A061 FTAI, FTET and AI

Factors that influence the results after TAI protocols in Nelore and Nelore x Angus heifers inseminated between 12 and 14 months old

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Keywords: heifers, induction of puberty, TAI.

The response of heifers from different breed submitted to protocols for induction of puberty and TAI between 12 and 14 month old was evaluated. At the beginning of the induction of puberty protocol, Nelore heifers (NE; n=433) with 11.4 ± 0.05 months (mo) old, weighted 278.1 ± 1.6 Kg and presented a body condition score (BCS; 1 to 5 scale) of 3.6 ± 0.02; and Nelore x Angus heifers (AN; n=414) with 9.2 ± 0.26 mo old, weighted 306.9 ± 1.7 Kg and presented a BCS of 4.1 ± 0.02. All heifers received a previously used for 27 days (4th use) intravaginal progesterone (P4) device (CIDR®; 1.9 g of P4, Zoetis) on D-22 (22 days before the onset of the synchronization protocol). On D-10, the CIDR was removed and all heifers received 0.5 mg of estradiol cypionate I.M. (ECP®, Zoestis). On D0, heifers were evaluated by transrectal ultrasonography (Aloka SSD 500) to detect the presence of corpus luteum (CL). All heifers had their ovulation synchronized with the following protocol: insertion of a previously used CIDR for 18 days (3rd use) and 2.0 mg I.M. of estradiol benzoate on D0; 12.5 mg I.M. of dinoprost tromethamine (Lutalyse®, Zoetis) on D7; on D9 the CIDR was removed and the heifers received 0.5 mg I.M. of ECP and 200 IU of eCG. The TAI was performed on D11, 48 h after CIDR withdrawal. Pregnancy diagnosis was performed on D41. Data were analyzed using GLIMMIX procedure of SAS. The analysis for the presence of CL on D0 included in the model the effects of age, breed, BCS and weight. To analyze pregnancy per AI (P/AI) it was included in the model the effects of age, breed, presence of CL on D0, AI technician and semen. The data were reported as least square means and significance was defined when $P<0.05$. The presence of CL on D0 did not differ between breeds (NE: 79.9% and AN: 78.8%). Regardless breed, heavier heifers were more likely ($P<0.05$) to have CL on D0. There was an effect of presence of CL on D0 ($P<0.05$) for P/AI (With CL: 57.5% [370/650] vs. Without CL: 44.3% [74/169]). There was no interaction between presence of CL on D0 and breed affecting the P/AI. The NE heifers had lower ($P<0.05$) P/AI (46.9%; 199/433) than AN heifers (62.6%; 259/414). A significant interaction between presence of CL on D0 and weight was found for P/AI. In heifers with CL, the weight did not affect ($P>0.1$) P/AI. However, among heifers without CL, heavier heifers were more likely ($P<0.05$) to become pregnant. The BCS influenced the heifers response to induction of puberty protocols. In summary, heifers can be inseminated with younger ages; however, the presence of CL at the beginning of the TAI synchronization protocol and the BCS of heifers can determine the reproductive success. Furthermore, under the same conditions Nelore x Angus heifers have higher reproductive outcomes than Nelore heifers.

A062 FTAI, FTET and AI

Effect of the moment of insemination after progesterone device removal on conception rate in lactacting dairy cows

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Keywords: conception rate, insemination momento, TAI.

The aim of this trial was to determine whether delaying of the time to perform the timed artificial insemination (TAI) from 60 to 72 h after progesterone device removal affect the conception rate in lactating Holstein cows. The trial was conducted in three commercial dairies located in Cuenca Mar and Sierras region in the southeast of Buenos Aires province, Argentine [Herd 1 (n=72), Herd 2 (n=76) and Herd 3 (n=104 cows)]. The total milk production of 305 days, DIM and number of lactation were respectively: Herd 1 = 8,295±750; 170±88.4 e 2.6±1.3; Herd 2 = 8,069±1,050; 202.8±80 e 2.4±1.4; and Herd 3 = 8,264±1,021; 106±1.7 e 2.2±1.2. On the first day of the synchronization protocol (D0), cows received an intravaginal progesterone device (DIB, Syntex, Argentina) and 2 mg I.M.of estradiol benzoate (EB, Syntex, Argentina). On D7, the device was removed and 0.5 mg Clorprostenol (Cyclase, Syntex) I.M. was administered and all cows received 1 mg of EB 24 h later. After the last EB treatement, cows were randomly assigned in one of two groups (TAI60h and TAI72h), and were timed inseminated at 60 or 72 h after progesterone device removal. All cows received 10 ug of busereline acetate (Gonasyn, Syntex) at the same moment of the TAI. Pregnancy was diagnosed 32 days after TAI using transrectal ultrasonography. Data was analysed using PROC CATMOD of SAS. There was no effect (P>0.05) of TAI moment (60 and 72 hours) on the conception rate, which on the Herd 1 was 32.5% and 40.0%; on the Herd 2, 38.9% and 32.5%; and on Herd 3, 37.7% and 37.3%, respectively. In conclusion the timing to performe the TAI (60 or 72 h) after the progesterone device removal did not affect the conception rate of lactating dairy cows.

A063 FTAI, FTET and AI

Profile of progesterone releasing of intravaginal device (Vallée ®) used for synchronization of ovulation protocols for TAI

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Keywords: fertility, progesterone, synchronization of ovulation.

The current study evaluated the protesterone (P4) blood releasing of intravaginal devices containing 1.2 (DMax; Dispocel Max®; Vallée S.A.) or 0.6g of P4 (DMonouso; Dispocel Monouso®; Vallée S.A.). Also, it was tested the number of use of each device (new; 1X=used for 8 days or 2X=used for 16 days). Thirty six Holstein cows were allotted in six treatments groups (n=6 in each group): P4Control new (DIB®; MSD Saúde Animal); DMax new; DMax 1X; DMax 2X; DMonouso new e DMonouso 1X. Blood was collected at: 0, 6, 12, 18, 24, 48, 72, 96, 120, 168, 192, 198, 204 e 216 h after device insertion to quantify the circulating P4 concentration. Data were tested according to the normality of residues and homogeneity of variances using the Guided Data Analysis of the SAS. Posteriorly, data were analyzed by Proc GLIMMIX using repeated measures analysis. The averages of P4 concentrations were compared by LSmeans. Significance level was set at 5%. The profile of P4 releasing was similar among P4Control new Group (1.6 ± 0.3^{ab} ng/mL) and new devices groups (DMax new=1.9 ± 0.1^{a} ng/mL; and DMonouso new=1.6 ± 0.1^{ab} ng/mL) or DMax 1X Group (1.2 ± 0.9^{bc} ng/mL). Devices containing 1.2g previously used twice and 0.6 g used once showed lower concentration of P4 than control group (DMax 2X=1.1 ± 0.7^{c} ng/mL and DMonouso 1X=1.0 ± 0.8^{c} ng/mL). All groups presented similar pattern of P4 releasing curve, characterized by and elevation on the circulating P4 to maximum level at six hours after device insertion, which was maintained until the moment of device removal. The present data indicate that Dispocel Max® 1.2g (new, previously used once or twice) or Dispocel Monouso® 0.6g (new or previously used once) can release sufficient quantity of P4, enabling the use in timed artificial insemination programs in cattle.

Acknowledgments: Apta Dairy Cattle, Nova Odessa-SP.

A064 FTAI, FTET and AI

Different sources of equine chorionic gonadotropin (eCG) on follicular growth and ovulation rate of *Bos indicus* cattle

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Keywords: eCG, ovulation, synchronization of ovulation.

The objective of this study was to evaluate the effect of different sources of equine chorionic gonadotropin (eCG) on the dominant follicle growth and ovulation rate of lactating beef cows submitted to synchronization of ovulation program. Forty-eight lactating *Bos indicus* cows (multiparous with 30 to 45 days postpartum) with body condition score greater than 2 (1 to 5 point scale) were used. At random stage of the estrous cycle (D0), cows received 2mg of estradiol benzoate (Sincrodiol®, Ourofino, Brazil) and an intravaginal progesterone device (Sincrogest®, Ourofino, Brazil). On the same day, an ultrasonographic examination (US; Aloka SSD500) was performed to verify the absence of corpus luteum (only cows without CL were used). At the P4 device removal (D8), cows were submitted to the US examination to measure the diameter of the larger follicle, and then were homogeneously allocated in one of three treatment groups [SincroeCG Group; Positive Control Group (PC Group) and Negative Control Group (NC Group)] according to follicular size. On the same day, the cows received 500µg of $PGF_{2\alpha}$ (Sincrocio®, Ourofino, Brazil) and 1.0 mg of estradiol cipionate (ECP®, Zoetis, Brazil). Cows from PC Group received 300IU of eCG (Folligon, MSD®, Netherland) and cows from SincroeCG Group received 300IU of eCG (SincroeCG®, Ourofino, Brazil). The animals from NC Group did not receive any additional treatment. On D10 and D12, US were accomplished to evaluate the follicular growth (mm/day) and ovulation rate (%). Data were analyzed by GLIMMIX of SAS. There was a treatment effect (P=0.02) on dominant follicle growth (0.85 ± 0.22^{b} mm/day on NC Group; 1.66 ± 0.29^{a} mm/day on PC Group and 1.80 ± 0.23^{a} mm/day on SincroeCG Group) and on ovulation rate [NC Group=50% $(8/16)^{b}$; CP Group=87.5% $(14/16)^{a}$ and SincroeCG Group=81.3% $(13/16)^{a}$; P=0.04]. In conclusion, both eCG sources are efficient to improve the final dominant follicle growth and ovulation rate in anestrous lactating beef cows submitted to synchronization of ovulation.

A065 FTAI, FTET and AI

Conception rate in *Bos indicus* beef cows submitted to TAI protocols using a new or previously used progesterone devices, and different body condition scores

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Keywords: body condition score, estrus synchronization, insemination.

This study aimed to verify the effects of the number of uses of intravaginal progesterone devices and body condition score (BCS) on conception rates of *Bos indicus* beef cows submitted to timed artificial insemination (TAI) protocols. Thus, 1,122 multiparous Nelore cows were subjected to a TAI protocol using new or second and third use intravaginal progesterone device. All females were raised on pasture management in a farm in southern of Para state. At the beginning of the synchronization (D0), cows received an intravaginal progesterone device (DIB® 1.0 g of P4; (Intervet/Schering-Plough) and 2 mg intramuscular (IM) of estradiol benzoate (EB; Gonadiol®, Intervet/Schering-Plough); on D8, the intravaginal device was removed and 300 IU of eCG IM (Folligon®, Intervet/Schering-Plough), 7.5 mg of PGF2α (Veteglan Lueolítico®; Hertape Calier Animal Health), and 1 mg of EB (Gonadiol®, Intervet/Schering-Plough) were administrated. The TAI was performed 48 hours after progesterone device removal. Cows were catergorized according to their BCS at the first day o synchronization using a 1 to 5 scale (Freitas J. R. Bras. Zootec. 37, 116-121). To analyze the effect of BCS on pregnancy success, cows were classified into one of two groups: 2.0 to 2.5 or 2.75 to 4.0 BCS. The data were analyzed by ANOVA, using the Statistical Analysis System version 9.2. Difference was considered when $P<0.05$. Overall conception rate was 61.1% 573/1,122). There was a significant effect ($P<0.0001$) of number of uses of the intravaginal device on the conception rate [New = 60.00%^{a} (198/330), Second use = 51.71%^{b} (227/439) and Third use = 41.93%^{b} (148/353)]. Females with BCS between 2.75 and 4.0 showed higher ($P<0.0001$) conception rate (69.75%^{a}; 385/552) than cows with BCS between 2.0 and 2.5 (32.98%^{b}; 188/570). It was concluded that the use of new intravaginal devices resulted in greater conception rates than previously used progesterone devices in synchronization of ovulation programs for TAI. Also lower conception rate was obtained when the TAI protocols were applied in low BCS cows (i.e BCS $\leq$2.5).

A066 FTAI, FTET and AI

Plasma progesterone concentration and luteolysis rate in crossbreed females at different stages of the estrous cycle

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Keywords: bovine, cloprostenol, luteolysis.

Induction of luteolysis using analogs of prostaglandin F2α (PGF2α) has a fundamental role to synchronize estrous cycle in oocyte and embryo donors and embryo recipients. It is also essential in protocols for timed artificial insemination. Besides the presence of a functional corpus luteum, the efficiency of the luteolytic process varies according with the phase of the estrous cycle when PGF2α is administered. (Fernandes, 2007. Rev. Bras. Reprod. Anim. 31, 406-414). The aim of the study was to compare the changes in progesterone (P4) concentration and the efficiency of luteolysis when PGF2α was administered at different stages of the estrous cycle in bovine crossbreed females. The study was conducted in the southern of Minas Gerais State, a region with mesothermic climate. Thirty crossbred cows and 30 crossbred heifers between the 6th and 17th days of the estrous cycle (estrus = day 0) were homogenously allocated to one of three groups according to the period of the estrous cycle. P1: between the 6th and 9th days, P2: between the 10th and 13th days, and P3: between the 14th and 17th days. Animals were kept in pasture (Brachiaria brizanta). They received 0.5 mg of Sodic Cloprostenol (Sincrosin®, Vallée, São Paulo, Brazil) I.M. Blood samples were collected immediately before PGF2α administration and then 36 hours after. The concentration of progesterone (P4) was evaluated by RIA. Luteolysis was considered effective if the difference between the two analyses of P4 was greater than 75%. The concentration of P4 between the initial and final periods (ANOVA) and the effectiveness of luteolysis between periods and between animal categories (Fisher Test) were compared. Differences were considered at the 5% level. There was no effect of category (cow or heifer) in the studied variables. The mean initial concentrations of P4 did not differ between the three periods of the estrous cycle (2.42 ± 1.02, 2.77 ± 1.25 and 2.18 ± 0.62 ng/mL), as well as P4 concentrations 36 hours after PGF2α administration (0.29 ± 0.16, 0.34 ± 0.18 and 0.26 ± 0.15 ng/mL) for P1, P2 and P3, respectively. Also no difference was found in luteolysis rate: P1 = 80.0% (17/20); P2 = 90.0% (18/20) and P3 = 100.0% (20/20; $P > 0.05$), showing that the PGF2α was similarly effective in all periods of the estrous cycle, regardless of the animal categories. We concluded that the treatment with 0.5 mg of Sodic Cloprostenol is effective to induce luteolysis in different periods of the estrous cycle of crossbred cows and heifers.

Acknowledgments: Fapemig.

A067 FTAI, FTET and AI

Fertility of lactating dairy cows submitted to TAI protocols with GnRH or estradiol compared to a pre-synchronization or AI after estrus detection

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Keywords: artificial insemination, bovine, fixed-time.

