on D0, 12.5 mg i.m. of dinoprost tromethamine (Lutalyse®, Zoetis) on D7, on D9 the CIDR was removed and cows received 0.5 mg i.m. of ECP and 300 IU of eCG. The TAI was performed on D11, 48 h after CIDR withdrawal. Pregnancy diagnosis was conducted on day 28 of gestation and a blood sample collected for bPAG analysis. Data were analyzed using GLIMMIX procedure from SAS. Although there was variation in sire conception rate to FTAI (44 to 64%), there was no linear relationship between sire pregnancy rate and circulating concentrations of bPAGs. However, there were significant differences in circulating concentrations of bPAGs among sires. There were 39 cows that established a pregnancy to FTAI and had a viable embryo on day 28 of gestation; but failed to maintain pregnancy to day 100 of gestation. These cows had lower bPAGs concentration (6.4 ± 0.9 ng/mL vs. 15.4 ± 0.5 ng/mL) compared to cows that pregnancy was maintained until day 72. Three sires, in this experiment, accounted for over 70% of the late embryonic mortality. After removing all cows that lost a pregnancy after day 28, the preceding three sires with the highest incidence of late embryonic mortality resulted in pregnancies that produced significantly ($P < 0.05$) lower (10.8 ± 0.4 ng/mL) maternal circulating concentrations of bPAGs on day 28 of gestation compared to the five remaining sires with low embryonic mortality (14.8 ± 0.3 ng/mL). In summary, decreased concentration of bPAGs on day 28 of gestation can be due to the sire used for FTAI.



A096 FTAI, FTET and AI

Panoptic staining for counting of embryonic cells

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Keywords: embryo, cellular staining, panoptic.

The embryonic cell counting is a method to assess embryo quality, especially for those produced in vitro. The trophoblast cells are responsible for secretion of interferons, which signal the pregnancy, being their measurement of major importance. The choice of the staining for embryo evaluation depends on the cell type to be evaluated, and the availability of equipment for evaluation. Panoptic staining enable to quantify embryonic cells using simple equipment such as stereoscopic microscope, and glass slides can be read several days after staining. The objective of this study was to evaluate the methodology proposed by Costa et al (Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v.62, n.6, p.1507-1510, 2010), for in vitro produced bovine embryos. Oocytes from slaughterhouse were matured, fertilized and cultured according to the IVP methodology described by Vajta. (Theriogenology, v.45, p.683-689, 2006). After seven days of culture, the embryos were classified as expanded blastocyst (BX) were fixed in individual glass slides. Initially, the embryos were transferred and maintained for 5 minutes in a 1:1 solution of sodium citrate 0.88% diluted in distilled water at room temperature. After this time, embryos were transferred and maintained for 30 seconds in a fixing solution composed of methanol: acetic acid: distilled water (3:2:1) at 5°C. The embryo was then transferred to the slide with minimum amount of solution in order to fasten and adhere the embryo on it. Then the slide was stained with fast Panoptic kit (Instant Prov®) during 5 seconds in each staining. Subsequently, the slide was washed in distilled water and air dried. Finally, the counting of cells was conducted under 40 x stereoscopic microscope or conventional microscope. This technique allows counting the cells in a simple and rapid method when compared to other techniques using some stainings such as Hoechst 33342 and Giemsa, which require fluorescence, inverted or phase contrast microscopy.



A097 FTAI, FTET and AI

Fertility of Holstein cows submitted to GnRH- or estradiol-based FTAI protocols

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Keywords: *Bos taurus*, hormone, synchronization.

The objective of this study was to compare the reproductive efficiency of high-producing dairy cows submitted to four protocols based on GnRH and/or estradiol for fixed time artificial insemination (FTAI). A total of 611 lactating Holstein cows was used, 239 primiparous and 372 multiparous, with BCS 3.1 ± 0.02 , 155.6 ± 4.20 DIM and 37.1 ± 0.36 kg/d of milk (least squares means \pm SE). Cows were kept in free stall barns, fed a TMR diet and had free access to water and mineral salt. In a completely randomized design with a 2x2 factorial arrangement, the cows were subjected to two treatments at the beginning of the FTAI protocol (GnRH; Fertagyl®, MSD or EB; Gonadiol®, MSD) and two treatments at the end of the protocol (EB; Gonadiol® or ECP MSD®; Zoetis), resulting in four treatments: GB (n=149), GC (n=170), BB (n=150) and BC (n=142). At the beginning of the protocol (D0) cows received 100 µg GnRH or 2 mg estradiol benzoate (EB) and an intravaginal P4 device (CIDR®, Zoetis), which remained for 8 d. Cows received two 500 µg injections of PGF2α (Sincrosin®, Vallée) on D7 and D8. At the time of intravaginal device removal (D8) cows received 1.0 mg estradiol cypionate (ECP) or at the next day (D9) they were treated with 1.0 mg EB. The FTAI was 48 or 24 h after administration of ECP or EB, respectively. Statistical analysis was performed using Proc GLIMMIX of SAS 9.3. There was no interaction between treatments on conception at 30 and 60 d ($P > 0.05$), nor effect of EB or GnRH at the beginning of the protocol at 30 d (35.4% and 38.0%, respectively; $P = 0.52$). However, there was effect of treatment at the end of protocol (31.7% and 42.0% for EB and ECP, respectively; $P < 0.01$). At 60 d after FTAI, there were no treatment effects at the beginning (31.9% and 31.5% for EB and GnRH, respectively; $P = 0.93$) nor at the end of the protocol (28.6% and 35.0% for EB and ECP, respectively; $P = 0.11$). In relation to embryonic/fetal loss between 30 and 60 d of conception, there was no effect of treatments at the beginning ($P = 0.53$) or at the end of the protocols ($P = 0.17$). However, there was a tendency ($P = 0.06$) of interaction between treatments at the beginning and at the end of the protocols (11.3%, 9.0%, 6.5% and 24.7% for BB, BC, GB and GC, respectively). Regardless of treatments, parity affected conception at d 30 (43.1% and 30.7%; $P < 0.01$), d 60 (40.7% and 23.9%; $P < 0.0001$) and influenced embryonic/fetal loss (7.4% and 17.4% for primiparous and multiparous, respectively; $P = 0.03$). We concluded that in high producing lactating Holstein cows, the use of ECP instead of EB at the end of the protocol provided greater conception at d 30 after FTAI, but not at d 60 due to increased embryonic/fetal loss between d 30 and 60 in GC cows. Moreover, regardless of the type of treatment, primiparous cows had higher conception rates at d 30 and 60 and lower embryonic/fetal losses than multiparous cows.

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A098 FTAI, FTET and AI

Resynchronization in dairy cows 13 days after TAI followed by pregnancy diagnosis based on corpus luteum vascularization by color doppler

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Keywords: estradiol benzoate, lactating cow, resynchronization.

In the present study 2 experiments were performed aiming to evaluate the reproductive efficiency of dairy cows undergoing resynchronization 13 d (Day 13) after TAI (Exp 1) and the effect of the resynchronization on the synchronization of the new follicular wave emergence and on the CL activity (Exp 2). The Exp 1 enrolled 183 lactating cows (25.2±8.8 L milk/d) distributed into two groups: control (CON, n=93) and resynchronization (RES, n=90). On a random day of the estrous cycle, all animals were submitted to the same TAI protocol [2mg of estradiol benzoate (EB)+progesterone device (P4)-8d (device removal+1mg estradiol cypionate (EC)+0.150mg of cloprostenol (PGF2 α)-TAI 48 h later]. The females of the CON group were inseminated after detection of estrus between Days 18 and 25 after TAI. The cows of the RES group were resynchronized with the insertion of a P4 device (Primer®, Agener-Tecnopec) and 1.5mg of EB (RIC-BE®, Agener-Tecnopec) on Day 13. On Day 21 the P4 devices were removed. At the same time, the females from both experimental groups underwent a color Doppler ultrasonography in order to assess the vascularization of the CL. All animals of the RES group classified as non-pregnant based on the CL vascularization (CL area <3cm², peripheral vascularization \leq 25% and central with reduced signals) or absence of CL received 1mg of EC (ECP®, Zoetis), 0.150 mg PGF2 α (Estron®, Agener-Tecnopec) and were inseminated 48 h later. The pregnancy diagnosis of the 1st TAI was performed on Day 30 in all females. Statistical analysis was performed using the GLIMMIX procedure of SAS®. The vascularized CL rate on Day 21 (P=0.003) and pregnancy rate (PR) on Day 30 after the 1st TAI (P=0.02) were lower in the RES group (40.0%, 36/90 and 14.9%, 13/87) compared to the CON group (62.4%, 58/93 and 32.6%, 28/86; respectively). However, there was no difference (P=0.98) in the PR after the 2nd AI between the CON (25.0%, 3/12) and RES group (22.6%, 12/53). In Exp 2, 17 lactating cows were submitted to the same treatments described in Exp 1 (CON, n=9 and RES, n=8). All cows were submitted to ultrasound exams every 24 h and evaluated by Doppler every 48 h from Day 13 to 21. Statistical analysis was performed using the MIXED procedure of SAS. An interaction (P=0.07) between treatment and time was observed for the vascularization rate of the CLs. Although a new follicular wave emergence was induced 3.2 \pm 1.4 d after EB treatment (RES group) and similar vascularization rate of the CLs was observed on Day 13 (CON: 87.8 and RES: 84.4%) and Day 15 (CON: 90.6 and RES: 86.9%); the vascularization of the CLs from animals of the RES group was reduced from Day 17 onwards (Day 17: 71.9, Day 19: 51.9 and Day 21: 40.6%) compared to the CON group (Day 17: 89.4, Day 19: 88.9 and Day 21: 76.1%). In conclusion, the resynchronization using 1.5 mg EB 13 d after the 1st TAI reduces the vascularization of the CL and consequently, the pregnancy rate to the 1st TAI in dairy cows.