In recent years, there has been a significant reduction in conception rates of dairy cows. This reduction is apparently linked to the increase in milk production per cow, resulting in drastic decrease in estrus expression, and consequently the efficiency of conventional artificial insemination (AI). Therefore, more effective timed AI (TAI) protocols have been developed. Thus, the first aim of this study was to compare the conception rates of TAI protocols based on GnRH vs. estradiol benzoate (EB) associated with an intravaginal progesterone (P4) device on the first day of the protocol (D0). The second objective was to compare the protocols mentioned to one that includes a pre-synchronization, but without P4. The third objective was to compare the three TAI protocols vs. AI after estrus detection. Between 41 and 47 d postpartum, 789 cows were randomly divided into four groups: G1 (n=187) pre-synchronization with PGF2α on D-8 and GnRH on D-6, followed by synchronization with GnRH on D0, two injections of PGF2α on D7 and D8, EB on D8, and TAI 36 h later; G2 (n=215) synchronization with EB+P4 on D0, P4 withdrawal on D8 and other procedures were identical as in G1; G3 (n=192) synchronization with GnRH+P4 on D0, P4 withdrawal at D8 and other procedures same as G1; and G4 (n=195): AI after estrus detection induced by two PGF2α treatments 14 d apart. Cows not observed in estrus were treated with GnRH and PGF2α at 7 and 14 d after the second PGF2α, respectively. All injections were I.M.. Transrectal ultrasonographic examination was performed 30 d after AI to evaluate the conception rates. Statistical analysis was performed by logistic regression using PROC GLIMMIX of SAS. The first comparison between G2 and G3 showed higher (P=0.02) conception rates in G2 (42.8%) compared to G3 (32.3%). When the comparison was between G1 vs. G2+G3, G1 (44.4%) and G2+G3 (37.8%) were not different (P=0.13). Finally, conception rates of G4 (55.2%; 64/116) when only bred cows were considered in the analysis, were higher (P=0.002) than the conception obtained by all TAI groups combined (39.9%). However, when considering pregnancy out of all the cows available for G4 (64/195), pregnancy rate (32.8%) tended to be lower (P=0.07) than in TAI groups (39.9%). It is concluded that a EB+P4-based TAI protocol for first service in lactating cows presented better results than the GnRH+P4-based protocol. It can also be concluded that, because no difference was detected in relation to the pre-synchronization strategy in fertility, the use of EB+P4 was more effective by allowing AI earlier. In addition, although the TAI protocols of this trial had lower conception rates than AI after estrus detection, they resulted in a better pregnancy rate.

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A068 FTAI, FTET and AI

Fertility of Nelore females with different antral follicles counts in a FTAI program

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Keywords: antral follicle population, *Bos taurus indicus*, conception rate.

The objective of this study was to compare conception rates between Nelore females with different categories of antral follicle count (AFC) submitted to a protocol for fixed-time artificial insemination (FTAI). A total of 481 Nelore multiparous cows aging 6.7±3.6 years, with body condition score (scale 1-5) of 2.7±0.4 and postpartum interval of 92.0±49.9 days were submitted to a protocol for synchronization of the follicular wave emergence and ovulation beginning on random day of the estrous cycle (D0). At this moment, cows received an intravaginal progesterone releasing device (Sincrogest®, Ouro-Fino, São Paulo, Brazil), associated with an intramuscular injection of 2.0mg of estradiol benzoate (Sincrodiol®, Ouro-Fino, São Paulo, Brazil). On D8, the progesterone devices were removed and cows were treated with 300 I.U. of equine chorionic gonadotropin (eCG; Novormon®, MSD Saúde Animal, São Paulo, Brazil) I.M., 500µg of cloprostenol (Sincrocio®, Ouro Fino, São Paulo, Brazil) I.M. and 1.0 mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) I.M.. On D10, the FTAI was performed. The antral follicular population was assessed on D4 of the synchronization protocol by ultrasonography using a 8.0 MHz linear transducer (Pie-Medical, Falco 100, Maastricht, Holanda), antral follicles with diameter ≥3mm were counted in both ovaries, and cows were grouped into three categories according to the AFC: low (≤ 34 follicles), intermediate (between 34 to 53 follicles) and high AFC (≥ 53 follicles). Pregnancy diagnosis was performed 55 days after the TAI by transrectal ultrasonography. Conception rates among AFC groups were compared with the chi-square test ($P<0.05$). The overall conception rate was 29.3% (141/481). There was no statistical difference on conception rates among the AFC categories (Low=29.34%;49/167; Intermediate=29.33%;44/150, and High=29.27%;48/164). These results suggest that the AFC does not affect conception rates following FTAI programs. Nevertheless, the use of eCG in synchronization protocol may have minimized the influence of AFC on reproductive outcome in this study. Further studies are necessary to cover the issue in FTAI protocols without the use of this drug, as well as in artificial insemination upon estrus detection programs.

A069 FTAI, FTET and AI

Productive aspects and beta-hydroxybutyrate concentrations of high production Holstein cows, diagnosed as pregnant or non pregnant until 150 days in milk

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Keywords: beta-hidroxibutyrate, conception, dairy herd.

The aim of the present study was to evaluate the differences between milk production and blood beta-hydroxybutyrate (BHB) concentration of high producing Holstein cows that become or not pregnant until 150 days in milk (DIM). It was used a database of biochemical and productive measures of 250 females (121primiparous and 129 multiparous) from a commercial farm (Agrindus S/A). Body condition score (BCS) and milk production were recorded at calving (D0) and at 30, 60 and 90 DIM. Blood concentrations of beta-hydroxybutyrate (BHBA) were analyzed using a handheld meter Precision XTRA® at calving (D0), 15 and 30 DIM. After 60 days of voluntary waiting period, cows were artificially inseminated upon detection of estrus. The interval from calving to first service was recorded and the pregnancy diagnosis was performed 45 days after insemination by transrectal palpation. Retrospectively, cows were grouped according to the 150 DIM pregnancy status (130 pregnant and 120 non pregnant cows) and the averages of BCS, milk production, BHBA and interval calving-first service were compared. All variables were analyzed by the GLIMMIX procedure of SAS®. The statistical models included the classificatory variable pregnancy at day 150 – and, linearly, by BCS (0, 30 and 90 DIM), milk production (30, 60 and 90 DIM) and BHBA (0, 15 and 30 DIM) variables. The data are presented as average ± standard error mean (SEM), considering $P<0.05$. There was no difference between pregnancy status among the different time points on BCS (0 - 3.16±0.01; 30 – 2.91±0.01; 60 – 2.90±0.01 and 90 DIM – 2.90±0.01) and milk production (30 – 37.6±0.5 liters; 60 – 39.8±0.4 liters and 90 DIM – 40.8±0.5 liters). Regardless pregnancy status at 150 DIM, the concentrations of BHBA were similar both at calving (0.42±0.01 mmol/L) and 60 DPP (0.73±0.06 mmol/L). However, pregnant cows had lower blood BHBA concentrations at day 15 (0.62±0.07 vs. 0.82±0.09 mmol/L; P=0.04) and shorter calving/first service interval (65.3±1.7 vs. 76.2±2.9 DPP; P=0.02) than non pregnant cows. Therefore, it was concluded that high production Holstein cows that become pregnant until 150 DIM have lower blood concentrations of BHBA at 15 DIM and a shorter calving/first service interval than non pregnant cows until this period.

A070 FTAI, FTET and AI

Seasonal variation in pregnancy rate and weight gain in embryo recipient manteined in pasture condition

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Keywords: bovine, nutrition, recipients.

The pregnancy rate of recipients has the greatest impact on the cost of a commercial embryo transfer program in cattle (VARAGO, et al., 2008. Rev. Bras.Reprod. Ani, 32, 100-109). The aim of this study was to evaluate the seasonal weight gain and the effect on pregnancy rate in crossbred embryo recipients manteined in pasture condition. It was conducted in a farm located in the Southwest of Minas Gerais State - Brazil. The region has climate classification CW, according to Koepen. A total of 484 *in vitro* produced (IVP) embryos from Gyr donors using sexed sorted semen were used. Heifers have their estrus synchronized using intramuscular injection of 0.530mg of cloprostenol. The recipients were weighed on the same day of the embryo transfer and again at 23 to 25 days later, when the pregnancy diagnosis was performed by ultrasonography. According to the daily weight gain, heifers were divided into four groups: G1 (n=132): below 150g, G2 (n=133): from 151 to 250g; G3 (n=119): from 251 to 350g and G4 (n=100): above 350g. After embryo transfer, the recipients were kept on *Brachiaria brizanta* pasture with water and mineral supplementation *ad libitum*. It was compared the pregnancy rate among the different weight gains and the proportion of heifers in each weight gain group in two seasons (compared by Fisher test): P1 – rain season (November to April), with a stocking rate of 1.3 animal unit/ha and P2 – dry season (May to October), with a stocking rate of 1.05 AU/ha. In the rain season, there was equitable distribution of the recipients among the different weight gain groups ($P>0.05$). However, during dry season, it was observed a greater frequency ($P< 0.05$) of heifers presenting lower daily weight gains (i.e. below 250g). In this last season, diferent ($P<0.05$) distribution (%) of heifers according to the daily weight gain was found ($G1=33.2^{a}$, $G2=31.4^{a}$, $G3=21.8^{b}$ and $G4=13.6^{c}$). There was no difference between seasons on pregnancy rates (Dry season = 42.3 vs Rain season 45.8%). Recipientes that presented greater weight gain (i.e. above 250g G3= 52.1% and G4= 51.0%) showed higher ($P<0.05$) pregnancy rate than those with lower weight gain (G1= 35.6% and G2 = 40.6%). For the same weight gain, there were no differences between seasons ($P>0.05$). It was concluded that weight gain is an important parameter to be considered in the management of IVP embryo recipients. Furthermore, the reduction in the stocking rate during the dry season is not sufficient to avoid the impact of weight gain on the pregnancy rates of the crossbreed embryo recipients.

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A071 FTAI, FTET and AI

Comparison between the animal category on the occurrence of estrus, ovulatory follicle diameter and conception rate in Nelore cattle

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Keywords: *Bos indicus*, estrus detection, TAI.

The aim of this study was to compare the effect of animal category on the occurrence of estrus, on the ovulatory follicle diameter and on conception rate of females submitted to a fixed-time artificial insemination (FTAI) protocol. A total of 200 female Zebu, with a body condition score of 3.19 ± 0.42 (scale 1-5) and divided according to animal category in lactating multiparous cows (n = 141; 64.58±18.42 days postpartum) and heifers (n = 59). Prior to the beginning of the synchronization protocol, heifers were submitted to clinical gynecological examination using ultrasound and only pubertal heifers (presence of corpus luteum) were used. On a random day of the estrous cycle (D0), the animals received an intravaginal progesterone (P4) device (PRIMER®, Tecnopec, São Paulo, Brazil) associated with 2.0mg estradiol benzoate (RIC-BE®, Tecnopec, São Paulo, Brazil) I.M.. On D8, the intravaginal P4 device was removed and 300 IU of equine chorionic gonadotropin (Folligon®, Intervet, São Paulo, Brazil), 150µg of d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) and 1.0 mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) were intramuscular adminstrated. The animals were then marked with a chalk maker (RAIDEX®, Walmur, Porto Alegre, Brazil) between the sacral tuberosity and the tail insertion. On D10, the animals were categorized into two groups according to the occurrence of estrus: Group 1 (G1) - No estrus, presence and permanence of color ink intensity (n=45); Group 2 (G2) - intermediate estrus, loss of ink color intensity (n=38) and Group 3 (G3) - estrus, complete removal of ink (n=117). Immediately after, the diameter of the ovulatory follicle (DFOL) was meansured by transrectal ultrasonography using a 5.0MHz linear transducer (Mindray, DP2200vet, São Paulo, Brazil) and FTAI was performed in all animals. Pregnancy diagnosis was performed by ultrasonography 45 days after FTAI. The program SPSS (version 19) was used to perform the statistical analysis ($P<0.05$). The overall conception rate was 49.0% (98/200). There was no significant difference between the occurrence of estrus and animal category in the experimental groups. The occurrence of estrus among groups was 46.0% (G1-cows), 54.0% (G2-cows), 33.3% (G1-heifers) and 66.7% (G2-heifers). Regarding DFOL, it was found that cows have a greater diameter (12.51±2.98mm) than heifers (9.54±3.61mm). Furthermore, there was statistical difference ($P=0.01$) on the conception rate between the animal categories (Cows=54.6%, 77/141 and heifers=35.6%, 21/59). It is possible to conclude that the animal category did not influence the occurrence of estrus; however, cows have greater ovulatory follicle diameter and greater conception rates than heifers. Furthermore, the occurrence of estrus is not a good parameter to predict the ovarian follicular and pregnancy response to FTAI protocols in heifers.

A072 FTAI, FTET and AI

Follicular diameter at insemination moment as a predictor of ovulatory response and pregnancy success in dairy buffaloes submitted to TAI

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Keywords: fertility, ovulatory follicle, ultrasonography.

This study aimed to establish the probability of ovulation and pregnancy per AI (P/AI) through the diameter of the largest follicle at the moment of artificial insemination (AI) of lactating buffaloes subjected to timed artificial insemination (TAI) protocols during the nonbreeding season (spring-summer). Thus, it was measured the diameter of the largest follicle at the moment of TAI (øFolAI) of 175 dairy buffaloes raised in six farms of the Ribeira Valley, State of São Paulo. All females were submitted to the same synchronization protocol. On D-12 all animals received a new or used once or twice intravaginal progesterone (P4) device (1 g of P4; Sincrogest®, Ourofino Agronegócio) plus 2.0 mg I.M. of estradiol benzoate (Sincrodiol®, Ourofino Agronegócio). On D-3 (PM), females received I.M. 0.53mg of PGF2a (Cloprostenol, Sincrocio®, Ourofino Agronegócio) and 400 IU of eCG (Novormon®, MSD Saúde Animal), followed by P4 device removal. On D-1 (PM), 10µg I.M. of buserelin acetate (GnRH, Sincroforte®, Ourofino Agronegócio) were administrated. The TAI was performed 16 hours after GnRH treatment (D0; am). A single bull was utilized for all inseminations. Ultrasound exams (Chison D600Vet, China) were performed to evaluate the øFolAI (mm), occurrence of ovulation rate (CL presence at day 10) and pregnancy 30 days after TAI. The prediction of the occurrence of ovulation (Ov) and pregnancy (P30) relative to øFolAI was obtained by the regression *Logit*=intercept+slop*(øFolIA) calculated through the solution *Interactive Data Analysis* of SAS®. The probability could be obtained by the formula: [EXP(*Logit*)/1+EXP(*Logit*)]*100. The adjusted *odds ratio* for the risk of pregnancy 30 days after AI relative to the classes of øFolAI were evaluated (<12.0; 12.0 to 15.0; and >15.0 mm). Variables were considered different when P<0.05. The overall means observed were øFolAI - 13.3±0.2 mm; ovulation rate - 84.0% (147/175); and pregnancy rate on D30 - 67.4% (118/175). The logistic curves obtained were: Logit (Ov)=-5.71+0.60*øFolAI (P<0.0001); and Logit (P30)=-2.23+0.22*øFolAI (P=0.0007). Pregnancy rates and adjusted *odds ratio* (confidence interval) for P30 in each class of øFolAI were: <12.00 mm - 46.3% (25/54)^{b} - reference; 12.01 to 15.00mm - 77.0% (57/74)^{a} - 3.889 (1.807 to 8.372) and >15.00 mm - 76.60% (36/47)^{a} - 3.796 (1.594 to 9.039) P=0.0007. It was concluded that the øFolAI can be consider a predictor to ovulatory responses and pregnancy success in dairy buffaloes submitted to TAI synchronization protocol during nonbreeding season, in which females that had larger follicle at TAI are associated to higher ovulation and pregnancy rates.