A099 FTAI, FTET and AI

The "bull effect": comparative results between the reproductive performance of semen batches at IVP and timed-AI

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Keywords: bovine, fertility, semen.

It has been reported that sperm cells of different bulls may differ in their ability to fertilize oocytes and/or to develop until blastocyst stage. Likewise, different conception rates (CR) can be obtained according to the semen utilized, evidencing the "bull effect". However, few studies have utilized the same semen batches for *in vitro* embryo production (IVP) and Timed-AI (TAI). Hence, this study aimed to compare the reproductive performance of seminal batches used in these both reproductive programs. Frozen semen doses of nine Angus bulls and a heterospermic Angus bull semen were utilized. One batch of each bull was evaluated. To assess field fertility, the 10 batches were randomly assigned to insemination of 1024 multiparous Nelore cows, which were submitted to the same TAI protocol. The CR was assessed by ultrasonography 45 days after AI. To assess *in vitro* fertility, the same semen batches used in the field (TAI) were used for IVP. In order to perform the IVPs, only grade I oocytes obtained from slaughterhouse ovaries were matured and fertilized with Percoll-selected semen with a final concentration adjusted to 1×10^6 sperm/mL. Sixty oocytes were fertilized for each semen dose. The presumptive zygotes were cultured until day 7, when blastocyst rates were evaluated. Three replicates of IVP were performed for each semen batch. Statistical differences for CR at TAI between bulls were analyzed by logistic regression (GLIMMIX). The IVP data were analyzed by ANOVA and Tukey test. Significance level was 5 %. Overall CR at TAI was 48.1% (n = 1024) and IVP outcome was 36.6% (n = 30). Regarding to field fertility (TAI) results, semen from bull 2 (40.0%; n = 120) presented lower ($P < 0.05$) CR when compared to bulls 3 (53.6%; n = 84), 7 (54.2%; n = 83) and the heterospermic semen (58.4%; n = 101). Additionally, the heterospermic semen also presented higher ($P < 0.05$) CR than bulls 6 (40.9%; n = 93) and 8 (44.3%; n = 140). The bulls 1 (48.1%; n = 104), 4 (46.6%; n = 131), 5 (51.3%; n = 80) and 9 (50.0%; n = 88) presented similar field fertility results ($P > 0.05$) compared to the bulls mentioned above. On the other hand, at IVP, bulls 1 ($23.7 \pm 0.06\%$) and 2 ($23.9 \pm 0.03\%$) demonstrated lower ($P < 0.05$) blastocyst rates compared to bulls 8 ($42.2 \pm 0.02\%$), 9 ($44.3 \pm 0.12\%$) and heterospermic semen ($50.0 \pm 0.05\%$). However, for bulls 3 ($31.2 \pm 0.11\%$), 4 ($35.6 \pm 0.13\%$), 5 ($35.0 \pm 0.08\%$), 6 ($39.4 \pm 0.18\%$) and 7 ($42.2 \pm 0.14\%$) no differences ($P > 0.05$) were observed for *in vitro* fertility compared to the animals mentioned above. It was concluded that only the heterospermic semen presented higher reproductive performance for both *in vivo* and *in vitro* fertility outcomes. Additionally, it was observed that bulls which demonstrated better blastocyst rates at IVP not necessarily presented better CR at TAI. Therefore, our efforts remain focused on understanding the underlying physiology involved in the "bull effect" as well as in sperm characteristics most closely correlated with sire fertility.



A100 FTAI, FTET and AI

The use of color doppler ultrasonography as a method of pregnancy diagnosis 22 days after FTAI in Nelore beef cows

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Keywords: doppler, early pregnancy diagnosis, beef cows.

The aim of the present study was to evaluate the accuracy of verifying pregnancy status on day 22 post-TAI by detection of luteolysis through color Doppler ultrasonography in *Bos indicus* beef cows. On a random day of the estrous cycle, 434 females were submitted to the same TAI protocol: 2mg of estradiol benzoate (EB) and the insertion of a progesterone device (P4). On day 8, the P4 device was removed and cows received 1mg of estradiol cypionate (EC) plus 0.530mg of cloprostenol sodium (PGF2 α). The TAI was performed 48 h after PGF2 α . Twenty two days later, the presence of the CL as well as CL size and blood flow in all females was evaluated by color Doppler ultrasonography (Doppler-US; Mindray, M5Vet). The females were classified as non-pregnant based on the detection of luteolysis characterized by the CL area < 2cm², peripheral vascularization \leq 25%, reduced central vascularization, and by the absence of CL. Transrectal ultrasonography (US) diagnosis was performed on Day 30 and considered the gold standard pregnancy diagnosis method in beef cows. The diagnosis methods were evaluated considering all the females (ALL; n=434) or only those cows with a CL (CL; n=320) on Day 22 and, therefore, the accuracy of pregnancy diagnosis (Doppler-US or US) was assessed within these two groups. The Doppler-US 22 post-TAI resulted in similar sensitivity [positive / (positive + false negative)] when performed only in females with CL (99.4%) or in all females (99.4%); however, lower specificity [non-pregnant / (false positive + non-pregnant)] was observed when only cows with CL were considered (75.9% vs. 85.9%). Additionally, greater true positive values [positive / (positive + false positive)] were observed when only females with CL were evaluated [With CL: 49.1% (157/320) vs. ALL: 36.2% (157/434)]; whereas, we also had greater true negatives [negative/ (negative + false negative); With CL = 49.1% (157/320) vs. ALL = 36.2% (157/434)] and false positives [false positive/ (positive + false positive); With CL: 9% (39/320) vs. ALL: 12.2% (39/434)]. Finally, similar false negative values were observed between females with CL [0.3% (1/320)] or females overall [0.2% (1/434)], as well as, equivalent accuracy of the diagnosis method (With CL: 87.5% vs. ALL: 90.8%). In conclusion, high detection of luteolysis by Doppler imaging on day 22 post-TAI can be feasible and used for earlier detection of non-pregnant cows in reproductive programs.



A101 FTAI, FTET and AI

Different hormonal stimulation protocols can affect the number of ovulations, volume of corpora lutea and progesterone production in sows?

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Keywords: swine, FTAI, corpus luteum.

The use of fixed-time artificial insemination (FTAI) in swine aims to minimize errors and labor associated with estrus detection, to reduce the variation of estrus and ovulation interval (KNOX, *Theriogenology*, v.75, p.308-19, 2011), possibly decreasing the number of doses of semen used per female in heat, consequently reducing production costs. However, there are evidences that ovarian stimulation with exogenous hormones can be detrimental to reproductive function in sows. As an example of that, concentration of progesterone (P4) is lower in the uterus of gilts stimulated with gonadotropins on day 12 of pregnancy (BLITEK, *Dom. Anim. Endocrinol.*, vol.38, p.222-34, 2010) may be due to low capacity of the corpus luteum (CL) to produce P4 in treated animals. Most of the FTAI protocols (with the interval 72-80 hours between the eCG and GnRH) has insufficient success to synchronize estrus in order to use frozen semen (HÜHN, *Theriogenology*, v.46, p.911-24, 1996). Therefore, reducing the interval to 56 hours is an alternative to stimulate lower amplitudes of ovulations (CANDINI, *Braz. J. Vet. Res. Anim. Sci.*, v.41, p.124-30, 2004). The aim of this study was to evaluate the effect of different FTAI protocols regarding the number of ovulations, volume of CL and P4 production. Thirty-eight sows were randomly assigned into groups: control, eCG (eCG im 600IU at weaning), GnRH56h (600IU eCG IM at weaning, 50 µg GnRH IM 56h after eCG) and GnRH80h (600IU eCG IM at weaning, 50 µg GnRH IM 80h after eCG). At day 7.5 after the beginning of heat or application of GnRH, animals were euthanised and blood and ovaries samples were collected. Serum progesterone levels were measured using radioimmunoassay. The number of ovulations were counted and two CLs from each ovary were collected and their volume were estimated (calculated by $V = 4/3 \pi \text{radius}^3$). The obtained value were multiplied by the total number of CLs present in each female. Data were analyzed by One-way ANOVA and Tukey test. No significant differences were observed between experimental groups. The results are shown as mean \pm SE. The number of ovulations were 23.9 ± 1.3 (control); 22.2 ± 1.1 (eCG); 25.8 ± 1.4 (GnRH56h) and 23.9 ± 1.0 (GnRH80h) ($P = 0.25$). The total volume of CL were $12.1 \pm 1.7 \text{ cm}^3$ (control); $12.0 \pm 0.8 \text{ cm}^3$ (eCG); $13.1 \pm 1.6 \text{ cm}^3$ (GnRH56h) and $14.8 \pm 1.7 \text{ cm}^3$ (GnRH80h) ($P = 0.52$). The P4 concentrations were $25.1 \pm 2.2 \text{ ng/mL}$ (control); $26.9 \pm 2.5 \text{ ng/mL}$ (eCG); $25.2 \pm 2.8 \text{ ng/mL}$ (GnRH56h) and $24.5 \pm 2.8 \text{ ng/mL}$ (GnRH80h) ($P = 0.93$). The P4 production per cm^3 of CL was $2.3 \pm 0.2 \text{ ng/mL}$ (control); $2.3 \pm 0.2 \text{ ng/mL}$ (eCG); $2.1 \pm 0.3 \text{ ng/mL}$ (GnRH56h) and $1.9 \pm 0.3 \text{ ng/mL}$ (GnRH80h) ($P = 0.63$). These data show that hormonal stimulation has no effect on ovulation rate, volume of CL and P4 production. Moreover, the reduction in the interval between the application of eCG and GnRH from 80 to 56 hours does not affect the number of ovulations neither the functionality of CL.



A102 FTAI, FTET and AI

Expression of estrus increases fertility and decreases pregnancy loss in timed-AI or timed-ET protocols

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Keywords: estrus, TAI, TET.

The objective was to evaluate if expression of estrus in dairy cattle altered fertility in timed AI (TAI) or timed ET (TET) protocols that used estradiol and progesterone (E2/P4) to synchronize ovulation. A retrospective analysis was performed of past studies in which lactating dairy cows received the following protocol: 2mg Estradiol Benzoate (d-11 Estrogin/Farmavet/SP/Brazil) +intravaginal P4 device (1.9g CIDR®/Zoetis/SP/Brazil) 7d later 25mg of PGF (d-4 Lutalyse/Zoetis/SP/Brazil) 2d later CIDR removal+1mg of ECP (d-2 ECP/Zoetis/SP/Brazil). Two days after CIDR removal, cows received TAI (d0; n=5,430) or on d7 TET (n=2,003). Binomial variables were analyzed using PROC GLIMMIX. Ovarian ultrasonographies (US) were performed on d0 and d7 to determine ovulatory follicle diameter. Only cows with a visible CL on d7 were used in this study. At the time of CIDR removal, all cows received tail-chalk and cows with complete removal of tail chalk by d0 were considered to have expressed estrus. Circulating P4 concentrations were evaluated on d7. Pregnancies per AI (P/AI) or ET (P/ET) were determined by US on d32 and d60. At d32 pregnancy diagnosis (PD), cows with expression of estrus had increased (P<0.01) P/AI (no estrus=25.5[846] vs. estrus=38.9[4,584]) and P/ET (no estrus=32.7[606] vs. estrus=46.2[1,397]). Similarly, at 60d PD, expression of estrus increased (P<0.01) P/AI (no estrus=20.1[846] vs. estrus=33.3[4,584]) and P/ET (no estrus=25.1[606] vs. estrus=37.5[1,397]). In addition, pregnancy loss was lower in cows that expressed estrus (P=0.01) on TAI (no estrus=20.1[222] vs. estrus=14.4[1,785]) or TET (no estrus=22.7[193] vs. estrus=18.6[645]). There was a positive effect of P4 concentrations at d7 on P/AI in cows that either expressed (P=0.01) or did not express (P=0.02) estrus. In contrast, there was no effect of P4 at d7 on P/ET (no estrus P=0.76; estrus P=0.52). Independent of expression of estrus (P<0.01), cows ovulating either too small (<11 mm) or too large (>17 mm) of follicles had lower P/AI. There was no effect of ovulatory follicle diameter on P/ET in cows that expressed estrus (P=0.34); although, cows that did not express estrus had lower P/ET if they ovulated larger follicles (P=0.05). In cows that showed estrus, follicle diameter did not affect pregnancy loss after TAI (P=0.43) or TET (P=0.34), but in cows that showed no estrus, cows that ovulated larger follicles had greater pregnancy loss after TAI (P=0.04) or TET (P=0.04). Expression of estrus during protocols for TAI or TET is associated with an increase in fertility. In TAI programs, optimizing follicle diameter, increasing circulating P4 on d7 after AI and expression of estrus were associated with increased fertility. However in cows with TET, the association of fertility with either ovulatory follicle diameter or P4 on d7 was less dramatic and seemed to be related to whether cows expressed estrus.



A103 FTAI, FTET and AI

Serum concentrations of progesterone in cows treated with injectable progesterone

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Keywords: *Bos taurus*, hormone, synchronization,

The aim of the study was to evaluate the profile of circulating progesterone (P4) induced by treatment with injectable P4 in cows. Two products were used: Afisterone® (1 g/100 mL, Hertape Calier, Brazil) and Progestar® (2.5 g/100 mL, Syntex, Argentina). Non-lactating and non-pregnant Holstein cows (n = 20) were remained for 7 d with an intravaginal P4 device (Sincrogest®, Ourofino, Brazil). After withdrawal (D-8), a norgestomet ear implant (Crestar®, MSD, Brazil) was inserted and maintained for 8 d. At D-8 and D-7, 0.526 mg of PGF2 α (Sincrocio®, Ourofino, Brazil) was applied. At D0, cows were randomized to receive Afisterone (50 mg, n = 10) or Progestar (50 mg, n = 10). From the time of P4 injection, blood samples were taken at 0, 2, 6, 12, 24, 48, 72, 96, 120, 144, 168 and 192 h to verify the profile of serum P4 by enzyme immunoassay (ELISA, Enzyme-Linked immunosorbent Assay, adapted Rasmussen et al., J. Dairy Sci, v.79, p.227-234, 1996). Statistical analysis was performed using Proc-Mixed of SAS (9.2; repeated measures), accepting significance at P < 0.05. Data are shown as least squares means \pm SEM. There was no effect of treatment, but there was effect of time. At 2 h, Progestar treatment reached the highest concentration of P4 (1.21 \pm 0.18 ng/mL), while for Afisterone, the peak occurred at 6 h (0.95 \pm 0.16 ng/mL). The average circulating P4 concentration over all periods after treatments were 0.41 \pm 0.04 and 0.44 \pm 0.04 ng/mL for Afisterone and Progestar, respectively. At other periods, circulating P4, regardless of treatment, was: 12 h (0.70 \pm 0.13 ng/mL), 24 h (0.43 \pm 0.08 ng/mL), 48 h (0.30 \pm 0.09 ng/mL), 72 h (0.20 \pm 0.05 ng/mL), 96 h (0.20 \pm 0.03 ng/mL), 120 h (0.20 \pm 0.04 ng/mL), 144 h (0.18 \pm 0.03 ng/mL), 168 h (0.19 \pm 0.04 ng/mL) and 192 h (0.20 \pm 0.08 ng/mL). Due to the low serum concentrations of P4 obtained by using 50 mg of injectable P4, independent of the commercial product used, it is suggested that this dose routinely recommended in protocols for synchronization of wave emergence is insufficient to aid regression (turnover) of follicles at the beginning of the protocols, in combination with estradiol esters.

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A104 FTAI, FTET and AI

Efficiency of a single use progesterone intravaginal device for cattle

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Keywords: hormones, reproductive performance, TAI.

Hormonal protocols used in FTAI programs involve considerable financial figures, in which progesterone (P4) devices represent 43% of the total cost. P4 releasing from vaginal implants happen by passive diffusion, i.e., the drug release is driven by a gradient concentration (Rothen-Weinhold et al. 2000, J. Dairy Sci., v.83, p.2771–2778). Given the importance of P4 in the protocols and the importance of this steroid in the treatment costs, studies described the reutilization of P4 vaginal implants as an alternative to make this technology more viable (Almeida et al. 2006, Braz. J. Vet. Res. Anim. Sci., v.43, p.456-465). However, the results of reutilization are controversial. Reducing the amount of active substance in the implants in so called Single Use Devices is an alternative to reduce costs and prevent reutilization, which in addition to technical problems can represent a biosecurity risk for the herd. The objective was to verify the efficacy of a single use implant with 0.55g of P4, measuring serum P4 concentrations by radioimmunoassay in bovine females with absence of this natural hormone. We used 14 crossbred females, 7 heifers with body weight from 320 to 365kg and 7 cows weighing between 455 and 612kg. To avoid the effects of endogenous P4 results, females underwent a protocol using norgestomet (Crestar™, MSD), which prevents further ovulation and does not interfere with P4 measurement. Blood samples were initially collected immediately before insertion of the device. The second sample was taken at 12 hours followed by seven other daily samples, in an interval of 24 hours. Twelve hours after removal of the device, another sample was collected, totaling 10 samples per animal. The hormone assays were performed with radioimmunoassay (Imunotech™). The concentrations of P4 on different days were compared using the Tukey test at 5% probability. All animals were with baseline levels of P4 before inserting the device. The plasma P4 concentration increased rapidly after implant placement, being significantly ($P < 0.05$) greater at 12 hours after device insertion. Mean progesterone concentrations remained above 1.7 ng/mL throughout the device insertion period, level which suppresses ovulation and manifestation of estrus. Mean P4 concentrations were: 0.35 ± 0.13^d ; 2.99 ± 1.51^{ab} ; 3.27 ± 1.32^a ; 3.48 ± 1.09^a ; 2.49 ± 0.99^{abc} ; 2.42 ± 0.95^{abc} ; 2.04 ± 0.75^{bc} ; 1.73 ± 0.61^c ; 1.97 ± 0.69^{bc} and 0.56 ± 0.24^d (sequentially for 10 periods of blood sampling). The profile during the 8 days shows a rapid increase after insertion (D0 to D1) and fast decline after removal of the device. It appears to produce an ideal P4 profile, since the purpose of P4 devices for TAI programs is standardize follicular development and block ovulation. It was concluded that the studied device is effective in maintaining circulating levels of P4 over a period of use of up to 8 days.