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A073 FTAI, FTET and AI

The use of a GnRH analog at the time of the artificial insemination in dairy cattle

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Keywords: bovine, buserelin acetate, conception rate.

The GnRH belongs to a group of decapeptides isolated from the hypothalamus, acting as a controller of the anterior pituitary gland, which has influence on reproductive processes mainly by regulating the release of follicle stimulating hormone and luteinizing hormone, which regulates the folliculogenesis. This function has been used for veterinary purposes, including the induction of ovarian activity, optimizing fixed-time artificial insemination, ovulation induction, and in the treatment of follicular ovarian cysts in cattle. The present study aims to evaluate the influence of GnRH analogue buserelin acetate (Sincroforte™, Ouro Fino, Cravinhos, SP, Brazil) applied at the time of artificial insemination in dairy cows (range of milk production from 10 to 15kg per day). A total of 92 animals (Gir, Girolando, Holstein and Jersey) were randomly assigned into two groups. Group I (n=48) received 0.01 mg of buserelin acetate, IM at the time of AI and Group II (n=44) received saline solution, IM at the time of AI. All cows were inseminated 12 hours after detection of estrus by the same techinitian, using semen obtained from certified company. Pregnancy diagnosis was performed at 40 days after the AI by transrectal palpation performed by trained veterinarian. Data were analyzed by Chi Square, with 5% of significance level. The conception rate in group I was 54.17% (26/48), and in group II was 65.91% (29/44). No significant difference was found between groups ($p > 0.05$).

A074 FTAI, FTET and AI

Progesterone suplementation with 2 CIDR in lactating Holstein cows without corpus luteum submitted to E2/P4 based protocol

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Keywords: FTET, progesterone, TAI.

The objective was to evaluate the effect of the utilization of two intravaginal progesterone devices (CIDR®, Zoetis, SP, Brazil) in lactating Holstein cows without a corpus luteum (CL) at the beginning of the synchronization of ovulation protocol, on pregnancy per AI (P/AI) and pregnancy per ET (P/ET). This experiment was performed in 9 farms, 5 of them destined to fixed time embryo transfer (FTET; n=534) and 4 of them to timed AI (TAI; n=433). The protocol used was: D-10 2mg IM EB (2.0mL de Estrogin®, Farmavet, SP, Brazil) + CIDR (1.9g of P4); D-3 PGF 25mg IM (5.0 mL de Lutalyse®, Zoetis, SP, Brazil); D-2 removal of the CIDR + 1mg IM de ECP (0.5 mL, ECP®, Zoetis, SP, Brazil); D0 TAI or D7 FTET. In the D-10, cows were randomly assigned to receive1 CIDR (CON) or 2 CIDR (2CIDR). The diameter of the ovulatory follicle was evaluated by ultrasound (US) in D0. Blood samples were collected for P4 meansure on D-10, D-3 and D7, being considered synchronized animals with P4 ≥1.0 ng/mL in D7 (TAI) or CL visible in the US D7 (FTET). Pregnancy diagnosis was performed by US at 32d to verify the presence of the embryo and at 60d for the presence of the fetus. For evaluate binomial variables was used PROC GLIMMIX and continuous variables PROC MIXED. It was considered significant when P <0.05 and tendency if P <0.10. The P4 on D0 did not differ between groups (CON = 0.81 ± 0.09 ng/mL and 2CIDR = 0.88 ± 0.09 ng/mL). On the D-3 2CIDR group had higher P4 concentration (2.05 ± 0.22 ng/mL) compared to the CON group (1.65 ± 0.22 ng/mL).On D7, P4 of the CON (2.76 ± 0.16 ng/mL) was higher than the 2CIDR group (2.35 ± 016 ng/mL). The diameter of the ovulatory follicle (CON = 15.6 ± 0.36 mm and 2CIDR = 15.2 ± 0.37 mm) and ovulation (CON = 82.8% and 2CIDR = 81.0%) did not differ between treatments. The P/AI was similar at 32d (CON = 38.7% x 2CIDR = 42.1%) and 60d (CON = 32.9% x 2CIDR = 37.4%). In synchronized cows there was no difference between treatments on P/AI at 32d (CON = 42.6% x 2CIDR = 50.0%); however, the 2CIDR group tended to have higher P/AI at 60d (CON = 36.5% x 2CIDR = 45.8%). The P/ET did not differ between treatments at 32d (CON = 29.2% x 2CIDR = 32.2%) and 60d (CON = 24.3% x 2CIDR = 25.8%). The pregnancy loss did not differ between treatments in AI (CON = 14.4% x 2CIDR = 8.7%) and ET (CON = 17.9% x 2CIDR = 20.8%). The use of two P4 devices in lactating cows without CL increased P4 concentration during follicular growth and tended to increase P/AI in synchronized cows, but did not affect P/ET, suggesting that the concentrations of P4 can improve the P/AI due to improvement in oocyte quality.

A075 FTAI, FTET and AI

Efficiency of anticipation of the application of prostaglandin F2α in the Ovsynch protocol for ovulation synchronization and TAI in bufallo heifers during the breeding season

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Keywords: bufallo heifers, Ovsynch, ovulation.

The present study aimed to evaluate the efficiency of the application of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) twenty-four hours before the recommended period for *Ovsynch* protocol. It is known that buffalo heifers present three waves of follicular growth and it was believed that the administration of $PGF_{2\alpha}$ 24h earlier could increase ovulation rate due to anticipated *turnover* compared to animals of two waves of follicular growth. Previous study demonstrated that buffalo heifers subjected to 24h in advance at application of $PGF_{2\alpha}$ showed numerically greater ovulation rate at the end of the *Ovsynch* protocol (Gimenes *et al*., 2005, In: 3rd Congresso Nazionale sull Allevamento del Bufalo and the 1st Buffalo Symposium of the Europe and the Americas, 238). Therefore, it could be expected to obtain higher conception rate in heifers that received this treatment. Thus, a total of 160 buffalo heifers homogenously distributed into two groups (Control Group - GPGFD7 and $PGF_{2\alpha}$ anticipated Group - GPGFD6), according to ovarian activity, the body condition score and the age were used in this study. The heifers from GPGFD7 group received intramuscularly (im) the application of 10mg of GnRH (Sincroforte®, Ourofino Agronegócio) at random stage of the estrous cycle (D-1) and the animals from GPGFD6 group received the same hormone on D0. On D6, all heifers received im 0.53mg of $PGF_{2\alpha}$ (Cloprostenol sodium, Sincrocio®, Ourofino Agronegócio). On D8, 10mg of GnRH (Sincroforte®, Ourofino Agronegócio) was intramuscular administered, and heifers were inseminated 16 hours after the application of the GnRH (D9). Ultrasound evaluations (Mindray DP2200Vet, China) were performed on D -1 to verify the ovarian activity and on the D39 for the pregnancy diagnosis. The data were analyzed using the GLIMMIX procedure of SAS®. The results obtained showed that there was no difference on the conception rate [35.9% (28/78) vs. 34.1% (28/82); $P > 0.05$] between the experimental groups GPGFD7 and GPGFD6, respectively. It is concluded that reducing 24 h of the interval between the applications of the first GnRH and $PGF_{2\alpha}$ of the *Ovsynch* protocol did not improve the conception rate in buffalo heifers submitted to TAI during the breeding season.

A076 FTAI, FTET and AI

Ovarian and endocrinology responses in Taurus and Zebu heifers submitted to different nutrition challenges

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Keywords: Holstein, Nelore, progesterone.

The aim of this study was to evaluate circulating progesterone concentration (P4) and ovarian follicular dynamics in *Bos indicus* and *Bos taurus* heifers under high (ACMS; weight gain of 900g per day) and low (BCMS; mantenance, NRC 2001) consumption of dry matter/energy. Cycling Holstein (n=16) and Nelore (n=16) heifers were used in a 2x2 factorial arrangement (cross-over). The experimental diet was given during 32 days (15 days before and 17 days during the hormonal protocol). The animals were pre-synchronized with two applications of cloprostenol (0.53mg, i.m. $PGF_{2\alpha}$, Sincrocio®, Ourofino Agronegócio) 12 days apart. To ensure that the progesterone from device is the unique source of P4, two doses of $PGF_{2\alpha}$ were given 18 and 12 hours before device insertion. At onset of synchronization protocol (D0), heifers received a new intravaginal P4 device (CIDR®, Zoetis Brasil), 2mg of estradiol benzoate i.m. (BE, Sincrodiol®, Ourofino Agronegócio) and a dose of $PGF_{2\alpha}$. After 8 days, the P4 device was removed and 1mg of BE was administered 24 hours afterward. Ultrasonographic exams were performed every 24 hours during P4 device treatment (D0 until D8), every 12 hours from P4 device removal until ovulation and on D17 to evaluate the corpus luteum (CL) volume (mm^3). Blood samples were collected daily from D0 to D10. The results were analysed using PROC MIXED of SAS 9.2 and presented as mean ± standard error (SEM). The new follicular wave emergence occurred at 3.3±0.1d, regardless of the breed and diet (P=0.22). Regardless of diet (P= 0.93), number of recruited follicles were higher in Nelore than in Holstein heifers (46.9±4.1 vs. 26.3±3.5, respectively; P<0.001). Breed and diet affected the ovulatory follicle size (P=0.04 and P=0.0002) and CL volume (P<0.001 and P=0.002), respectively. The maximum diameter of ovulatory follicle was higher in Holsein heifers (14.9±0.7 and 12.7±0.5) than in Nelore heifers (13.4±0.4 and 11.6±0.4) receiving ACMS and BCMS diet, respectively. Furthermore, Holstein heifers had larger CL volume (6525.3±502.8 and 4653.4±503.8) than Nelore heifers (4188.1±531.7 and 2698.4±252.5). Regardless of the breed (P=0.29), heifers that received ACMS diet had greater follicular growth rate than heifers receiving BCMS diet, respectively (1.4±0.1 and 1.2±0.1 for Holstein; 1.3±0.1 and 1.1±0.1 for Nelore, P=0.002). The circulating P4 concentrations were greater in Nelore than in Holstein from D2 to D9 of protocol (P=0.001) and heifers receiving BCMS than those receiving ACMS from D1 to D8 (P=0.0002). Regardlless of the breed, higher dry matter consumption during the hormonal protocol resulted in a greater follicular growth rate, a larger ovulatory follicle and a larger CL. (Preliminary data).

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A077 FTAI, FTET and AI

Impact of the moment of TAI and rumen protected fat supplementation for cows submitted to early weaning in native grasslands in the pantanal

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Keywords: postpartum supplementation, production system, TAI moment.

The Pantanal is a biome with a natural aptitude for beef cattle due to the fields of native grasslands, however the herds' reproductive rates are low due to the variability of the diet (composition and availability of forage) and the physical and structural characteristics of farms. Alternatives such as food supplementation, fixed time artificial insemination (FTAI) and early weaning of calves can be used as strategies to increase reproductive efficiency of livestock. Thus, two experiments were conducted in fields of native pastures during the breeding season of 2012/2013, to assess: a) Experiment 1 - The effect of supplementation of postpartum cows on pregnancy rate to TAI and late breeding season (BS) was evaluated. In Treat 1 (CONTROL), cow [n = 130, 364.9 kg of body weight and 4.7 of BCS (1 to 9 scale)] received Ureado 50 P® (In-Vivo NSA, Paulínia, SP, Brazil). In Treat 2 (ENERGY) cows (n = 130, 364.1 kg of body weight and 4.8 of BCS) received energy supplement (MEGALAC-E®; Elanco, Nova Ponte, Brasil). The TAI was performed at 70 days postpartum. After TAI, cows were placed with bulls until the end of the 120 days of BS. Cows had their calves weaned at 110 days postpartum. b) Experiment 2 - The effect of the postpartum moment to perform the TAI on pregnancy rates of cows that had their calves weaned early was evaluated. In this experiment we used data from CONTROL group (TAI at 70 days postpartum) of the previous experiment and an additional group: MOMENT treatment cows [n = 65, weight = 369.7 kg, BCS = 4.8 (1-9)] received the same supplementation postpartum as Control, and TAI was performed 10 days after early weaning (at 110 days postpartum, the implant was placed on the day the calves were separated from their mothers). All cows were inseminated at fixed time with the protocol: D0: BE-2mg (RIC-BE ®, Farmavet, Sorocaba, Brazil) and Primer ® (Tecnopec-Agener União, São Paulo, Brazil); D8: device removal + d-cloprostenol-150µg (Prolise ®, Arsa, Argentina) + 1mg of EB and D10: TAI using semen from the same batch of a single bull. Data were subjected to analysis using the GLIMMIX SAS version 9.12. The fixed variables were considered treatment, the category of differences were found, cow (primiparous or multiparous), inseminator and BCS were excluded from the model. In exp 1, similar (P>0.01) pregnancy per TAI (Control=38.5% and Energy=40.8%) and pregnancy rates at the end of BS was observed (Control=97.4% and Energy=98.3%). In Exp 2, also no diference between groups on pregnancy per TAI (Control=38.5% and Moment=41.5%) and on pregnancy rates at the end of BS was observed (Control=97.4 and Moment=91.5%). In conclusion, the postpartum moment to perform the TAI did not influence the pregnancy per AI of Nelore cows submitted to early weaning. Also, the energy supplementation by rumen-protected fat did not alter pregnancy per TAI and pregnancy rate at the end of BS in Nelore cows manteined in native pastures of the Pantanal.

A078 FTAI, FTET and AI

Effects of exogenous progesterone on luteal function of high production dairy cows

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Keywords: corpus luteum, dairy cattle, progesterone.

We evaluated the effect of administration of injectable progesterone (P4; Sincrogest® injectable Ourofino, Brazil) on luteal function of lactating Holstein cows. A total of 83 cows, at a random stage of the estrous cycle, had their ovulation synchronized. Cows that ovulated after the synchronization (n=75; milk production of 32.5±0.6 kg/day, 95.8±4 days in milk, 1.5±1,0 lactations and body condition score of 2.9±0.6 - scale 1-5) were used in this study. On the third day of the estrous cycle, cows were allocated evenly according to the daily milk yield, days in milk, number of lactations, body condition score and follicle diameter at the time of ovulation into four experimental groups: Control (n=20) cows received no treatment; P4 (n=18) cows received 900mg im P4; P4+hCG (n=18) cows received 900mg P4 and 2000IU of hCG (Chorulon, MSD, Netherlands) im.; hCG (n=19) cows were treated with 2000IU i.m. of hCG. Blood samples were collected on D3, D5, D9, D13 and D17 of the estrous cycle to meansure the plasma P4 concentration (ng/mL) and ultrasonographic examinations were performed on D5, D9 and D13 of the estrous cycle to measure the volume (mm^3) of the corpus luteum (CL) excluing the cavities. Statistical analysis was performed using the SAS procedure GLIMMIX. The plasma P4 concentration among the control, P4, P4 + hCG and hCG groups were respectively; D3 (0.6±0.2; 0.7±0.06; 0.6± 0.05 and 0.5±0.08), D5 ($1.5±0.1^{b}$; $2.4±0.2^{a}$; $3.0±0.3^{a}$ and $1.2±0.1^{b}$), D9 ($3.4±0.4^{b}$; $4.0±0.4^{b}$; $5.9±0.9^{a}$ and $4.2±0.4^{b}$), D13 (4.4±0.5; 4.5±0.5; 5.2±0.6 and 5.13±0.5) and D17 (4.12± 0.4; 3.3±0.5; 4.0±0.6 and 4.5±0.4). The CL volumes were respectively: D5 (2721±38; 2451±26; 3165±38 and 2645±39), D9 ($6399±71^{b}$; $6661±69^{b}$; $8932±93^{a}$, and $8751±83^{a}$) and D13 ($6007±62^{ab}$, $5417±36^{b}$, $7497±61^{ab}$ and $7980±64^{a}$). There was an interaction between treatment and day of the estrous cycle (P=0.001) on plasma P4 concentration in P4 treated groups compared to the control group and hCG in D5 and D9. There was no interaction between treatments and day of the estrous cycle on CL volume, but there was a statistical difference (P=0.002) among groups treated with hCG compared to control and P4 in moments D9 and D13. We conclude that treatment with injectable P4 adminstration on D3 of the estrous cycle increases plasma P4 concentration from D5 to D9 of the estrous cycle without any influence on CL volume. The hCG treatment increased the volume of CL on D9 and D13 of the cycle.