Suport: Agener União, Biotran and Fapemig.



A105 FTAI, FTET and AI

Effect of body condition score on diameter of the dominant follicle, estrous behaviour, ovulation rate and fertility in Nelore cows submitted to timed artificial insemination

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Keywords: animal reproduction, *Bos indicus*, nutritional status.

This study aimed to evaluate the effect of body condition score (BCS) on the diameter of the dominant follicle, estrous behaviour, ovulation rate and fertility of *Bos indicus* cows submitted to fixed-time artificial insemination (TAI). Data were collected from different farms in Mato Grosso, São Paulo and Minas Gerais states in Brazil. To evaluate the BCS, a 5-point scales was used and the procedure performed by a single technician. The TAI protocol used in this study consisted of administration on day 0 (D0) of an intravaginal device containing 1 g of P4 (Sincrogest®, Ouro Fino Agronegócio, São Paulo, Brazil) and 2 mg of estradiol benzoate (Sincrodiol®, Ouro Fino). On day 8 (D8), the P4-device was removed and the animals were treated with 1 mg of estradiol cypionate (ECP®, Pfizer, Brazil) and 500 µg of PGF2α (sodium cloprostenol; Sincrocio®, Ouro Fino). The insemination was performed 48 hours after the P4-device removal (D10). Ultrasonographic examinations were performed (Chison 600 VET, 5 MHz linear transducer, China) at every 12 h from P4-device removal to ovulation to evaluate timing ovulation (n=168), on D8 and D10 ultrasound exams were used to calculate follicle growth (n=989) and pregnancy diagnosis occurred on D40 (n=878). Also on D8, a subgroup of animals (n=414) had their tail-head marked with chalk paint to evaluate estrus following the synchronization protocol. Estrus was assumed to have occurred in cows without a tail-head chalk mark at TAI. Statistical analysis was performed using the GLIMMIX procedure of SAS. The pregnancy rate was analyzed by logistic regression for binomially distributed data. The evaluation of the follicular diameter at the time of P4 removal, the maximum diameter of the dominant follicle (P = 0.002) and the ovulation rate (P = 0.04) were less in cows with BCS ≤ 2.5 [7.4 ± 0.4; 9.7 ± 0.8 and 57.1 % (40/70)] than in cows with BCS ≥ 3.0 [9.4 ± 0.3; 12.7 ± 0.5 and 72.4 % (71/98)]. In addition, the diameters of the dominant follicle on D8 (P = 0.0001) and D10 (P = 0.0006) were less in cows with BCS ≤ 2.5 (9.7 ± 0.2 and 11.3 ± 0.3) than in cows with BCS ≥ 3.0 (10.7 ± 0.1 and 12.3 ± 0.2). Furthermore, cows with BCS ≤ 2.5 had lower estrus expression (47.6 %; 50/105 vs. 62.1 %; 192/309; P = 0.01) as well as conception (27.1 %; 76/280 vs. 39.4%; 236/598; P = 0.0005) than cows with BCS ≥ 3.0. In conclusion, cows with BCS ≤ 2.5 had lower estrus expression and poorer fertility following the FTAI protocol likely due to lower follicle growth and ovulation rates.



A106 FTAI, FTET and AI

Effect of oxytocin administration at the time of TAI in buffaloes submitted to synchronization of ovulation during the non breeding season

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Keywords: ovulation, oxytocin, pregnancy.

The effect of oxytocin administration was evaluated in buffaloes submitted to ovulation synchronization and fixed-time artificial insemination (TAI) during the non-breeding season. It was hypothesized that oxytocin administration at the time of TAI increases pregnancy rate. At a random day of the estrous cycle (D0), 249 buffaloes received an intravaginal progesterone device (750mg P4; Proclinar®, Hertape Calier Saúde Animal S.A., Brazil) and 2mg im of estradiol benzoate (EB, Benzoato HC®, Hertape Calier Saúde Animal S.A., Brazil). All animals were subjected to ultrasonographic evaluations (Mindray DP2200Vet, China) performed on D0 to verify ovarian activity and D9 to measure the follicular diameter (Ø). At this time, females that lost the P4 device were removed from the experiment (n = 24). The other buffaloes received 150µg im of D+cloprostenol (Veteglan® Luteolítico, Hertape Calier Saúde Animal S.A., Brazil) and 30IU of FSH/LH (Pluset®, Hertape Calier Saúde Animal S.A., Brazil), followed by P4 device removal. On D10, buffaloes received 1mg im of EB (Benzoato HC®, Hertape Calier Saúde Animal S.A., Brazil). On D11, all animals were again subjected to ultrasonographic evaluations and those with follicles ≤ 9.5mm were removed from the experiment (n = 33). The other buffaloes were distributed according to age, number of calvings, body condition score, ovarian activity and the largest follicle diameter in one of two groups (Control Group - GCon, n=96 and Oxytocin Group - GOci, n=96) and a new ultrasonographic evaluation was performed 64h after the P4 device removal (D12). All buffaloes were submitted to TAI on D11. Simultaneously to AI, animals in GOci received 50IU of Oxytocin (Ocitocina HC®, Hertape Calier Saúde Animal S.A., Brazil). In a subgroup of animals (GCon, n=13 and GOci, n=14), we evaluated the ovaries by ultrasound every 12h from D11 to D14 to determine the time of ovulation and the diameter of the ovulatory follicle. In addition, it was assessed by ultrasonography the diameter of the CL on D19 and the pregnancy in D42. Statistical analysis was performed by the GLIMMIX procedure of SAS®. There was no difference between experimental groups (GCon vs. GOci) for any variables (P > 0.05), as follows: follicle Ø at the time of TAI (12.9 ± 0.2 vs. 12.8 ± 0.2 mm); ovulatory follicle Ø (13.0 ± 0.6 vs. 12.8 ± 0.4 mm); time of ovulation (67.3 ± 1.0 vs. 69.6 ± 1.5 h); ovulation rate [69.2% (9/13) vs. 71.4% (10/14)]; CL Ø (16.7 ± 0.3 vs. 16.5 ± 0.3 mm) and pregnancy rate [42.7% (41/96) vs. 36.5% (35/96)]. In conclusion, oxytocin administration at the time of TAI failed to increase pregnancy rate in buffaloes submitted to ovulation synchronization during the non breeding season.



A107 FTAI, FTET and AI

Evaluation of sexed semen deposition site on the *in vivo* embryo production of Nelore breed

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Keywords: *Bos indicus*, embryos, superovulation.

This study's aim was to verify if the site of sexed semen deposition influences the quantity and quality of embryos from superovulated Nelore cows. Twelve cycling females were used and went through a superovulation protocol (SOV), FTAI and embryo collection. The females received an intravaginal progesterone-releasing insert (1.9g P4, CIDR®, New York, USA) and 2 mg (I.M.) of estradiol benzoate (Estrogin®, São Paulo, Brazil) at a random day of the estrous cycle, day 0 (07h). Superstimulation was induced with 133 mg of FSH-p (Folltropin-V®, Ontario, Canada) in 8 decreasing doses at every 12h, I.M., beginning at D4. At D6, two 25 mg treatments of PGF2 α were administered (07 and 19h, I.M., Lutalyse®, New York, USA). The progesterone devices were removed 36h after the first PGF2 α , and a treatment of 0.25 mg of gonadorelin (I.M., Fertagyl®, Millsboro, USA) was given 48h after the first PGF2 α . FTAI was performed at 18 and 30h after the gonadorelin treatment, with two straws, each containing 2.1×10^6 sperm cells, and the embryo collection was done 7 days after the gonadorelin treatment. Two replicates were performed, in a crossover design, according to semen deposition site, body or uterine horns, reaching a total of two collections per treatment. Images of the ovaries were taken and transferred to a computer, for measurement and analysis of the follicles, using the IMAGEJ® software (National Institute of Mental Health, Bethesda, USA). For statistical analysis, the PROC MIXED OF SAS® 9.0 (Statistical Analysis System, Cary, EUA) was used. There were no effect of treatment in any response variables analysed. At the beginning of the superstimulatory treatment, 5.2 ± 2.4 and 5.5 ± 1.9 follicles were identified, with mean diameter of 5.4 ± 1.7 and 5.1 ± 1.9 mm, for the groups inseminated in the uterine body and horns, respectively. At the end of the treatment, 12.0 ± 3.0 and 12.3 ± 4.4 follicles were identified, with mean diameter of 8.6 ± 2.5 and 8.7 ± 2.4 mm, for the groups inseminated in the uterine body and horns, respectively. It was noticed that 66.7% (8/12) of the animals had CL at the day of FTAI, been four animals in each group, and ultrasonography indicated possible failure to respond to the PGF2 α . The group inseminated in the uterine body had embryo recovery rate of 41.1% (23/56) and the group inseminated in the uterine horns had 35.7% (15/42). It was observed a decrease in the embryo recovery rate across repetitions, where the first repetition achieved 44.3% (27/61) while the second showed embryo recovery rate of 29.7% (11/37). Yield was 52.2% (12/23) of morulas code 1 and 2 and early blastocyst (viable embryos) for the group inseminated in the uterine body and 60.0% (9/15) for the group inseminated in the uterine horns. In conclusion, based on the mean and standard deviations obtained, semen deposition site in the uterus following timed AI of superovulated cows did not affect quantity or quality of embryos recovered.



A108 FTAI, FTET and AI

Effect of Prostaglandin F2 α on ovulation in dairy cows and buffaloes

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Keywords: buffaloes, cattle, ovulatory stimulus.

The objective of this study was to evaluate the efficiency of a Prostaglandin F2 α analogue (PGF) as ovulatory stimulus in dairy cows and buffaloes. In Experiment 1, 16 lactating dairy buffaloes received 2 mg estradiol benzoate (EB; Bioestrogen, Biogenesis-Bagó, Curitiba, Brazil), im, on Day 0. A progesterone-releasing device (CIDR, Pfizer Animal Health, São Paulo, Brazil) was intravaginally placed from Day 0 to Day 9. On Days 8 and 9 cows were given 500 μ g of d-Cloprostenol (PGF analogue, Croniben, Biogenesis-Bagó, Curitiba, Brazil), im. On Day 10, buffaloes were assigned into two groups to receive 500 μ g of PGF (PG group, n = 8) or no treatment (CTL group, n = 8). There were no differences in ovulation rate (75.0 % vs 62.5 %; P = 0.59) and time of ovulation (96.0 \pm 7.9 vs 91.2 \pm 8.6 h; P = 0.69) between CTL and PG groups, respectively. In Experiment 2, 16 lactating dairy cows (Girolando crossbred) received 2 mg EB on Day 0. A CIDR was intravaginally placed from Day 0 to Day 8. On Days 7 and 8, cows were given 500 μ g of d-Cloprostenol. On Day 9, the cows were allocated into two groups to receive 500 μ g of d-Cloprostenol (PG group, n = 8) or no treatment (CTL group, n = 8). Only one cow, in the PG Group, did not ovulate. There were no differences in the diameter of the ovulatory follicle (14.1 \pm 0.5 vs 13.9 \pm 0.58 mm; P = 0.79) and the time of ovulation (84.0 \pm 5.7 and 80.6 \pm 6.1 h; P = 0.69) between CTL and PG groups, respectively. In Experiment 3, 16 lactating cows (Girolando crossbred) were handled similarly to the Experiment 2, however, all cows were given d-Cloprostenol only on Day 7. On Day 9, cows were assigned into two groups similarly as in the Experiment 2. All cows ovulated and there was no difference in the diameter of the ovulatory follicle (14.9 \pm 0.5 vs. 16.0 \pm 0.9 mm), between CTL and PG groups, respectively (P = 0.34). Cows treated with PGF ovulated earlier than CTL group (62.5 \pm 5.8 vs 94.5 \pm 13.5 h; P = 0.04). These results suggested that PGF hasten ovulation in lactating dairy cows, however, in lactating buffaloes, treatment with PGF after progesterone device removal did not anticipate ovulation.



A109 FTAI, FTET and AI

Effects of supplemental progesterone after AI on expression of Interferon-stimulated genes and fertility in dairy cows

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Keywords: dairy cow, interferon-stimulated gene, progesterone.

Objectives were to evaluate the effects of supplemental progesterone after artificial insemination (AI) on expression of interferon-stimulated genes (ISG) in blood leukocytes and fertility in lactating dairy cows. Weekly cohorts of Holstein cows (n = 1498) were blocked by parity (575 primiparous and 923 multiparous) and method of insemination as timed AI or AI on estrus and allocated randomly within each block to untreated controls, a controlled-internal drug release (CIDR) containing 1.38 g of progesterone from d 4 to 18 after AI (CIDR4), or a CIDR on d 4 and another on d 7 after AI and both removed on d 18 (CIDR4+7). Blood was sampled to quantify progesterone concentrations in plasma and mRNA expression in leukocytes for the ubiquitin-like IFN-stimulated gene 15-kDa protein (ISG15) and receptor transporter protein-4 (RTP4) genes. Pregnancy was diagnosed on d 34 and 62 after AI. Statistical analyses were performed with Glimmix procedure of SAS. Treatment increased progesterone concentrations in a dose-dependent manner (control = 3.42^c, CIDR4 = 4.97^b, and CIDR4+7 = 5.46^a ng/mL; P = 0.05). Cows supplemented with progesterone tended (P = 0.09) to have increased luteolysis by d 19 after AI (Control = 17.2^y vs. CIDR4 = 29.1^x vs. CIDR4+7 = 30.2^x %), which resulted in a shorter AI interval for those re-inseminated after study d 18. Pregnancy upregulated expression of ISG in leukocytes on d 19 of gestation, but supplementing progesterone did not increase mRNA abundance for ISG15 and RTP4 on d 16 after insemination, and on d 19 tended (P = 0.10) to reduce mRNA expression of ISG15 and reduced (P = 0.01) mRNA expression of IRTP4. This negative effect of supplemental progesterone was observed only in the nonpregnant cows. No overall effect of treatment was observed on P/AI on d 62 after insemination and averaged 28.6, 32.7, and 29.5% for control, CIDR4 and CIDR4+7, respectively. Interestingly, an interaction between level of supplemental progesterone and method of AI was observed for P/AI. For cows receiving exogenous progesterone, the lower supplementation with CIDR4 increased P/AI on d 62 in cows inseminated following timed AI (Control = 31.3^b vs. CIDR4 = 39.2^a vs. CIDR4+7 = 27.5^b %; P < 0.05) but, in those inseminated following detection of estrus, there was no difference among treatments (Control = 26.0 vs. CIDR4 = 26.9 vs. CIDR4+7 = 31.5%). Pregnancy loss did not differ among treatments. However, when type of AI was analyzed, cows inseminated in estrus tended (P = 0.10) to have higher pregnancy loss (10.0 vs. 5.7%). Supplemental progesterone post-AI using a single intravaginal insert on d 4 was beneficial to pregnancy in dairy cows inseminated following timed AI.

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A110 FTAI, FTET and AI

Relationship between antral follicle population and pregnancy results following fixed-time AI in Nelore (*Bos indicus*) cows

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

The present study aimed to evaluate the association between antral follicle count (antral follicle population, AFP) on estrus and pregnancy rates of Nelore cows. This study was performed in a commercial beef herd (Corrego do Meio), located at Alvorada-TO. A total of 291 cows were submitted to the same protocol to synchronize ovulation allowing for fixed-time artificial insemination (FTAI), starting on a random stage of the estrus cycle (Day 0). On Day 0, all females received an intravaginal progesterone (P4) device (DIB®, MSD Animal Health) and 2.0 mg intramuscular (IM) of estradiol benzoate (EB; Gonadiol®, MSD Animal Health). On Day 8, the P4 devices were removed simultaneously to 1.0 mg IM of estradiol cypionate (EC; E.C.P.®, Zoetis Animal Health), 0.530 mg IM of cloprostenol sodium (Ciosin®, MSD Animal Health) and 300 IU IM of equine chorionic gonadotropin (eCG; Folligon®, MSD Animal Health). All cows were fixed time artificially inseminated 48h after the P4 device removal (Day 10). Ultrasonography examinations (Mindray M5vet) were performed on Days 4, 8 and 10 of the FTAI protocol in order to count detectable follicles (>2.0 mm; AFP) and to measure the largest follicle at the P4 device removal (FOLD8) and immediately before the artificial insemination (FOLD10), respectively. To evaluate the occurrence of estrus, a tail-head chalk marker was used at the time of the P4 device removal, estrus occurrence was considered in females that had the chalk removed on Day 10 (FTAI). The pregnancy diagnosis was performed by transrectal ultrasonography exam 40 d after FTAI. Records were analyzed with the GLIMMIX procedure of SAS 9.3.®. Females were classified into three categories according to the AFP: 1. Low (28.8 ± 0.8), n=97; 2. Average (50.3 ± 0.7), n=97; and 3. High (74.7 ± 0.6), n=97. It was found that females with high AFP presented lower ($P=0.002$) diameter of FOLD8 (9.8 ± 0.3) compared to cows with average (11.0 ± 0.2) and low AFP (10.7 ± 0.3). Additionally, reduced diameter of FOLD10 ($P = 0.03$) and estrus rates ($P = 0.02$) were observed among females with high AFP (11.7 ± 0.3 and 54.7%, 47/86) compared to females with average AFP (12.9 ± 0.3 and 76.6%, 59/77), with low AFP-cows having intermediate results (12.1 ± 0.3 and 73.7, 61/83). However, similar pregnancy rates ($P=0.44$) were observed among the different APF categories (high: 45.4%, 44/97; average: 51.6%, 50/97; and low: 52.6%, 51/97). In conclusion, although cows with average AFP had follicles with greater diameter on Days 8 and 10 and greater estrus rates compared to females with high AFP, AFP was not associated with pregnancy results of Nelore cows following timed artificial insemination.