A079 FTAI, FTET and AI

Evaluation of ovulation time of suckled Nelore cows treated with eCG or calf removal in a nine day-based TAI protocol using different uses of CIDR

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Keywords: cow, reproduction, TAI.

The aim of this study was to evaluate different used CIDRs associated with different gonadotropic stimuli (GS) on follicular dynamic of suckled Nelore cows. A total of 117 cows received the following TAI protocol - D0: CIDR+2mg EB; D7: 12,5mg PGF; D9: Remove CIDR+0,5mg ECP. The cows were randomized to receive difference uses of CIDRs (1^{st}: n=33; 2^{nd}: n=33; 3^{rd}: n=19 e 4^{th}: n=32) and eCG at device removal (n=60) or calf removal (CR) during 48 hours from device removal to timed artificial insemination (n=57), 4x2 factorial arrangement. At the time of CIDR remove the cows had the tailhead painted with chalk marker, and had their estrus behavior monitored. Cows had their ovaries evaluated by ultrasound at Days 9 (0h), 11 (48h), 11.5 (56h) and 12 (72h), to follow the dominant follicle (DF) growth and to determine the moment of ovulation, and at Day 18 to detect the CL presence. The variables analyzed were DF 0h, DF 48h and follicular growth, FG), time of ovulation, P4 concentration and occurrence of estrus. The FG was calculated by the DF between 0 and 48h. Blood samples for P4 dosage were taken on 0h, 48h and Day 18. Data were analyzed using PROC MIXED of SAS. Significant effects were considered $P \leq 0.05$. There was no interaction between CIDR use and GS in analyzed variables ($P > 0.1$). The follicle diameter at 0 and 48h and the difference between 48 and 0h were affect ($P < 0.01$) by CIDR use: 1^{st} use ($8.2 \pm 0.29mm^{a}$; $10.57 \pm 0.31mm^{a}$; $2.25 \pm 0.23mm^{a}$), 2^{nd} use ($10.05 \pm 0.29mm^{b}$; $12.07 \pm 0.31mm^{b}$; $2.05 \pm 0.23mm^{a}$), 3^{rd} use ($10.63 \pm 0.38mm^{b}$; $11.97 \pm 0.39mm^{b}$; $1.33 \pm 0.28mm^{b}$), 4^{th} use ($10.84 \pm 0.30mm^{b}$; $12.33 \pm 0.31mm^{b}$; $1.41 \pm 0.23mm^{b}$); and no effect of GS in diameter of the DF at 0 and 48h, however tended to differ in FG (1,98±0,17mm vs. 1,64±0,17mm) for eCG and CR respectively. CIDR use affected P4 concentration ($P < 0.01$) on day 9 (1^{st}: $5.06 \pm 0.24ng/mL^{a}$, 2^{nd}: $3.80 \pm 0.24ng/mL^{b}$, 3^{rd}: $3.02 \pm 0.30ng/mL^{c}$, 4^{th}: $2.23 \pm 0.23ng/mL^{d}$), and there was no differences at 48h. Concentration of P4 on day 18 of ovulated cows only (P4 ≥ 1ng/ml and presence of CL) was greater ($P < 0.01$) on eCG treated (6.55 ± 0.23 ng/mL^{a}) when compared to CR treatment (5.39 ± 0.23 ng/mL^{b}). Cows that receive the 4^{th} use CIDR had a higher proportion of estrus ocurrence at 48h (87.5%; $28/32^{a}$) compared with 1^{st} use (51.8%; $18/33^{b}$), 2^{nd} use (60.6%; $21/33^{b}$) and 3^{rd} use (53.3%; $11/19^{b}$). Cows treated with CR had higher proportion of estrus occurrence (39.2%; $22/57^{a}$) compared with eCG treated cows (16.5%; $11/60^{b}$). The proportion of cow that presented a ovulation with ≤48h was 5.9% (7/117), between 48-60h was 15.3% (17/117), between 60-72h was 51.2% (60/117), and more than ≥72h was 16.2% (19/117), and cows that did not ovulate was 11.1% (13/117). Greater proportion of cows synchronized with 4th use CIDR ovulated at 56h (40.6 %; $13/32^{a}$) when compared to the cows using CIDR 1^{st} use (15.0%; $5/33^{b}$), 2^{nd} use (15.2%; $5/33^{b}$) and or 3^{th} use (11.1%; $2/19^{b}$). Higher proportion of cows treated with CR ovulating at 56hrs (28.9%; $17/57^{a}$) compared with eCG (12.0%; $8/60^{b}$). Synchronized cows with lower concentrations of P4 (i.e. 4th use of CIDR) and cows stimulated by CR had a higher proportion of premature ovulations after the CIDR removal.

A080 FTAI, FTET and AI

FTAI with injectable progesterone: ovarian follicular dynamics and pregnancy rates of Nelore (*Bos indicus*) cows, with and without corpus luteum

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Keywords: follicular growth, injectable progesterone, ovulation.

The aim of this study was to evaluate the ovarian follicular dynamics and pregnancy rate of Nelore cows, with and without CL, synchronized for fixed-time AI (FTAI) with a parenteral injectable P4. Forty-four multiparous Nelore (72-84 months of age), 2.8±0.1 of BCS and 45 to 65 days postpartum were used. The experimental design was a 2x2 factorial, where 11 cows with and 11 without CL, received an intravaginal device with 1g P4 (DIB®, Syntex, Argentina) and 2mg EB (Syntex®, Syntex, Argentina) IM on D_0. The device was removed on D_8 and animals were injected with 500µg cloprostenol (Cyclase®, Syntex, Argentina) and 300IU eCG (Novormon®, Syntex, Argentina) IM. On D_9 1mg EB was given IM and FTAI performed 24h later. Eleven other animals with and 11 without CL received two applications of injectable P4 on D_0 (300mg[1] SC and 50mg[2] IM) and 2mg EB IM. On D_6 were applied 500µg cloprostenol and 300IU eCG IM. On D_8 1mg EB was given IM and FTAI performed 24h later. The design of injectable protocol was performed according to previous studies (Unpublished data). Ultrasonography was performed on D0, D4 to FTAI every 24h, from FTAI until ovulation every 12h and 12 days after ovulation. The presence of follicles ≥ 5 mm on D4, follicular growth rate, behavioral estrus rate, diameter of the dominant follicle (DF) at FTAI, ovulatory follicle (OF), ovulation rate, diameter of the CL and pregnancy rate were evaluated. The results were analyzed by ANOVA and Tukey test or by Chi-square test ($P \leq 0.05$). The presence of follicles ≥ 5mm on D4 was similar between CL group - CLG (50% and 6.1±0.8mm) and group without CL - GwCL (55% and 6.6±0.8mm), and between the injectable group - IG (55% and 6.5±0.9mm) and the device group - DG (50% and 6.2±0.8mm). The follicular growth rate was similar between CLG (1.1±0.3mm/day) and GwCL (1.1±0.4mm/day), and between the IG (1.2±0.4mm/day) and the DG (1.1±0.3mm/day). The diameter of the DF at FTAI and OF were similar between CLG (10.5±1.8 and 11.4±1.3mm, respectively) and GwCL (10.8±2.3 and 11.1±2.2mm, respectively) but DG was higher than IG (11.7±2.0 and 11.8±1.7 *vs.* 9.7±1.7 and 10.6±2.0 mm, respectively; $p<0.05$). The CL size was similar between CLG (17.3±3.8mm) and GwCL (16.6±3.2mm), and between DG (17.6±3.5mm) and IG (16.1±3.4mm). The ovulation rate was similar between CLG (59%) and GwCL (77%) but higher in the DG (91%) than in the IG (45.5%; $p<0.05$). The pregnancy rate was similar between CLG (36%) and GwCL (27%) but higher in the DG (45%) compared to IG (18%; $p<0.05$). The CL present or absent did not effected the ovarian follicular dynamics and pregnancy rate. However, the synchronization of ovulation with intravaginal device showed higher diameter of the DF at FTAI, OF and ovulation and pregnancy rates compared to synchronization using P4 injectable.

[1]150mg/ml of natural P4 in base vehicle sesame and peanut oil (slow absorption).[2]50mg/ml of natural P4 in base vehicle sesame and peanut oil (fast absorption). * Prepared in the laboratory only for studies.

A081 FTAI, FTET and AI

eCG influence the intracellular signaling pathways in bovine corpus luteum

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Keywords: corpus luteum, eCG, follicular stimulation.

Using timed artificial insemination (TAI) techniques allied with equine chorionic gonadotropin (eCG) aims to increase the size of the dominant follicle and consequently the volume of the corpus luteum (CL), the progesterone (P4) production and conception rates. Changes in the regulation of the function and development of the CL in females stimulated with eCG were previously reported (Baruselli et al., 2011). This fact drove the hypothesis that treatment with eCG alters intracellular signaling pathways in the formed CL. To test the hypothesis 11 cows (*Bos indicus*) were divided into two groups: control and stimulated. Cows from both groups were treated with the same protocol for synchronization of follicular wave emergence, except for the administration of 400 IU of eCG (Novormon, Syntex, Buenos Aires, Argentina) on Day 8, which was only done in stimulated cows. At a random day of estrous cycle, animals all cows received an intravaginal device containing 1g of P4 (Primer, Technopec Brazil) and 2mg of estradiol benzoate (Estrogin, Farmavet, São Paulo, Brazil) intramuscularly. On Day 8, the intravaginal devices were removed, and 0.150 mg of d-cloprostenol (Prolise, Arsa, Buenos Aires, Argentina) was administrated. Forty eight hours after P4 devices removal, cows received 0.025 mg of Lecirelin (Gestran Plus, Arsa, Buenos Aires, Argentina). Six days after estimated ovulation (i.e. lecirelin treatment), all cows were slaughtered. Their CL were collected, weighed, measured, and frozen in liquid nitrogen. Partial results were collected from microarrays which were performed to identify differentially expressed genes between the two groups. The relative expression of genes involved in steroidogenesis signaling (*ADM, MMP9, NOS2*), activation of matrix metalloproteinases and protease activated receptor that regulates hemostasis and inflammation (*PRSS2*), angiogenesis (*ANG and ANGPT1*) and luteolysis (*PLAU*) were evaluated by qPCR. Decreased expression of genes related to the steroidogenesis (*MMP9*; P=0.01) was found in the CL of eCG-treated cows, but expression of *ADM* did not change (P=0.41) and the expression of *NOS2* was unaltered (P=0.87), but was increased in stimulated group contrasting to the result of the microarray. On inflammation pathway gene expression, *PRSS2* (P=0.05) was decreased. Regarding genes that interact in the angiogenesis pathway, the expression of *ANG* (P=0.02) was increased in the CL of eCG-treated cows, while the expression of *ANGPT1* (P=0.11) was similar in the CL of control and stimulated cows, like *PLAU* (P=0.41) for luteolysis. In summary, the results showed herein are indicative that eCG changes the relative expression of genes that play important roles in the signaling pathways of the inflammation and angiogenesis. Thus, they may alter cellular responses related to the improvement of the CL function and, consequently, improve reproductive outcomes of cows submitted to TAI techniques.

A082 FTAI, FTET and AI

Effect of different P4 devices over follicular dynamics of pubescent Holstein heifers submitted to timed artificial insemination

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Keywords: follicular dynamics, progesterone, TAI.

In heifers, high serum progesterone (P4) suppresses follicle growth by decreasing the LH pulsatile secretion. The aim of this study was to evaluate the effect of different P4 devices over follicular dynamics of pubescent Holstein heifers submitted to timed artificial insemination (TAI). A total of 20 pubescent Holstein heifers, in a random day of estrous cycle (D0) received an intravaginal devices containing 0.75 g of P_4 (*Prociclar®, HertapeCalier, Minas Gerais, Brazil)* in G1 (n = 10) and 1g of P4 *(Primer®, Tecnopec, São Paulo, Brazil*) in G2 (n = 10) associated to 2mg of estradiol benzoate, im *(EB, Estrogin®, Farmavet, São Paulo, Brazil)*. On D8, intravaginal P4 devices were removed, 500µg of cloprostenol were administered, im *(Ciosin®, Schering-Plough Animal Health, São Paulo, Brazil)*, and 300 IU of eCG im *(Novormon®, Schering-Plough Animal Health, São Paulo, Brazil)*. On D9, induction of ovulation was proceeded with 1mg BE, im *(Estrogin®, Farmavet, São Paulo, Brazil)* in both groups and TAI were done 54 hours after P4 device removal. The ultrasound examination was performed on D10, 4 hours after the TAI for measuring the diameter of the preovulatory follicle followed by examinations each 6 hours from D11 to time of ovulation. Data were analyzed using SPSS 16.0. There was no significant effect of treatment on these variables, the average diameter of the preovulatory follicle was 15.7 ± 0.45 mm. vs 14.2 ± 0.58 mm, for G1 and G2, respectively (P=0.08). Ovulation rate 70% vs 80% (P>0.05), mean time between removal of the intravaginal P4 device and ovulation 60.57 ± 3.17 hours vs. 70.0 ± 0.0 hours (P = 0.09) between G1 and G2, respectively. It can be verified that the concentration of P4 in the intravaginal devices with 0.75g and 1.0g did not influece the ovulatory follicle diameter nor the ovulation rate or the interval between the P4 devices removal and ovulation of pubescent Holstein heifers submitted to TAI.

A083 FTAI, FTET and AI

Conception rates following FTAI of Nelore cows (*Bos indicus*) with high, intermediate and low numbers of antral follicles

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Keywords: antral follicle count, conception rate, FTAI.