Acknowledgment: Fazenda Corrego do Meio.



A111 FTAI, FTET and AI

Histological analysis of the cervix in Santa Inês sheep in different phases of the estrous cycle

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Keywords: histology, ovine, reproduction.

The sheep industry has some obstacles to genetic expansion due to difficulty in performing the artificial insemination (AI) due to anatomical and physiological limitations mainly involving the cervix. Therefore, this study aimed to evaluate the histology of the cervix in Santa Inês sheep at the luteal and follicular phases. In this experiment, twenty cervixes of Santa Inês sheep (ten in the follicular phase and ten in the luteal phase) from abattoir were used. The luteal phase was defined when the sheep had at least one corpus luteum in the ovary and the follicular phase with the absence of corpus luteum and presence of dominant follicles. The reproductive tracts were kept on ice during transportation from the abattoir to the animal reproduction laboratory. At the laboratory, the cervixes were divided into three portions (proximal, middle and distal), fixed in 10% buffered formalin solution and stained with hematoxylin-eosin, obtaining photomicrographs for some histological features analysis. Statistical analysis was performed using the GLIMMIX procedure of SAS, comparing the frequency of observation of some histological features in different phases. It was found that the thin epithelium in the distal areas of the cervix had the highest frequency in the luteal phase ($P = 0.001$) and the thick epithelium was more frequent in the follicular phase. There was no statistical difference between phases of the estrous cycle in all others parameters. However, the proximal portion of the cervix during follicular phase was characterized by the presence of small intestine epithelium undeveloped and cubic aspect with goblet cells, a few glands in the lamina propria, small, thin and unbranched folds, and luteal phase protruding fine folds and branched, numerous goblet cells, lamina propria glands producing mucus and areas with stratified squamous epithelium at the end of the fold. In the middle portion of the follicular phase was observed pseudostratified ciliated epithelium, lamina propria containing thin vessels and agglomerated glands secreting mucus, branched and thin folds of epithelium. In the luteal phase there were large folds, glands that extend into the mucosa, thick epithelium, numerous goblet cells and thickening muscle layer. It is concluded that there are small differences between the microscopic features of the cervix in luteal and follicular phases of Santa Inês sheep.



A112 FTAI, FTET and AI

Impact of the nutritional reserves and body development on the cyclicity of Nelore heifers of 13 months of age

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Keywords: cyclicity, FTAI, beef heifers.

The aim of this study was to evaluate different parameters of corporal and reproductive development at the sexual precocity in Nelore heifers. Between October and December 2013, 740 Nelore heifers aged 13.0±0.85 months were enrolled from three commercial farms located in the cities of Camapua, Tres Lagoas and Agua Clara – MS. The following parameters were evaluated: age, weight, body score condition (BCS, 1 to 5 scale), subcutaneous fat thickness of rump (SFTR), height/depth of rib (PROF). Subcutaneous fat thickness was measured by ultrasound examination (Aloka SSD500, Aloka, Japan) on the gluteal muscle biceps. The cyclicity was evaluated by two ultrasound exams within 10 days, using a device with a 7.5-MHz transducer (Aloka SSD-500, Aloka, Japan). Heifers with luteal tissue in one of the examinations were considered cyclic. The body condition score was assessed during the ultrasound examination. Cutoff values for the variables previously described were determined through the ROC curve (Receiver Operating Characteristic). The variables were analyzed using the GLIMMIX procedure of SAS 9.3. Heifers were classified into categories: ≤ 13.0 months (≤ 13m) and > 13 months of age (> 13m); ≤ 248 kg of body weight (LIGHT) and > 248 kg of body weight (HEAVY); low BCS (LBCS; ≤ 3.0) and high BCS (HBCS; > 3.0); low SFTR (LSFTR; ≤ 2.47 mm) and high SFTR (HSFTR; > 2.47 mm), low (LPROF; ≤ 44%) and high depth of rib (HPROF; > 44%). There was relationship only between cyclic heifers and BCS [LBCS = 9.59% (45/469) vs. HBCS = 16.97% (46/271); P < 0.04] and farms [P = 0.0075]. However, there was no relationship between cyclicity and the other evaluated variables, such as age [≤ 13m = 10.0% (30/300) vs. > 13m = 13.86% (61/440); P = 0.35], body weight [LIGHT = 9.64% (30/311) vs. HEAVY = 14.21% (61/429); P = 0.69], SFTR [LSFTR = 8.60% (27/314) vs. HSFTR = 15.09% (62/411); P = 0.45] and PROF [LPROF = 8.93% (31/347) vs. HPROF = 15.27% (60/393); P = 0.49]. Therefore, it was possible to conclude that the body condition score was positively correlated with cyclicity in Nelore heifers aging 13 months.

Acknowledgment: CRV Lagoa and MSD Animal Health.



A113 FTAI, FTET and AI

Effect of low or high-dose intravaginal progesterone releasing devices on pregnancy rates in Nelore heifers submitted to fixed-time artificial insemination

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Keywords: FTAI, heifer, progesterone.

It is well known that progesterone (P4) concentrations are able to modulate LH pulsatility, follicle growth, and ovulatory response in cattle. Thus, differences in plasma concentrations of P4 during an ovulation synchronization protocol could influence the likelihood of pregnancy after fixed-time artificial insemination (FTAI), as suggested previously in cattle (Dias CC et al. 2009, *Theriogenology* 72, 378–385) and buffalo cows (Carvalho NAT et al. 2014, *Theriogenology* 81, 490–495). This study aimed to evaluate the use of low or high-dose intravaginal P4 releasing devices on pregnancy rate of zebu heifers submitted to FTAI. Pubertal *Bos indicus* (Nelore) heifers were injected at day-0 (Day 0) with 2 mg estradiol benzoate (EB, Estrogin, AUSA, São Paulo, Brazil) and assigned randomly to three groups. Groups 1 (n=80) and 2 (n=78) were treated with an intravaginal device containing 1.9 g (CIDR-B®, MSD Saúde Animal, São Paulo, Brazil) or 0.75 g (Prociclar®, Hertape Calier, Juatuba, MG, Brazil) P4, while Group 3 (n=76) was treated with a P4-inactivated device (Placebo group, Primer, Tecnopec, São Paulo, SP, Brazil). All intravaginal devices were removed on D8 and administered one injection of 125 µg cloprostenol (Veteglan®, Hertape Calier, Juatuba, MG, Brazil) and 300 IU of eCG (Novormon® MSD Saúde Animal, São Paulo, Brazil). Twenty-four hours later, heifers received a second injection of 1 mg EB. Heifers were FTAI (semen from four sires) 54 hours after P4 device removal. Estrus was monitored after cloprostenol injection and pregnancy status was evaluated via transrectal ultrasonography on day 40 after TAI. Data were analyzed by Chi-square test. The conception rates for the groups 1, 2 and 3 were 27.5%, 29.5% and 28.9%, respectively ($P>0.05$). The presence and absence of CL at time of device insert, sire or estrus expression at AI had no effect on pregnancy rate to FTAI. Furthermore, an unexpected finding of this study was the relatively high pregnancy rate of placebo group (28.9%), which might be attributed to EB injections. In conclusion, treatment of Nelore heifers with CIDR-B or Prociclar to synchronize ovulation did not improve pregnancy rate to FTAI.

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A114 FTAI, FTET and AI

Effect of the administration of PGF2 α at the onset of TAI protocol for cyclic Nelore heifers treated with Cronipres® mono dose device (0,558g P4)

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Keywords: heifers, progesterone, prostaglandin.

This study was conducted in three commercial farms located at the South of Bahia during the 90 days of the breeding season of 2013-14. Five hundred and eight Nelore heifers with 25.9 ± 0.1 months of age, 349.6 ± 1.2 kg of body weight and BCS of 3.19 ± 0.01 (average \pm SE) were evaluated by ultrasonography to assess presence of CL and uterine maturity (grade 1 = well developed to 3 = juvenile). Only heifers with a CL were included in the trial. They were homogeneously allocated in one of two treatment groups (with and without PGF2 α administration on D0). All heifers were treated with the same synchronization protocol for TAI, except for the administration or not of PGF2 α at the onset of the protocol (D0). On D0, all heifers received an intravaginal device with 0.558g of P4 (Cronipres® Mono Dose, Biogênese-Bagó, Curitiba, PR, Brazil) and 2 mg estradiol benzoate (Bioestrogen®, Biogênese-Bagó) IM. At the same time, half of the heifers were also treated with 0.150 mg D-Cloprostenol (Croniben®, Biogênese-Bagó) IM. On D8, the device was removed and 0.150 mg D-Cloprostenol IM plus 300 IU eCG (Folligon®, MSD, São Paulo, SP, Brazil) IM were administered in all heifers. On D9, heifers from both groups were treated with 1 mg estradiol benzoate IM and TAI was performed 54h after device removal (D10). Heifers that showed estrus after TAI were inseminated again and then left with clean up bulls until the end of the breeding season. Pregnancy diagnosis was done by ultrasonography 30d after TAI. Data was analyzed by logistic regression, using the procedure GLIMMIX of SAS. On average, heifers had good uterine maturity on D0 (1.04 ± 0.02), regardless of experimental group. Similar P/AI after TAI were observed for heifers treated or not with PGF2 α on D0 [46.9% (90/192) vs 54.1% (100/185), respectively; P = 0.16]. Pregnancy rate at the end of the breeding season was also similar among the aforementioned groups [94.3% (181/192) vs 93.5% (173/185), respectively; P = 0.76]. Thus, these results suggest that when cyclic Nelore heifers are treated for TAI using intravaginal device with 0.558g of P4 (Cronipres® Mono Dose M-24) as described herein, the use of PGF2 α on D0 of the protocol is not necessary.