The objective was to evaluate the influence of the population of antral follicles on the conception rate of *Bos indicus* cows with high, intermediate and low numbers of antral follicles during the follicular waves. Multiparous, suckling (40-60 days postpartum) Nelore cows (*Bos indicus,* n=701) with BCS 3.0 ± 0.5 (1-5 scale) were submitted to a synchronization of ovulation protocol. Randomly during their estrous cycle (D0), they received an intravaginal progesterone (P4) device (CIDR®, Pfizer, São Paulo, Brazil) and 2 mg EB (Estrogin®, Farmavet, São Paulo, Brazil), i.m. At P4 device removal (D8), they were injected with 0.53 mg of cloprostenol (Ciosin®, Intervet-Schering Plough, Cotia, Brazil), 300 IU of eCG (Novormon®, Syntex SA, Buenos Aires, Argentina) and 1 mg of EC (ECP®, Pfizer, São Paulo, Brazil), i.m. Cows were timed inseminated 48h after P4 device removal. Antral follicles ≥ 3 mm were counted (D8) using an intravaginal microconvex array (Áquila PRO, Pie medical, Maastricht, The Netherland) and cows were assigned into groups with high antral follicular count (AFC; G-High, ≥25 follicles, n = 149), intermediate AFC (G-Intermediate, 11-24 follicles, n = 400) or low AFC (G-Low, ≤10 follicles, n = 152). Numbers of follicles were compared using the Kruskal-Wallis test and conception rates were compared using the Qui-square test, using a significance level of 0.05 (Bioestat 5.0). The overall mean number of antral follicles (mean ± SD) was 17.93 ± 8.45 and the overall conception rate was 51.49% (361/701). The mean population of antral follicles was 30.70 ± 5.66 (G-High), 17.03 ± 3.28 (G-Intermediate) and 7.83 ± 2.42 follicles (G-Low, $P<0.05$). There was no difference on the conception rate among the groups of high and low AFC (51.67 versus 60.50%), however, conception rate of cows with low AFC was greater compared to the intermediate AFC group (60.50 versus 48.00%, $P<0.05$). Thus, it is concluded that Nelore cows with low AFC had greater conception rate following FTAI protocol compared to cows with intermediate AFC.

A084 FTAI, FTET and AI

Pregnancy rates following FTAI of Nelore heifers (*Bos indicus*) with high, intermediate and low numbers of antral follicles

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

The objective of this study was to evaluate the influence of the population of antral follicles on the conception rate of Nelore heifers submitted to fixed-time AI (FTAI). Two hundred and eight pubertal Nelore heifers with body condition score (BCS) of 3.0 ± 0.5 were submitted to a synchronization of ovulation protocol. On a random day of the estrous cycle (D0), heifers received an ear implant containing norgestomet (Crestar ®, Intervet Schering-Plough, Brazil), 2mg BE (Estrogin ®, Farmavet, Brazil) and 1 mg cloprostenol (Ciosin ®, Intervet Schering-Plough, Brazil), IM. At implant removal (D8), heifers received 1mg of cloprostenol (Ciosin ®, Intervet Schering-Plough, Brazil), 300IU eCG (Novormon ®, Syntex SA, Argentina) and 0.5 mg of estradiol cypionate (ECP ®, Pfizer, Brazil) IM. Heifers were timed inseminated 48 hours after the implant removal. Antral follicles ≥ 3 mm were counted by using ultrasound transducer microconvex of 5 MHz and heifers were divided into groups according to the antral follicle count (G-High, ≥ 30 follicles, n = 38, G-Intermediate, 13-29 follicles, n = 143, G-Low, ≤ 12 follicles, n = 27). Pregnancy diagnosis was performed by transrectal ultrasonography (5 MHz), 30 days after FTAI. The number of follicles was evaluated by Kruskal-Wallis and pregnancy per AI (P/AI) were compared by Chi-square test ($P \leq 0.05$). There was no interaction between BCS and AFC. The overall average number of antral follicles (mean ± SD) was 21.48 ± 9.47 and the overall P/AI was 44.71% (93/208). The average of follicular population observed in groups G-High, G-Intermediate, and G-Low was 37.73 ± 7.05, 19.23 ± 4.29 and 10.55 ± 2.17 follicles, respectively. There was no difference on P/AI among groups (G-High: 44.73%, G-Intermediate: 43.35%, G-Low: 51.85%, $P>0.05$). The present data suggest that the variations in the population of antral follicles do not influence P/AI of heifers submitted to FTAI.

A085 FTAI, FTET and AI

Circulating progesterone concentrations according to ovarian luteal activity of heifers treated with intravaginal devices

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Keywords: FTAI, progesterone, reproduction.

Progesterone (P4) is an important hormone in fixed-time artificial inseminstion (FTAI) programs (Costa et. al. 1999, Braz J AnimSci, 28, p.1243-1247). Hormonal protocols used in FTAI programs involve considerable financial values, in which P4 represents 43% of the total cost. The P4 releasing from intravaginal devices occurs by passive diffusion (Brunton et. al. 2008, Manual of Pharmacology and Therapeutics. 4th Ed., 1254p), i.e., the hormone release is driven by a concentration gradient and enhanced by the contact area between the device and vaginal epithelium (Rothen-Weinhold et al. 2000, J Dairy Sci, 83, p.2771–2778). Given the importance of P4 in the protocols (Savio et. al. 1993, J. Reprod.Fertil. v. 98, p. 77-84) and the representativeness of this steroid in the synchronization treatment costs, several studies described the reutilization of P4 devices as an alternative to make this technology viable (Almeida et. al. 2006, Braz. J. Vet. Res. Anim. Sci., 43, p. 456-465). However, the results are controversial and the P4 releasing pattern from intravaginal devices in animals with different luteal activity (amount of endogenous P4 synthesis) is not described. The aim of the present study was to evaluate the P4 profile in heifers with different ovarian luteal activity treated with a new intravaginal device (1.0 g of P4) for 8 days. Animals were allocated into 3 groups: Group 1 – with corpus luteum (CL) during all treatment period; Group 2 – with CL during half of treatment period; Group 3 – without CL. At the beginning of the treatment (D0), the animals from G1 and G2 had a functional CL formed 8 days before implant insertion. In G2 animals, 150µg of D-cloprostenol (Veteglan, Hertape Calier, Brazil) was administrated 3 days after implant insertion (D3). Animals from G3 did not have luteal function (i.e. absence of CL) at the beginning of the treatment. Blood samples were collected on D0 (moment of intravaginal P4 device insertion), D3, D5, and D8. Serum P4 concentration was determined by radioimmunoassay. Mean plasma P4 concentration on each day was compared by Tukey's test. Serum P4 concentration in the groups G1 and G2 was greater than in G3 on D0 (5.34^a; 5.30^a and 0.64^b ng/mL, respectively; $P<0.05$) and also on D3 (5.76^a; 5.46^a and 3.61^b ng/mL, respectively; $P<0.05$). On D5, 36 hours after D-cloprostenol treatment in G2, serum P4 concentration of those heifers was similar to G3, and both were lower than G1 (3.36^a, 2.46^b and 2.15^b ng/mL, for G1, G2, and G3 respectively; $P<0.05$). Similar result was observed on D8 (3.19^a, 1.84^b and 1.58^b ng/mL for G1, G2, and G3 respectively; $P<0.05$). Moreover, difference of the serum P4 concentration between D3 and D0 in G1 and G2 were different when compared to G3 (0.46^a; 0.19^a and 2.83^b ng/mL, respectively; $P<0.05$). In conclusion, the present results suggest that P4 release from intravaginal device is directly influencied by blood P4 concentration of the treated animal. Thus, animals with a functional CL during FTAI protocol may consume less P4 from the intravaginal P4 device when compared to those without CL.

A086 FTAI, FTET and AI

Effects of the components of artificial insemination technique on fertility of white Leghorn hens

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Keywords: artificial insemination, fertility, white leghorn chickens.

Artificial insemination (AI) of chickens in cages has been shown to be technically feasible. However some of the technical procedures could contribute to the variation in the fertility results. To ascertain the effects of some components factors of the technical protocols of AI, in poultry fertility rates, were comparatively evaluated data in the file of Pesagro-Rio. We used the records of 480 White Leghorn chickens (two per cage) and 50 roosters selected with routine procedures for semen collection and AI performed as described in the artificial insemination manual of hens of the PESAGRO-RIO (Resende et al. 1983 PESAGRO-RIO, Technical Bulletin, n. 6. 16p.). The design was a randomized block in a factorial of two levels of factors of the number of AI by week (two and one); semen concentration (undiluted and diluted 1:1 in Locke-Ringer solution), dose volume (0.05 and 0.025 mL), semen preservation temperature (37 to 15°C) and in blocks periods during the process of AI (0-15, 15-30 and 30-45 minutes). The data were subjected to analysis of variance and t test in the Assistat Program (Silva, F. A. S., 2013. Assistat-Statiscal Assistence Software – UFCG, Paraíba, Brasil. http://www.assistat.com). The evaluation results showed that the fertility rates in relation of the levels of isolated factors were, respectively, 96.1 and 89.1% for two and one AI by week ($P<0.01$); 93.2 and 91.8% for the undiluted and diluted semen ($P>0.05$); 92.0 and 93.1% for doses of 0.05 and 0.025 mL ($P>0.05$); 91.5 and 93.3% for storage temperatures of 37 and 15ºC ($P>0.05$); 93.6, 91.6 and 92.3% for the preservation times of semen in periods of 0-15, 15-30 and 30-45 minutes ($P>0.05$). Interactions between treatments were not significant. Fertility rates were high, and only the number factor AI per week showed significant effect on fertility outcomes with the reproductive technique of AI in White Leghorn chickens.

A087 FTAI, FTET and AI

Effect of the number of daily observation on heat detection efficiency in dairy herds

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Keywords: dairy herd, detection efficiency, heat.

The aim of this trial was to compare the impact of the number of daily observation (2 vs 6 observation/day) on heat detection efficiency in Holstein dairy herds. The trial was conducted in three commercial herds located in Cuenca Mar and Sierras region in the south east of Buenos Aires province, Argentina. Herds 1, 2 and 3 had 74, 143 and 201 lactating cows, respectively. The average (±SE) production 305 days, DIM and number of lactation were: 7,536±1,565.2, 132.9±53.3 and 2.5±1.5 for herd 1; 7364±1242.3, 155.8±100.9 and 4.0±1.5 for herd 2; and 7,767.8±1,313.8, 89.5±46.2 and 2.3±1.7 for herd 3. The observations were done at 4:00 am; 08:00 am; 11:00 am; 03:00 pm; 06:00 pm and 09:00 pm during 45 min each. For the 2 observation group, only cows in heat at 8:00 am and 06:00 pm were considered. Tail paint was used as an auxiliary method for heat detection. The cow was considered in heat when standing heat was detected as well as if more than 50% of the tail paint has disappeared. Statistic evaluation was made evaluating proportion using the Test of Mac Nemar. Heat detection efficiency was increased when cows were observed 6 times daily (69.6%; 291/418) compared to two times daily (27.0%; 113/418: P=0.001). Considering each herd, heat detection efficiency for cows observed 2 and 6 times was respectively: 33.8% (25/74) and 75.7% (56/74) for herd 1 (P<0.001); 25.9% (37/143) and 50.3% (72/143) for herd 2 (P<0.001); and 25.4% (51/201) and 81.1% (163/201) for herd 3 (P<0.001). In conclusion 6 daily observation increases heat detection efficiency in dairy herds. This management can allow increased number of eligible cows to be inseminated (i.e. greater service rate) and thus improve reproductive efficiency in dairy herds.

A088 FTAI, FTET and AI

Supplementation with organic selenium source on the fertility of Nelore cows

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Keywords: FTAI, minerals, supplementation.

Selenium has been shown to be very important for reproduction. It has higher bioavailability in organic form. The aim of this study was to evaluate the influence of organic source of selenium composed by antioxidant, replacing the inorganic source of selenium on pregnancy rate Nelore cows. The experiment was realized in the Santa Marta farm, Campo Florido, MG. Nelore cows (n=338) were allocated into two groups: 1) Control- supplementation with inorganic source of selenium (Premiphos Monta®, Premix) and 2) Treatment- supplementation with organic source of selenium (EconamasE®, Alltech), both supplemented during all 90-days of the breeding season (BS). On day 50 of the BS a timed artificial insemination (TAI) was performed. Follicle diameter (ØDF) on D9 (i.e. one day after the intravaginal progesterone [P4] removal) of the TAI program, and corpus luteum diameter (ØCL), 7 days after TAI were measured by ultrassonography (DP-2200 Vet®, Mindray, China). Blood samples were collected for P4 concentration and lipid peroxidation (TBARS) analysis 7 days after TAI. Mean body weight before the beginning of supplementation, on day 50 (moment of TAI) and at the end of 90-d of the BS were evaluated. The TAI program consisted of D0: 2 mg estradiol benzoate (EB, RIC-BE®, Tecnopec-AgenerUnião) plus an intravaginal P4 device (Primer®, Tecnopec-Agener União); D8: P4 device withdrawal, 150 µg *d*-cloprostenol (Prolise®, Arsa, Argentina), 10 mg FSH*p* (Folltropin-V®, Bioniche, Canada) and 1 mg EB. On D10, cows were timed inseminated and pregnancy diagnosis was performed 45 d after TAI. The statistical analysis was accomplished by SAS program, using the variance analysis by Tukey and Chi-square test ($P<0.05$). Pregnancy per TAI and ØDF were similar between groups, respectively [Control = 42.2% (71/168) and 11.2±2.4 mm vs Treatment = 46.5% (79/170) and 10.9±2.1 mm]. However, ØCL (16.5±3.1 vs. 18.2±2.5 mm), P4 concentration (2.1±1.3.vs. 2.5±1.5 ng/mL), mean body weight (427.3±59 vs. 445.0±65 kg) and TBARS (0.097 vs. 0.039 n/mols) were different between groups (control vs. treatment, respectively). Organic mineral supplementation improved CL diameter, P4 concentration and lipid peroxidation levels; however, did not influence the pregnancy per TAI in Nelore cows.

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A089 FTAI, FTET and AI

Increasing the interval between luteolysis and AI decreases pregnancy loss in lactating dairy cows submitted to E2/P4 TAI program

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Keywords: estrus, luteolysis, pregnancy loss.