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A115 FTAI, FTET and AI

The use of estradiol valerate or benzoate at the beginning of the FTAI protocol results in similar pregnancy rate in suckled Nelore (*Bos indicus*) cows, regardless of progesterone/progestin source

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Keywords: beef cow, estradiol, synchronization.

The present study aimed to evaluate the efficiency of different estradiol esters (estradiol valerate, EV or benzoate, EB) and different progesterone/progestin sources [intravaginal progesterone (DIB, P4) device and Norgestomet ear implant (Crestar)] in multiparous Nelore (*Bos indicus*) cows with 3.3 ± 0.0 of BCS and 30 to 60 days postpartum, at the beginning of the fixed-time artificial insemination (FTAI) protocol. A total of 501 females was allocated into four different experimental groups: 1. EV+DIB (n = 117) – on a random day of the estrus cycle (Day -1), intramuscular (IM) injection of 5.0 mg of EV (Injectable solution of the Crestar®, MSD Animal Health, São Paulo, Brazil) and insertion of an intravaginal P4 device (DIB®, MSD Animal Health, São Paulo, Brazil). On Day 8, the P4 device was removed and 300 IU of equine chorionic gonadotropin (eCG; Folligon®, MSD Animal Health, São Paulo, Brazil) was administered IM; 2. EV+CRESTAR (n = 130) – the animals were submitted to the same protocol of group EV+DIB, however using the Norgestomet ear implant (Crestar®, MSD Animal Health, São Paulo, Brazil) instead of the P4 device; 3. EB+DIB (n = 122) – on a random day of the estrus cycle (day 0), IM injection of 2.0 mg of EB (Gonadiol®, MSD Animal Health, São Paulo, Brazil) and insertion of a P4 device. On Day 8, the P4 device was removed and 300 IU of eCG were administered IM; 4. EB+CRESTAR (n = 132) the animals were submitted to the same protocol of group EB+DIB, however using Norgestomet ear implant instead of the P4 device. Additionally, animals in groups 3 and 4 were treated with 1.0 mg IM of estradiol cypionate (EC, E.C.P®, Zoetis, São Paulo, Brazil) and 150 µg IM of D-cloprostenol (PGF; Preloban®, MSD Animal Health, São Paulo, Brazil) on Day 8 (as revised by Baruselli et al., Animal Reproduction, v.9, p.139-152, 2012). All females were timed artificially inseminated 48h after the device/implant removal (Day 10) and submitted to ultrasound evaluation (Aloka SSD 500, Aloka, Japan) for pregnancy diagnosis 30 d after FTAI. All data were analyzed by the GLIMMIX procedure of SAS 9.3. There was no interaction ($P = 0.48$) between the different estradiol esters (EV vs. EB) and the different sources of P4/progestin (DIB vs. CRESTAR). Therefore, as main effects, it was observed similar pregnancy rates regarding the two estradiol esters [EB = 58.3% (148/254) vs. EV = 53.9% (133/247); $P = 0.29$] and the different sources of P4/progestin [DIB = 56.1% (134/239) vs. CRESTAR = 56.1% (147/262); $P = 0.97$]. In conclusion, both estradiol esters (EV and EB) associated with either DIB or Crestar resulted in similar pregnancy rate after FTAI protocol in multiparous Nelore cows.

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A116 FTAI, FTET and AI

Influence of duration of treatment with medroxyprogesterone acetate sponge on reproductive parameters of Santa Inês ewes subjected to estrus synchronization

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Keywords: artificial insemination, estrus synchronization, sheep.

The aim of this study was to evaluate the effect of three different treatment periods with medroxyprogesterone acetate (MAP) sponge on reproductive parameters of Santa Inês ewes subjected to estrus synchronization. Thirty two ewes were divided into three groups. In D0, animals received intravaginal sponges containing 60mg of MAP (Progespon®, MSD Animal health, Brazil), what were kept for 6 (G1, n = 11), 9 (G2, n = 11) or 12 (G3, n = 10) days. All the ewes received 75 µg of D-Cloprostenol IM (Prolise®, Tecnopec, São Paulo, Brazil) and 300 IU of eCG IM (Novormon®, MSD Animal Health, São Paulo, Brazil) 24 hours before the sponge removal. Transrectal ovarian ultrasonography was performed for monitoring follicular dynamics and to determine ovulation time, procedure done once a day until sponge removal and twice a day afterwards. After sponge removal, all ewes were subjected to a teaser male twice daily, for three days, until detection of estrus. The qualitative variable was assessed by Fisher's exact test. The results for quantitative variables of the animals presenting estrous behavior were tested for normality by Lilliefors test, and then subjected to ANOVA and Tukey's tests. The variables also were tested concerning correlations among them (Pearson correlation - SAEG). In regards to estrus behavior, G1 had 72.7% (8/11), in G2 72.7% (8/11), and in G3 80% (8/10) ($P > 0.05$). The protocols affected the interval (h) from sponge removal to onset of estrus (IROE) and interval from sponge removal to ovulation (IRO) (respectively, G1: 46.0 ± 8.9^a and 71.9 ± 10.6^a ; G2: 31.0 ± 7.7^b and 58.7 ± 8.7^b ; G3: $34.6 \pm 15.3^{a,b}$ and $64.0 \pm 9.5^{a,b}$; $P < 0.05$). In terms of duration of estrus (DE), interval from sponge removal to end of estrus (IREE) and interval from onset of estrus to ovulation (IOEO), there were no differences among protocols (respectively, G1: 39.0 ± 14.0 / 85.0 ± 12.7 / 25.9 ± 6.1 ; G2: 42.0 ± 12.8 / 73.0 ± 14.2 / 27.7 ± 1.1 ; G3: 41.1 ± 21.7 / 75.8 ± 16.1 / 29.4 ± 11.3 ; $P > 0.05$). Positive correlations were found between the variables DE/IREE, DE/IOEO, IROE/IRO and IREE/IRO ($P < 0.05$; respectively, 0.67; 0.53; 0.82; 0.49), and negative correlations were detected between the variables DE/IROE and IROE/IOEO ($P < 0.05$; respectively, -0.47 and -0.49). Although the three protocols were equally effective in synchronizing estrus, there was a greater individual variation in the 12 days protocol. In addition, the 6 days protocol showed greater IROE and IRO than the 9 days protocol, which should be taken into account in reproductive management practices when these treatments are employed.



A117 FTAI, FTET and AI

GnRH use in different times on estrus synchronization and ovulation in Santa Inês ewes

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Keywords: hormonal protocol, induction of synchronized estrus, sheep.

The aim of study was to evaluate the use of GnRH at different times during a short protocol of synchronization and induction of estrus in Santa Inês ewes on different reproductive parameters. Ewes (n = 29) were divided according to weight and body condition score (49.5 ± 5.9 and 3.0 ± 0.4 , respectively) and divided into in three experimental groups according to the time of GnRH application. The estrus was induced and synchronized (D0) using intravaginal sponges impregnated with 60mg of MAP for 6 days. All ewes received 30 µg of d-cloprostenol IM and 300 IU of eCG IM 24 hours before sponge removal. At 12 hours after sponge removal, Gc (n = 10) 1.0 mL NaCL 0,9% solution IM. G24h (n = 10) received after 24 hours of sponge withdraw, 0.025 mg of GnRH and G36h (n = 9) received the same dosage after 36 hours of the withdraw of the sponge. Transrectal ovarian ultrasonography was performed always by the same operator twice a day since the insertion of the sponge for monitoring follicular dynamics and to determine ovulation time. Blood samples were taken daily to determine progesterone plasma concentration. Regarding to estrous behavior, 53% (16/30) of the ewes showed signs of estrus, although no ewe from G24h showed estrus, 90% (9/10) of ewes in control group and 70% (7/10) of ewes in G36h showed signs of estrus. The duration of estrus, interval from sponge removal to onset of estrus and interval from onset of estrus to ovulation was not different. The interval (h) from sponge removal to ovulation was 64.1 ± 9.7 ; 48.0 ± 10.2 ; and 56.7 ± 5.7 , respectively to Gc, G24h and G36h. The ovulation rate (100% vs 90% vs 90%) did not differ among treatments, although the average number of ovulations per ewe (1.9 ± 0.6 vs 1.2 ± 0.4 vs 2.0 ± 1.0) was smaller ($P < 0.05$) in G24h. Regarding maximum follicle size, there was a detectable difference ($P < 0.05$) between Gc and G24h (6.5 ± 0.4 vs 5.8 ± 0.7). In terms of circulating progesterone concentration, we could observe that only one ewe was in anestrus at the beginning of hormonal treatment (3.29% - 1/29). It was observed that circulating progesterone concentration decreased during the hormonal treatment and period and that the use of GnRH did not cause an increase in circulating progesterone after the ovulation. The use of a protocol of induction and synchronization used in the present study, apart from the GnRH application, synchronizes the estrus effectively. The use of GnRH 24 hours after the sponge removal is not indicated, since the estrus signs could not be observed, the use of GnRH 36 hours after the sponge removal showed to be effective, achieving results that were similar to the control group.