The hypothesis of this study was that increasing interval between luteolysis and artificial insemination (AI) would improve fertility to the timed AI (TAI) program. Lactating Holstein cows (n=759) yielding 30.5±8kg of milk/d, with a detectable CL at US (D-11) were randomly assigned to receive either one of the following treatments: 1) 3d interval between luteolysis and TAI: D-10 = CIDR insertion (CIDR®, 1.9g P4, Zoetis, São Paulo, Brazil) plus 2.0 mg im of estradiol benzoate (EB; Estrogin®, Farmavet, São Paulo, Brazil); D-3=$PGF_2\alpha$ im (25mg dinoprost tromethamine, Lutalyse®, Zoetis, São Paulo, Brazil); D-2 = CIDR removal plus 1.0 mg of ECP im (ECP®, Zoetis, São Paulo, Brazil); D0 = TAI; 2) 4d interval between luteolysis and TAI: D-11 = CIDR insertion plus 2.0 mg of EB im; D-4 = 25 mg of $PGF_2\alpha$ im; D-2 CIDR removal plus 1.0 mg of ECP im; D0 = TAI. Cows were considered synchronized following to the protocol when a CL was detected on D7 (i.e. 7 days after TAI). Pregnancy diagnoses were performed on D32 and D60. Data were analyzed by PROC GLIMMIX of SAS. Significance level was defined as $P<0.05$. The largest follicle diameter at TAI did not differ ($P=0.30$) between treatments (3d=14.7±0.39 x 4d=15.0±0.40mm). Synchronized cows treated with the 4d program tended ($P=0.06$) to have higher P4 concentrations at D7 (3.14±0.18ng/mL) than synchronized cows treated with the 3d program (3.05±0.18ng/mL). Although the P/AI at D32 [3d=45%(175/385) vs. 4d=43.9%(166/377)] and at D60 [3d=38.1%(150/385) vs. 4d=40.0%(154/377)] were not different between groups, the 4d program determined lower ($P=0.04$) pregnancy loss (7.6%;12/166) than the 3d program (14.7%;25/175). The P/AI at 60d was reduced ($P<0.01$) in cows that ovulated a smaller follicle (<11mm=37.2%; 22/66) or a larger follicle (>17mm=29.3%; 39/128), compared to cows that ovulated follicles between 11 and 17mm (49.1%; 197/395). The 4d program-treated cows were more likely (P<0.01) to display estrus (73.0%; 269/374) than 3d program-treated cows (63.4%; 240/385). The occurrence of estrus improved (P<0.01) the synchronization (97.4%; 489/501 vs. 81%; 202/248), P4 concentrations at D7 (3.22±0.16 vs. 2.77±0.17ng/mL), P/AI at D32 (51.2%; 252/489 vs. 39.4%; 81/202), P/AI at D60 (46.3%; 230/489 vs. 31.1%; 66/202); and reduced (P<0.01) the pregnancy loss (9.3%; 22/252 vs. 19.8%; 15/51), compared to cows that did not display estrus, respectively. Cows that did not display estrus within small (<11mm) or big follicles (>17mm) at TAI had higher pregnancy loss (P=0.01). However, in those cows that displayed estrus, the follicle diameter at TAI did not affect (P=0.97) the pregnancy loss. In conclusion, increasing the interval between luteolysis and TAI increased the estrus occurrence and reduced the pregnancy loss of lactating dairy cows submitted to E2/P4 TAI program.

A090 FTAI, FTET and AI

Progesterone treatment during the periovulatory period decreases embryo production in superovulated buffaloes

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Keywords: buffaloes, embryos, superovulation.

The present study evaluated the hypothesis that elevated progesterone (P4) during the expected multiple ovulations can improve the embryo recovery rate in superovulated buffalo donors due to decreased estradiol concentrations and contractility of genital tract. Buffalo donors cows were homogeneously assigned into 2 groups, according to age, body condition score, days postpartum and ovarian activity (presence of CL), in a cross over experimental design (35 d interval between superovulations): Control (C-G; n=8) and P4 treatment during the periovulatory period (P4-G; n=8). The follicular wave emergence was synchronized with an intravaginal P4 device (Sincrogest®, Ourofino Agronegócio, São Paulo, Brazil) plus 2 mg i.m. of estradiol benzoate (Sincrodiol®, Ourofino Agronegócio, Brazil) administrated at a random stage of the estrous cycle (Day 0=D0 AM). All buffaloes received 200 mg i.m. of FSH (Foltropin-V®, Bioniche Animal Health, Canada) twice-daily, in 8 decreasing doses starting on D4 AM. A dose of $PGF_{2\alpha}$ (530µg i.m. Sincrocio®, Ourofino Agronegócio, Brazil) was given on D6 PM and on D7 AM. The P4 device was removed from cows of the C-G on D7 PM and from cow of the P4-G on D10 PM. On D8 PM, all buffaloes received 25 mg i.m. of pLH (Lutropin-V®, Bioniche Animal Health). The timed inseminations were done 12 and 24 h after the pLH treatment. Blood samples were collected from D7 (PM) to D11 (AM) for further progesterone assay. The structures (ova/embryos) were collected nonsurgically 6 days after the second TAI (D14 PM). Transrectal ovarian ultrasound examinations (Mindray, DP2200Vet, China) were performed on D0 to verify ovarian activity, on D8 PM and on D14 to verify respectively, the superestimulation and the superovulatory responses. The variables were analyzed by GLIMMIX procedure of Statistical Analysis System (SAS®). On D8, was verified similar number of follicles >8mm in both groups (P4-G=12.1±3.2 vs. C-G=11.0±2.7; P=0.68). Buffaloes from P4-G group showed lower ovulation rate (13.5±4.9 vs. 71.5±16.1 %; P=0.002) and lower number of CLs on D14 (1.1±0.3 vs. 8.0±2.8; P=0.04) than buffaloes from C-G group, respectively. The number of recovered structures, transferable and freezable embryos to C-G and P4-G were: 0.0±0.0 and 1.9±0.7 (P=0.03); 0.0±0.0 and 1.6±0.7 (P=0.04); 0.0±0.0 and 1.6±0.7 (P=0.04). The serum progesterone concentration measured from D7 to D11 was greater in the P4-G (1.87±0.13) than in the C-G (0.48±0.10, P <0.0001). It is possible that the high serum progesterone concentration due to the maintenance of the intravaginal P4 device during the periovulatory period was responsible for reducing the ovulation and the absence of embryonic structures in P4-G, rejecting the present hypothesis.

A091 FTAI, FTET and AI

Cooled semen for fixed-time artificial insemination in cattle

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Keywords: *Bos indicus*, dilution, synchronization.

Cryopreservation process causes damage in the cells that affects the viability and may compromise the pregnancy per artificial insemination (P/AI) and reproductive efficiency. Thus, the aim of this study was to evaluate the possible use of cooled semen in fixed-time artificial insemination (FTAI) program compared with frozen semen to improve P/AI in beef cattle. Ejaculates of three bulls were collected and divided into two treatments: 1) frozen semen; and 2) chilled semen, maintained at 5°C, for 24 h. All proceedings were the same until the cooling process. Egg-yolk extender without glycerol was used for the cooling process. Straws of 0.25 mL with $25x10^6$ sperm were submitted to the cooling process in a cooled room for the cryopreservation process (-196°C); or in a Botu-Tainer® box for preservation at 5°C until 24 h later, when FTAI was performed. The straws were thawed at 35°C, for 20 sec. Chilled semen did not undergo the thawing process and it was used immediately after being removed from the box. Semen samples were evaluated after collection, dilution, post-thaw and cooling process for motility, termoresitance and hyposmotic test, by the same operator. Postpartum multiparous Nelore cows (n=838) were submitted to FTAI with the following protocol: D0: estradiol benzoate (EB, 2mg, RIC-BE®, im, Tecnopec-Agener União) and a progesterone device (Primer®, Tecnopec-Agener União); D8: device withdrawal, *d*-cloprostenol (PGF2α, 150µg, Prolise®, Arsa, Argentina), 1mg EB plus 300 IU eCG (Novormom®, Syntax, Argentina) im administrations; and D10: AI using frozen semen (n=408) or cooled semen (n=430). The statistical analysis was accomplished using the GLIMMIX procedure of SAS for pregnancy rate and with ANOVA followed by Tukey for complementary tests ($P<0.05$). There was ~20% increase in pregnancy rate with the use of cooled semen compared with frozen semen (59.9% vs. 49.4%; $P<0.05$). There was no difference among bulls for pregnancy ($P=0.39$). Pregnancy rates for bull semen frozen and cooled were, respectively, 45.8% vs 55.4% (bull 1, n=330), 56.4% vs 76.4% (bull 2, n=114) and 51% vs 56.2% (bull 3, n=394). Frozen semen had less viable sperm than cooled semen evaluated by sperm motility (61.7%±7.6 vs 81.0%±10.4), thermoresistance (41.7%±7.4 vs 66.7%±10.4) and hyposmotic (38.3%±7.6 vs 53.7%±9.9) tests. The use of cooled semen from bulls genetically evaluated in breeding programs, compared with frozen semen, increased the P/AI in multiparous postpartum Nelore cows submitted to a FTAI protocol. This is an interesting alternative to increase the reproductive outcomes and reduce the costs of the FTAI program.

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A092 FTAI, FTET and AI

The economic impact of the use of TAI or natural service to rebreed cows submitted to TAI programs on cow-calf operation

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Keywords: costs, Nelore, pregnancy.

The use of timed artificial insemination (TAI), associated with natural service for rebreed non-pregnant cows, adds the costs of the synchronization for TAI program with the necessity of maintenance of bulls, that could reduce the profitability of cow-calf production system. At the same time, there is a wide variety of hormonal protocols and cost of semen dose, which represent an alternative to improve the cost-effectiveness of the reproductive program. This experiment aimed to evaluate the cost-benefit ratio when using only TAI (three inseminations) and TAI (two inseminations) associated with the rebreeding using bulls. Herein was evaluated the pregnancy outcomes obtained and the cost per pregnancy produced in each system. One hundred cows and one hundred Nelore heifers were randomly divided into four groups: G1 - Heifers + three TAI; G2 - Heifers two TAI + rebreeding using bulls (NS); G3 - Cows + three TAI; G4 - Cows two TAI + NS. At a random day of the estrous cycle (D0), the animals received an ear implant containing 3 mg of norgestomet (Crestar®, MSD Animal Health, São Paulo, Brazil) and intramuscular injection of 2 mg of estradiol benzoate (Gonadiol®, IM, MSD Animal Health, Brazil). The ear implant was removed on D8, along with intramuscular administration of 300 IU of equine chorionic gonadotropin (Novormon®, MSD Animal Health, São Paulo, Brazil), 1 mg of estradiol cypionate (E.C.P®, IM, Zoetis, São Paulo, Brazil) and 350 mg Sodium cloprostenol (Ciosin®, MSD Animal Health, São Paulo, Brazil). The animals were timed inseminated on D10 and the pregnancy diagnosis was performed 28 days after each TAI. Non-pregnant females were submited to a new synchronization protocol. The groups 1 and 3 were submited to three TAI synchronization protocols. Groups 2 and 4 underwent two TAI synchronization protocols and eight days after the second TAI, females were placed together with bulls for adding 45 days. Twenty-eight days after the removal of the bulls the pregnancy diagnosis was performed by ultrasound (Mindray Vet-2200) in all breeding groups. Data were analyzed by ANOVA using F test (SISVAR). There was no difference (Tukey, $P<0.10$) in overall pregnancy rates obtained: 78.85% with production cost of R$ 66.91 per pregnancy in G1 group, 87.96% of pregnancy rate with cost per pregnancy of R$ 76.51 in the G2 group, 85.42% of pregnancy rate with a cost of R $ 56.36 in the G3 group, and 90.57% pregnancy rates with a cost of R$ 81.47 in the G4 group. In addition, the costs for production of calves did not differ among treatments. Considering the value of marketing on average R$ 700.00 for calves weaned at eight months old, the costs represent 9.5% of the calves produced for G1, 10% for G2, 8% for G3 and 12% for group 4. Therefore, it is possible to achieve good reproductive rates at the end of the breeding season with satisfactory cost-benefit ratio using only three TAI programs without the use of bull service.

A093 FTAI, FTET and AI

Which is the best protocol for cervical dilatation in sheep embryo collection?

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Keywords: ketamine, misoprostol, oxytocin.

The development of methodologies for the transcervical embryo collection in sheep is strongly encouraged by the sequelae caused by conventional surgical methodology. However, this technique is impaired by the fact that, on average, only 30-40% of the animals have a pervious cervix. Three transcervical approaches in ewes are described: prostaglandin E (PGE; Gusmão et al., 2007, Rev. Bras. Saúde Prod. An. 8, 1-10), estradiol+oxytocin (EO; Masoudi et al., 2012, Afr. J. Biotech. 11, 2803-2806) or subarachnoid anesthesia with ketamine (AK; DeRossi et al., 2009, Small Rumin. Res. 83, 74-78). However, there is no comparative information on what is the most efficient. Thus, this study was designed in order to compare the cervical dilator efficiency of treatment with PGE analogue (Misoprostol; Prostokos®, Hebron, BR), EO (Estradiol benzoate; Estrogin®, Farmavet, BR + Synthetic oxytocin; Placentex®, Agener União, BR) or AK (Ketamine; Dopalen®, Vetbrands, BR) in Crioula Lanada ewes. Initially, the cervical transposition of the animals (n=18) was evaluated under physiological dilation (estrus) using a Hegar's dilator № 2 in a maximum period of 7min. The cervixes were classified as fully pervious (FP, ≥6 cm), partially pervious (PP, from 4 to 5.9 cm) and non-pervious (NP, ≤ 3.9 cm) for homogeneous distribution of animals (n=6) across treatment groups in order to evaluate the effect of cervical dilator treatments at diestrus (Day 6). On Day 5, 12h before the evaluation, animals from PGE and EO groups received one intravaginal tablet of misoprostol (200µg) and an IM injection of estradiol benzoate (100µg), respectively. On the morning of Day 6, the animals from PGE group were immediately evaluated and those from EO and AK groups were evaluated 15min after an IM application of oxytocin (100 IU) and a subarachnoid injection of ketamine (1.5mg/kg), respectively. The evaluation during estrus determined that 44.4%, 22.2% and 33.3% of the animals had FP, PP and NP cervixes, respectively. During diestrus, in PGE group one animal maintained FP classification and another went from PP to FP while the others were NP. In this group it was observed that most of the intravaginal tablet was expelled by the animals, which probably impaired the effect on cervical dilatation. In the EO group, one of two FP animals went to PP and from two initially PP one had increased in the depth and the other became FP. From two NP animals one became PP and the other FP, resulting in 83% dilatation. In AK group, from three classified as FP, one became NP and the PP animal became NP. In this group, beyond the low cervical dilator response, one animal had complications from an unexpected reaction during insertion of the needle into the subarachnoid space. In conclusion, due to the increased possibility of cervical transposition, the treatment with a combination of estradiol benzoate + oxytocin is the most suitable for transcervical embryo collection in sheep.

A094 FTAI, FTET and AI

Ovulation and pregnancy rates in Nelore cows submitted to timed AI

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Keywords: FTAI, Nelore, ovulation.

The number of cows inseminated by the fixed-timed artificial insemination (TAI) in Brazil has been largely increased due to the simplification of the necessary management and the possibility of insemination with no needs of estrus detection, especially in large scale beef herds. Several pharmacological protocols for the control of the estrus and ovulation of cattle have been proposed to improve the TAI efficiency and facilitate the reproductive management. An important change in the traditional protocol called PEPE (progesterone [P4]-estradiol-prostaglandin-estradiol) is using estradiol cypionate on the same time of implant removal, reducing the managment with the animals. The aim of this field study was to compare two TAI protocols using estradiol cypionate or estradiol benzoate (EB) compared with the use of PEPE synchronization protocol during the 2012/2013 breeding season Also, it was evaluated the possibility to perform the AI after the detection of estrus signs on the day of the beginning of the protocol (D0). Multiparous Nelore cows (n=96) of a commercial herd, with more than 45 days postpartum, were assigned into one of two homogeneous groups and subjected to different TAI protocols. The two management protocol had P4 device insertion (Sincrogest, OuroFino Animal Health, São Paulo, Brazil) and 2.0mg im of EB (Gonadiol®; MSD, São Paulo, Brazil) on a random day of the estrous cycle (D0). Eight days later, de P4 device was removed and 1.0mg of EB and 12.5 mg of dinoprost (Lutalyse®, Pfizer, São Paulo, Brazil) were adminstred im. The TAI was performed 54 to 56 hours (D10) after the device removal. In the PEPE protocol, the P4 device insertion and 2.0mg EB was adminstred on D0. On D7, the P4 device was removed and dinoprost was administrated. Twenty four hours later, 1.0 mg of EB was administrated and the TAI 34 to 36 hours after the last EB treatment (D9). All animals underwent a gynecological examination by transrectal ultrasonography (US) to confirm the presence of a dominant follicle (DF) at the time of TAI. Pregnancy diagnosis was performed 30 days after TAI by US. In two management protocol, 10.41% (5/48) of the cows became pregnant and 30% (12/40) showed FD at the TAI. However, in the PEPE protocol, 43.75% (21/48) of the cows became pregnant and 91.89% (34/37) had FD at the TAI moment. In the beginning of the protocols, 20 cows showed estrus signs and were inseminated 10 to 12 h after (D0), but none became pregnant. A simple observation of estrus signs in the first day of the protocol is not sufficient to carry out the AI. Changes in pharmacological synchronization protocols should be carefully considered before its indication for commercial use.

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A095 FTAI, FTET and AI

Follicular dynamic of Holstein cows superstimulated with FSH associated with a slow release diluent for *in vivo* embryo production programs

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Keywords: donors, non lactating cows, superovulation.

The aim of the present study was to evaluate the follicular dynamic of Holstein cows submitted to superstimulation protocols with FSH (Folltropin, Agener Union, Brazil) associated with a diluent with slow release carrier (hyaluronic acid; MAP5, Bioniche). The cows (non-lactating) were randomly allocated into one of two treatments: Folltropin (FOLL, n=8) or Folltropin diluted in MAP5 (MAP5, n=8). In random day of the estrous cycle (D0) females received an intravaginal P4 device (Primer, Tecnopec) and 2.0 mg IM of EB (RIC-BE, Tecnopec). From D4 on, FOLL group received 300 mg of Folltropin diluted in 20 mL of saline and administrated in 8 decreasing doses, with an interval of 12h. The MAP5 group received the same dose of Folltropin diluted with MAP5 and fractionated into two applications in decreasing doses (5.0 and 2.5ml) on day D4 and D6. On D6 (am and pm) 0,150 mg of cloprostenol (Estron, Tecnopec) were administered. Still, P4 implants were removed on D7 pm and 12h later 62.5µg IM of Lecirelin (Gestran, Tecnopec) were administered. Ultrasonography examinations were performed on D4, D6 and D8 to quantify and measure the ovarian follicles. Follicles were classified as small (SF;Ø<8mm), medium (MF Ø≥ 8 and <10mm) and large (LF;Ø≥10mm). Still, on D15 the number of CL was recorded to evaluate the ovulation rate (number of CL on D15 per number of LF). Statistical analysis was performed using the GLIMMIX procedure of SAS. Data are presented as mean±SEM or percentage. Values with superscript letters within treatment ($^{A\neq B\neq C}$) and within time ($^{x\neq y}$) differ. In both treatments there was a reduction in the number of SF over time, however, the decrease was more accentuated in the FOLL group (MAP5- D4:16.1±2.7^{Ax}, D6:11.3±2.7^{Bx} and D8:6.3±3.0^{Cx}; FOLL-D4:18.4±4.0^{Ax}, D6:6.1±1.7^{By} and D8:2.1±0.9^{Cy}; P_{TREAT}=0.002, P_{TIME}<0.0001 and $P_{TREAT*TIME}$=0.0002). Regarding the number of MF, unlike MAP5 group (D4:0.9±0.4^{Bx}, D6:2.4±0.9^{Ax} and D8:2.1±0.9^{ABx}), FOLL group had an increased on D6 (9.7±3.2^{Ay}) compared to D4 (1.1±0.4^{Bx}) and D8 (0.1±0.1^{By}; P_{TREAT}=0.39, P_{TIME} <0.0001 and $P_{TREAT*TIME}$=0.0007). Also, the number of LF, unlike MAP5 group (D4: 1.3±0.4^{ABx}, D6: 0.9±0.2^{Bx} and D8: 2.6±0.5^{Ax}), in FOLL group was increased over time (D4: 1.9±0.3^{Cx}, D6: 3.9±0.8^{By} and D8: 16.1±4.2^{Ay}; P_{TREAT}<0.0001, P_{TIME}<0.0001 and $P_{TREAT*TIME}$=0.02). Further, an increased proportion of LF (number of MF and LF per total follicles) over time was observed for both treatments, however, the increase was more evident in the FOLL group (MAP5– D4:6.9%^{Bx}, D6:6.0%^{Bx} and D8:23.9%^{Ax}; FOLL–D4:8.7%^{Cx}, D6:19.6%^{By} and D8:87.6%^{Ay}; P_{TREAT}<0.0001, P_{TIME}<0.0001 and $P_{TREAT*TIME}$<0.0001). Finally, ovulation rate was lower in cows from MAP5 group (33.3%) compared to FOLL group (69.1%; P=0.01). In conclusion, the association of FSH with MAP5 does not promote a superstimulation response with the same efficiency as FSH in non-lactating Holstein cows submitted to superstimulation protocols.

Acknowledgments: Agrindus and Tecnopec.

A096 FTAI, FTET and AI

Progesterone plasma concentration in non-lactating Holstein cows during reuse of intravaginal progesterone device, previously autoclaved or disinfected

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Keywords: *Bos taurus,* device, hormone.

Intravaginal devices of progestogens/progesterone (P4) have been used for decades with the aim of controlling the estrous cycle in farm animals. Although the manufacturer`s recommendation be just one use, reuse has been widely reported, due to residual P4. Various ways have been used to process devices prior to reuse. However, no comparisons between different types of devices have been reported. Thus, the aim of this study was to compare plasma concentrations of P4 during the reuse of two types of intravaginal P4 devices previously autoclaved or disinfected with 8 d of use in 24 Holstein cows. Non-lactating multiparous cycling cows (~600 kg of BW and BCS 3) were used. They were kept in confinement, fed maintenance diet and water *ad libitum*. In a 2x3 factorial arrangement and two replicates, all animals underwent two treatments. Two sources of P4 (CIDR® [C], Zoetis, and Sincrogest® [S], Ourofino) and three types of processing: new (N), reused autoclaved (RA) and reused disinfected (RD) were used. The cows remained 8 d with a new device. At 7 and 8 d after device insertion, 25 mg of dinoprost ($PGF_2\alpha$; Lutalyse®, Zoetis) was administered and on Day 8, after the withdrawal of P4, a Norgestomet (Crestar®, MSD) ear implant was inserted, which was maintained for 24 h. On Day 9, the cows were randomized in one of six treatments (NC, RAC, RDC, NS, RAS and RDS). The devices were kept for 8 d and during this period blood samples were collected at the following times: 0, 2, 12, 24, 48, 72, 96, 120, 144, 168 and 192 h. At the last day, the P4 devices were removed and Norgestomet was inserted again, and maintained for 24 h, together with other $PGF_2\alpha$ treatment. Then, the second replicate began. Statistical analysis was done by the Proc-mixed and the averages and standard error of P4 concentrations were calculated by the Proc-means of SAS 9.2 ($P<0.05$). Differences were found between the types of devices (1.34±0.04 and 1.08±0.04 ng/mL; CIDR and Sincrogest, respectively) and interaction between time and type of processing. Regarding interaction, differences were observed for RA, RD and N, respectively, at 2 h (1.58 ± 0.23^{a}, 0.76 ± 0.11^{b} and 1.07 ± 0.11^{ab} ng/mL), 12 h (2.09 ± 0.20^{a}, 1.30 ± 0.10^{b} and 1.75 ± 0.17^{ab} ng/mL), 24 h (1.97 ± 0.16^{a}, 1.06 ± 0.10^{b} and 1.42 ± 0.10^{b} ng/mL), 48 h (2.01 ± 0.15^{a}, 1.27 ± 0.11^{b} and 1.77 ± 0.14^{a} ng/mL), 72 h (1.65 ± 0.11^{a}, 1.16 ± 0.11^{b} and 1.52 ± 0.09^{a} ng/mL) and 96 h (1.48 ± 0.11^{ab}, 1.18 ± 0.12^{a} and 1.56 ± 0.10^{b} ng/mL). It is concluded that in non-lactating Holstein cows, the mean plasma concentration of P4 was higher for CIDR than Sincrogest and regardless of the type of device, the autoclaving process provided higher circulating concentrations of P4, in relation to disinfected, and similar or higher compared to the new.

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A097 FTAI, FTET and AI

Effect of early or late resynchronization on reproductive performance of dairy cows observed for estrus

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Keywords: dairy cow, PAG, resynchronization.

The objective was to evaluate reproductive performance of dairy cows subjected to early (ER) or late resynchronization (LR) after nonpregnancy diagnosis. Holstein cows (n=972) were subjected to the Ovsynch protocol (D0 GnRH, D7 $PGF_{2\alpha}$, D9 GnRH, D10 AI) for first AI at 68 d in milk. Weekly cohorts of cows were blocked by parity and randomly assigned to ER, based on nonpregnancy diagnosis using pregnancy associated glycoprotein (PAG) ELISA in blood, or LR based on palpation. ER cows received GnRH 2 d before PAG testing between 27 and 33 d after the previous AI, and not reinseminated nonpregnant cows continued on the Ovsynch for timed AI (TAI). LR cows had pregnancy diagnosed by transrectal palpation between 36 and 49 d after AI and those not reinseminated nonpregnant were resynchronized with the Ovsynch starting on the day of nonpregnancy diagnosis. After the first AI, all cows were observed for estrus based on removal of tail chalk and those in heat were inseminated on the same day. The study lasted 70 d for ER and 112 d for LR to allow a maximum of two resynchronized timed AI for each treatment in cows not observed in estrus. A cow was considered pregnant at the end of the study based on palpation at 36 to 49 d after AI. Categorical and continuous data were analyzed with the GLIMMIX procedure of SAS. Time to pregnancy was analyzed using the Cox's proportional hazard model with the PHREG procedure of SAS. The sensitivity (Se), specificity (Sp), positive (PPV) and negative (NPV) predictive values of using PAG for diagnosis of pregnancy were calculated at different intervals after AI. Pregnancy per AI at first AI did not differ between treatments and averaged 28.9%. Cows in ER tended (P=0.09) to become pregnant faster after the first AI than LR cows (63 vs 73 d, respectively; adjusted hazard ratio=1.25; 95% CI=0.96-1.65). The proportion of cows resynchronized that were not pregnant to first AI was greater (P<0.01) for ER than LR (29.9 vs. 8.5%). A total of 2,129 test diagnostics for PAG were evaluated. Se (true pregnant) and Sp (true nonpregnant) of PAG for pregnancy diagnosis, according to days after AI, were: ≤ 27 d: 94.6% and 89.9%; 28-30 d: 96.1% and 90.7%; 31-35 d: 98.7% and 88.1%; > 35 d: 94.4% and 85.2%. Overall, Se was 95.1% (95% CI = 93.6-96.3), Sp was 89.0% (95% CI = 86.9-90.8), PPV was 90.1% (95% CI = 88.3-91.8), NPV was 94.5% (95% CI = 92.8-95.8), and accuracy was 92.1%. In summary, early diagnosis of nonpregnancy based on PAG with ER increased submission to TAI and tended to reduce interval to pregnancy in cows observed for estrus. The benefits of early resynchronization with a negative PAG diagnosis need to offset the 4.9% iatrogenic abortion.

A098 FTAI, FTET and AI

Effects of reutilization of a progesterone device on follicular dynamics in ewes

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Keywords: hormonal protocol, ovulation, sheep.

The present study aimed to evaluate the effect of reutilization of a progesterone (P4) device for small ruminants on follicular dynamics of crossbred Santa Inês ewes in 9 days-synchronization protocol. Two consecutive replicas were performed, with nine ewes on each. The animals were randomly divided into two treatments: Control Group (CG) and Reused Group (RG). CG ewes received new intravaginal device on day 0 (D0). The device was removed on day 9 (D9) when 35µg of d-cloprostenol (Prolise®, Syntex, Argentina) and 250 IU of eCG (Folligon®, Intervet, Holland) were administrated via IM. The RG ewes were submitted to the same protocol, although the P4 device had been previously used in a similar protocol. Ultrasound exams (Chison, 9300VET, Kylumax, Brazil) were performed every 24 h from D0 to D9, and every 12 h from removal of the device until ovulation time. Statistical analyses were performed by General Linear Models (GLM) - Statistical Analyses System (SAS) after residuals normality and homoscedasticity were tested. Level of significance of 5% was considered for statistical analyses. No difference was observed between the groups on series of comparisons regarding the size of the follicle: 1) diameters of the first largest follicle (CG: 4.0±0.2 *vs*. RG: 4.1±0.2 mm, P=0.75) and the second largest follicle (GC: 3.1±0.1 *vs*. GR: 3.1±0.4 mm, P=0.83) at the moment of device removal; 2) maximum diameters of the first largest preovulatory follicle (GC: 5.7±0.2 *vs*. GR: 5.5±0.2 mm, P=0.09) and the second largest preovulatory follicle (GC: 4.7±0.2 *vs*. GR: 4.7±0.1 mm, P=0.89). Also, no difference was observed between groups on the duration of the ovulatory follicular wave duration (CG: 5.0±0.4 *vs*. RG: 5.0±0.5 days, P=0.93) and; in the interval between device removal and second ovulation (CG: 70.5±5.3 *vs*. RG: 73.2±3.0 h, P=0.67) and the day of ovulatory emergency (CG: 7.1±0.4 *vs*. RG: 6.8±0.6 days, P=0.76). Only significant difference between groups was observed when comparing the interval from P4 device removal to first ovulation (CG: 71.7±2.5 *vs*. RG: 63.9±2.7 h, P=0.05). The reutilized device had similar results on ovarian follicular dynamics of ewes, although first ovulation may occur earlier on ewes that received the reused device than on ewes with new devices.

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A099 FTAI, FTET and AI

Relationship between bacterial isolation and somatic cell count on fertility of dairy cows receiving IVP embryos

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Keywords: bacterial isolation, IVF embryos, mastitis.