A118 FTAI, FTET and AI

Efficiency of TAI protocols using Cronipres® mono dose device (0.558g P4) for 8 or 9 days associated or not with the administration of eCG in Nelore heifers

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Keywords: eCG, progesterone device maintenance, reproductive efficiency.

This study was conducted in a commercial farm located in Barra do Garças, MT-Brazil during the breeding season of 2013-14. Six hundred Nelore heifers aging 17 to 24 months were induced cyclicity [10d P4 intravaginal device (Cronipres® Mono Dose M-24, Biogênese-Bagó Curitiba, PR, Brazil) previously used for 8d and 1 mg estradiol benzoate (Bioestrogen®, Biogênese-Bagó) at removal]. Only heifers with CL and/or developed uterus 30 days after treatment were selected to participate in the present study. All selected heifers (n = 497; average BCS 3.05 ± 0.02) were subjected to the same protocol for TAI, except for the duration of intravaginal device maintenance (8 or 9 d) and the use or not of 300 IU eCG at device withdrawal (2 x 2 factorial design). Briefly, at random days of the estrous cycle (D0 or D-1 for 8 or 9 d of P4 device maintenance, respectively), heifers received an estrus detection device (EstroTECT™ Heat Detector), 2 mg estradiol benzoate and a P4 intravaginal device with 0.558g of P4 (Cronipres® Mono Dose M-24), which was kept for 8 or 9 d. At device withdrawal, heifers received 0.150 mg D-Cloprostenol (Croniben®, Biogênese-Bagó) and 0 or 300 IU eCG (Ecegon®, Biogênese-Bagó). At this time, the estrus detection device was verified and replaced when activated. After 24h, another verification and replacement of estrus detection device was performed simultaneously to the administration of 1mg estradiol benzoate. Timed AI was done 48h after device removal, together with the last estrus verification. Pregnancy diagnosis was done by ultrasonography 35d after TAI. Data was analyzed by logistic regression, using the PROC GLIMMIX from SAS. No interaction between time of device maintenance and administration of eCG on P/AI was observed, thus data was grouped. Only few heifers have anticipated estrus, regardless of time of device maintenance [Estrus D8: Mono Dose 8d = 2.2% (5/225) vs 9d = 1.6% (4/250; P = 0.65); Estrus D9: Mono Dose 8d = 1.8% (4/228) vs 9d = 2.8% (7/249; P = 0.37)] and eCG administration [Estrus D8: without eCG = 1.7% (4/236) vs with eCG = 2.1% (5/229; P = 0.77); Estrus D9: without eCG = 2.5% (6/237) vs with eCG = 2.1% (5/240; P = 0.91)]. The greatest activation of estrus detectors was observed at TAI, regardless of time of device maintenance [8d = 61.1% (135/221) vs 9d = 63.1% (154/244; P = 0.54)] and eCG administration [without eCG = 60.3% (141/234) vs with eCG = 64.1% (148/231; P = 0.43)]. Similar P/AI was observed when heifers were kept with the intravaginal device for 8 or 9d [45.3% (110/243) vs 43.8% (109/249), respectively; P = 0.69]. Treatment with eCG also had no effect on P/AI [with eCG = 45.1% (110/244) vs without eCG 44.0% (109/248); P = 0.81]. Thus, TAI protocols for pubertal heifers can be successfully done using Cronipres® Mono Dose devices (0.558g P4) kept for 8 or 9d, without the need of eCG administration.

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A119 FTAI, FTET and AI

Effect of cyclicity, body condition score and antral follicle count on the conception rate of Nelore cows submitted to fixed-time artificial insemination

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Keywords: antral follicular count, *Bos indicus*, conception rate.

The present study aimed to evaluate the effect of cyclicity, antral follicles count (AFC) and body condition score (BCS) on the conception rate of Nelore cows submitted to fixed-time artificial insemination (FTAI). A total 550 Nelore cows, 30 to 45 days post partum with BCS of 2.7 ± 0.4 (range 1-5), in the region of Congoinhas, State of Parana was used. The animals were submitted to two ultrasound examinations with an interval of 14 days for evaluation of cyclicity by the presence of CL, and antral follicles ≥ 3 mm were counted by ultrasonography, using transrectal linear probe. The animals received an intravaginal progesterone device (CIDR®, Zoetis, Brazil) and 2mg of BE (Estrogin®, Farmavet, Brazil). Eight days later, the implants were removed and the animals received 12.5 mg of dinaprost (Lutalyse®, Zoetis, Brazil), 300IU eCG (Novormon®, Syntex SA, Argentina) and 0.5 mg EC (ECP®, Zoetis, Brazil). Forty-eight hours later the cows were inseminated and pregnancy diagnosis was performed 35 days later by ultrasonography examination. The results were subjected to analysis of nonparametric variance ($P < 0.05$). The proportion of cycling cows was 87.8% (483/550). Cows were classified in groups of high AFC (≥ 32 follicles, n=111), average AFC (11 to 31 follicles, n=343) or low AFC (≤ 10.5 follicles, n=96). The AFC was greater ($P = 0.003$) in anestrus cows (20.5 follicles) than in cycling cows (17.4 follicles). However, AFC was not affected by BCS (≤ 2.5 : 20.2 follicles vs. ≥ 2.75 : 20.0 follicles). Conception results were similar ($P > 0.05$) for cycling and anestrus cows (52.3%; 253/483 vs. 59.7; 40/67, respectively), as well as for those classified as high AFC (51.3%; 57/111), average AFC (54.2%; 186/343) or low AFC (52.0%; 50/96). Conception rate though was influenced by BCS (≤ 2.5 : 44.5%; 103/231; ≥ 2.75 : 57.7%; 190/329; $P = 0.03$). In conclusion, conception rate was not affected by AFC or cyclicity in postpartum Nelore cows. However, BCS had a significant effect in conception results, highlighting the importance of this parameter to choose cows that will be sent to FTAI programs.



A120 FTAI, FTET and AI

Artificial insemination in sheep with frozen semen using prostaglandin based protocol

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Keywords: GnRH, PGF2 α .

With the intense decrease in the space available for animal production is indispensable the use of technologies that allow manipulation of the reproductive cycle in sheep, maximizing productive capacity of these animals. An alternative to this technology is the use of protocols without steroid hormones such as GnRH and PGF2 α , which have the best responsiveness. The objective of this study was to compare pregnancy rate in ewes synchronized with PGF2 α , PGF2 α associated with GnRH and with the standard protocol (Control) consisting of insertion of vaginal device containing medroxyprogesterone acetate (MAP) and eCG. Ideal-breed ewes (n=279) maintained in extensive conditions in Rio Grande do Sul were randomly divided into three experimental groups. Group 1, ewes were (G1, n = 99) synchronized with two doses of 0.530 mg PGF2 α (Sincrocio®, Cloprostenol sodium, Ouro Fino, Brazil), within a 9 days interval (D0 and D9). In group 2, (G2, n = 92) ewes received two injections of 0.530 mg PGF2 α (Sincrocio®, Cloprostenol sódico, Ouro Fino, Brasil) 9 days apart (D0 and D9), and 24 h after the last PGF2 α they received 25 μ g of lecirelin, a GnRH agonist (Gestran Plus®, Tecnopec, ARSA S. R. L., Argentina). Group 3, control (G3, n = 88), the vaginal sponges containing MAP were inserted and remained for 12 days. At the day of the removal of the device (D12) 250IU of eCG (equine chorionic gonadotropin - Novormon®, Coopers, Argentina) was injected. Fixed time insemination was performed by intrauterine laparoscopy 54 h after the end of the protocol (D9 and D12). For insemination, frozen semen at a concentration of 100x10⁶ sperm per straw was deposited in both uterine horns. Data were analysed by Linear Generalized Model and Dunnett test (P < 0.05). The pregnancy rate was evaluated by ultrasonography 45 days after AI. Control protocol (G3) showed the highest pregnancy rate (31.8%) among the groups. No differences were observed on pregnancy rate between the other two protocols (G1= 16.2% and G2=13.0%). In conclusion, the control protocol, which is the most used, provided the greatest pregnancy rates following AI with frozen sperm. Moreover, the expected effect of GnRH increasing the synchronization of ovulation and pregnancy rate compared to a single PGF2 α treatment was not observed.