The aim of this study was to evaluate the relationship between bacterial isolation and somatic cell count (SCC) and its impact on maintenance of pregnancy in embryo recipient dairy cows. In experiment 1, two milk samples were collected from 2,532 Holstein and Girolando dairy cows (nine farms), with average DIM 175.1±9.2, for analysis of SCC and microbiological culture to determine bacterial growth. In experiment 2, 1,397 Girolando lactating cows with average DIM 132.9±6.2, were subjected to a fixed-time synchronization protocol for *in vitro* produced embryo (IVP) transfer (FTET). Milk samples were collected two days before the FTET for microbiological culture and SCC analysis. Pregnancy diagnosis was performed 28 days after FTET. The bacterial isolation was carried out in the Vidavet laboratory (Botucatu-SP, Brazil) and SCCS analysis was performed at the "Clínica do Leite" (Piracicaba-SP, Brazil). The animals were divided according to the bacterial isolation into 5 experimental groups according to the NMC: Gram-positive environmental (AB +; *Bacillus spp. Enterococcus spp. Streptococcus spp*.), Gram-negative environmental (AB-; Coliforms, *Enterobacter spp., Klebsiella spp., Proteus spp., Pseudomonas spp*.), Gram-positive contagious (C +; *Corynebacteriumbovis, Staphylococcus aureus, Streptococcus agalactiae*), CNS (Coagulase Negative Staphylococcus) and control (no bacterial isolation). The CNS were grouped with the control group in Experiment 2 analyzes, because the isolation of these agents is not usually associated with mammary gland disease. The animals were also classified into three groups according to the SCC: less than $2x10^5$ cells/mL, between $2x10^5$ and $4x10^5$ cells/mL, and greater than 4 $x10^5$ cells/mL. The SAS PROC GLIMMIX was used for statistical analysis. Results were considered significant if $P<0.05$ and have a tendency to differer if $P<0.10$. In experiment 1, the sensitivity of detection the causative agents of mastitis in samples with SCC lower than $2x10^5$ cells/mL was 60.32%. In cows with SCC less than $2x10^5$ cells/mL bacterial growth was detected in 27.2%, 51.4%, 38.9% and 51.4% of the samples in group C+, SCN, AB+ and AB-, respectively. In Experiment 2, there was an effect of bacteria group on the pregnancy rate ($P=0.0007$.) The pregnancy rate in [AB-] (30.1%; 68/201) and [AB +] (29.9%; 24/82) groups was lower than the control group (44.0; 367/869) and pregnancy rate of the [C +] (36.6%; 85/245]) group tended ($P=0.09$) to be lower than the control group. The group with SCC greater than 400,000 cells/mL had lower pregnancy rate ($P<0.01$) compared to those with less than $2x10^5$ SCC cells/mL. The group with SCC between $2x10^5$ and $4x10^5$ cells/mL tended ($P=0.09$) to have lower pregnancy rate than the group with SCC less than $2x10^5$ cells/mL. Even in milk samples with SCC considered low ($<2x10^5$ cells/mL) isolation of the causative agents of mastitis was detected. The presence of the causative agents of mastitis decreased pregnancy rate of dairy cows.

A100 FTAI, FTET and AI

Use of resynchronization as a tool to enhance the genetic improvement and reproductive performance in a *Bos indicus* beef cattle herd

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Keywords: pregnancy, resynchronization, TAI.

The economy efficiency of livestock is linked to calves production, which are destined for meat production or herd replacement. In this context, the reproductive efficiency of cows shows up as well an important objective to be achieved (Sá Filho *et al*., 2008, 3° Simpósio Internacional de Reprodução Animal Aplicada, 54). This study aimed to investigate the reproductive performance of a *Bos indicus* beef cattle herd submitted to two TAI protocols (synchronization and resynchronization) before the first 45 days of the breeding season (BS). Thus, a total of 333 multiparous beef cows between 30 and 60 days postpartum were used. All females were subjected to the same TAI protocol at the beginning of the BS. At the beginning of the treatment (D0), the animals received an intravaginal P4 device (1.9 g P4; CIDR®, Zoetis) and 2.0 mg estradiol benzoate i.m. (Ric-BE®, União Química). On D9, the females received 7.5 mg of PGF2α i.m. (Lutalyse®, Zoetis), 1.0 mg of estradiol benzoate i.m. (Ric-BE®, União Química) and 400 IU of eCG i.m. (Novormon®, MSD Animal Health), followed by removal of P4 device. The TAI was performed 48 h after the removal of the intravaginal device (D11). At the time of pregnancy diagnosis, which was carried out 30 days after TAI, the cows diagnosed as non-pregnant, were again subjected to the same TAI protocol (resynchronization). Pregnancy diagnosis of resynchronized cows was also performed 30 days after the second TAI. Pregnancy rates were calculated using the *Freq* procedure of SAS, and the difference between the rates of synchronization and resynchronization was calculated by Chi-square test (χ^2), with a significance level of $P<0.05$. It was observed similar pregnancy rate ($P=0.68$) among cows submitted to the first TAI (51.65%, 172/333) or resynchronization (49.69%, 80/161). However, when considering the cumulative pregnancy rate of the two TAI protocols performed in sequence, it was found that before mid-breeding season (41 days) 75.68% (252/333) of females available for reproduction had conceived. Therefore, it was concluded that the technique of TAI maintains the same efficiency to both, first synchronization and resynchronization. In addition, resynchronization could serve as a tool that enhances the reproductive performance and genetic improvement, since most of the females will create products of artificial insemination.

A101 FTAI, FTET and AI

Fixed-time transcervical artificial insemination in sheep using cooled semen

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Keywords: cooling, ram, spermatozoa.

Dilution allows fresh ram semen to be multiplied and used immediately in artificial insemination (AI). Cooling diluted semen to 5°C reduces the motility and metabolic activity of the spermatozoa and allows its storage for several days, which further favors its use in AI. The use of both fresh and cooled semen in AI routines presents, besides practical and economic advantages over frozen semen (Mara et al., 2005 Theriogenology 63, 2243-2253), greater sperm viability and better fertility rates (Wusiman et al., 2012 Asian J. Anim. Vet. Adv. 7, 299-308). The aim of this study was to evaluate the fertility of semen cooled up to 72 hours in Santa Inês ewes after fixed-time transcervical artificial insemination (FTTCAI). Semen samples from six rams were diluted in Glycine-yolk-milk extender (Rodello et al., 2011 Vet. e Zootec. 18, 239-248), packaged (100 x 10^6 spermatozoa/mL in 0.25 mL) and divided, part for immediate use as fresh semen (FS-control) and part stored at 5 ºC as cooled semen (CS) for 24 (CS-24), 48 (CS-48) and 72 hours (CS-72). A total of 242 nulliparous and multiparous ewes underwent estrus synchronization protocol (Biscarde et al., 2010 Rep. Fert. Develop., 22, 376-377): first day of synchronization (D0), 45 μg of prostaglandin; D3, insertion of sponge impregnated with 60 mg of medroxyprogesterone acetate; D7, sponge removal, 400 IU of equine chorionic gonadotropin and detection of estrus by teasing every 12 hours until the moment of AI; D8, 25 μg of gonadotropin-releasing hormone. The AI was performed, on average, 52 hours after sponge removal with use of cervical traction and Aplicador Expansor Ovino® (Alta Genetics, Brazil). Pregnancy rate and prolificacy data were analyzed by chi-square and Kruskal-Wallis test, respectively, both at a significance level of 5%. The overall pregnancy rate obtained (29%) was not significantly affected (P>0.05) by the semen cooling time: FS = 30%; CS-24 = 23%; CS-48 = 21%; and CS-72 = 26%. The semen cooling time did not affect (P>0.05) prolificacy (160%). It was concluded that it is possible to maintain ram semen fertility for at least three days under cooling at 5°C, enabling this practical technology to be used in semen transportation for artificial inseminations over long distances.

A102 FTAI, FTET and AI

Different hormonal stimulus during synchronized proestrus alter the ovarian follicle responses and subsequent luteal function in suckled anestrous zebu cows

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Keywords: estradiol, progesterone, reproduction.

The present study evaluated the effects of treatment with estradiol cypionate (ECP) or equine chorionic gonadotropin (eCG) during the synchronized proestrus on follicular and luteal characteristics and pregnancy rates in suckled anestrous zebu cows. A total of 172 suckled Nelore cows, received a used intravaginal progesterone (P4) device (Sincrogest®, Ouro Fino Agribusiness) and 2 mg im estradiol benzoate (Sincrodiol®, Ouro Fino Agribusiness). Eight days later, devices were removed and cows received 0.25 mg i.m. of cloprostenol (Sincrocio®, Ouro Fino Agribusiness). At this moment, females were randomly assigned into one of four treatments (control, ECP, eCG and ECP+eCG) in a 2x2 factorial design.Cows in the control group received no further treatment, while cows in the ECP group received 1mg ECP (ECP®, Zoetis Brazil) and those in the eCG group received 400 IU eCG (Folligon®, MSD Animal Health) at the same moment of the P4 device removal. Cows in the ECP+eCG group received both treatments at the same time previously mentioned. Cows had their tailhead painted using chalk marker at the time of P4 device removal to evaluate the occurrence of estrus. Immediately before TAI, all the cows were treated with 10µg im buserelin (Sincroforte®, Ouro Fino Agribusiness). Plasma P4 concentration (ng/mL) was evaluated 7 days after GnRH treatment. Data were analyzed using SAS Proc GLIMMIX. There was no interaction between treatments in any response variables (P>0.05). The DF diameter at device removal was similar between eCG (11.2±0.3 mm, n=86) and no eCG groups (10.9±0.3, n=86 P=0.38). However, an increased DF diameter at TAI (12.6±0.3 vs. 13.5±0.3 mm, P=0.03), dominant follicle growth rate (mm/day) from P4 device removal to TAI (0.9±0.1 vs. 1.2±0.1 mm, P=0.01), occurrence of estrus (46.4% vs. 63.7%; P=0.03), ovulation rate (82.6% vs. 96.7%, P=0.008), diameter of corpus luteum (CL; 18.2±0.4 vs. 19.4±0.4 mm, P=0.04) and plasma P4 concentration (3.9±0.2 vs. 4.8±0.2; P=0.001) was observed in the eCG group. Though, the eCG treatment did not affect the pregnancy per AI (P/AI; 36.9% vs. 43.1%, P=0.42). Similary, the DF diameter at P4 device removal was similar between ECP (11.1±0.3, n=85) and no ECP groups (11.1±0.3, n=87; P=0.90). Futhermore, the ECP treatment did not affect the DF diameter at TAI (13.0±0.3 vs. 13.1±0.3, P=0.90), dominant follicle growth rate (mm/day) from P4 device removal to TAI (1.1±0.1 vs. 1.0 ± 0.1, P=0.52), ovulation rate (90 6% vs. 93.6%, P=0.54), CL diameter (19.0±0.4 vs. 18.6±0.4, P=0.54) and plasma P4 concentration (4,4±0,2 vs. 4,4±0,2; P=0,94), however increased occurrence of estrus (44.6% vs. 65.4%, P=0.008) and P/AI (33.2% vs. 47.1%, P=0.07) was observed (no eCG and eCG groups, respectively). It is concluded that treatment with eCG but not with ECP given at the time of removal of P4 device can alter ovarian follicular response and subsequent luteal function in suckled anestrous zebu cows. Moreover, both treatments (eCG or ECP) increase the number of suckled cows displaying estrus following the synchronization protocol.

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A103 FTAI, FTET and AI

Leptospirosis serological identification and the possible interference of the disease in FTET protocols efficiency and in reproductive parameters of embryo recipient cows

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Keywords: embryos, leptospira, serologic test.

Leptospirosis is an acute infectious disease, zoonotic, cosmopolitan, which affects several species of domestic animals, and wild rodents. The incidence of leptospirosis is highest in tropical regions particularly during the rainy seasons. The possibility of its occurrence also in the state of Acre is not ruled out, though, to date, has not been diagnosed or reported before. A total of 235 crossbred cows (*Bos taurus* x *Bos taurus indicus*) as embryo recipients, subjected to identical synchronization of ovulation protocol for fixed time embryo transfer (FTET) were used. On the 16th day after the beginning of the protocol, an *in vitro* produced embryo was transferred to each recipient and, at the same time it was collected from each recipient a sample of blood by venous puncture of the coccygeal vein in tubes with vacuum without anticoagulant that were subsequently processed in attempt to obtain serum. The microscopic agglutination test (MAT) was used as diagnostic way in the presence of at least 18 leptospiras serovars grown in the laboratory. Pregnancy diagnosis and reevaluation of recipients pregnant for confirmation of pregnancy or abortion were performed on the 25th and 55th days after FTET,respectively both by ultrasonography (Aloka SSD 550, Aloka, Japan). Serological diagnosis was performed by R & D Center of Animal Biological Institute of São Paulo and statistical analysis used was the chi-square test at a significance level of 5%. As a result of testing, animals were identified 128 (128/235) reagents for at least one of serovars and 107 (107/235) of total non-reactive worked. Of the 158 recipients who responded to hormonal protocol (in D16, the presence of CL), 72 were positive and 86 were not, while of the 77 non-responders, 51 were positive and 26 were not. Of the 54 pregnant recipients, 27 were positive and of the 11 abortions observed on the 55th day after FTET, 3 were among reagents recipients for the following serovars (value of the titer): *L. icterohaemorrhagiae* (200), *L. wolffi* (200) and *L. hardjo* (400), one animal; *L. wolffi* (400) and *L. hardjo* (400) one animal; *L. wolffi* (1600) and *L. hardjo* (1600), one animal. It is concluded that the rate of utilization of protocols to FTET was dependent, statistically, on the recipient serum reagent condition ($P<0.05$), interfering in ovulation process. However, further studies are necessary to explain possible mechanisms of action of leptospirosis in the transfer/treated rate of FTET protocols.

A104 FTAI, FTET and AI

Conception rate in Nelore cows synchronized with different protocols of ovulation induction

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Keywords: females, FTAI, inducers.

Strategies have been developed to improve the responsiveness and efficiency of hormonal protocols, seeking a synchronized ovulation and better reproductive rate. The association of ovulation induction can be used to better timing of ovulation and improves in response to hormone treatments. Therefore, this study aimed to evaluate the effect of different protocols of ovulation induction about conception rate of Nelore cows submitted to a program of FTAI. Therefore, 196 cows multiparous Nelore were submitted to following protocol: At a random day of the estrous cycle, called day 0 (D0), they received an intravaginal progesterone device (PRIMER®, Tecnopec, São Paulo, Brazil) and an application of 2mg of estradiol benzoate (RIC-BE®, Tecnopec) intramuscularly (im). On D8, the intravaginal progesterone device was removed and 150µg of d-cloprostenol (Prolise®, Tecnopec) im. plus 10mg of FSHp (Folltropin®, Tecnopec) im were administered. At this time, the animals were assigned into three groups according to the protocol for synchronization of ovulation: Group EB (n = 62) - the animals received 1mg of estradiol benzoate (RIC-BE®, Tecnopec) im. on D8; Group GnRH (n = 62) - the animals received 25mg Lecirelin acetate (Gestran®, Tecnopec) on D10; and EB + GnRH group (n = 72) - the animals were treated with 1mg of estradiol benzoate on D8 and 25µg of Lecirelin acetate on D10. On D10 of the protocol, all animals were inseminated. The pregnancy diagnosis was performed by transrectal ultrasound 30 days after FTAI using 6.0MHz linear transducer (Pie-Medical, Falcon 100, Maastricht, The Netherlands). Statistical analysis was performed using SPSS, version 19. The conception rate was compared among groups using Chi-square (c^2), considering 5% as significance level. The overall conception rate was 62.0%. There was no significant difference among experimental groups on the conception rates [EB (59.71%), GnRH (61.32%) and BE + GnRH (54.23%)]. We conclude that the application of oestradiol benzoate, GnRH or association between these drugs promotes similars conception rates in females submitted to a FTAI protocol, demonstrating that all three synchronization of ovulation protocols can be used effectively in FTAI programs in Nelore cattle.

A105 FTAI, FTET and AI

Strategies to increase fertility in lactating dairy cows submitted to TAI and FTET E2/P4 based protocols

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Keywords: fixed-time artificial insemination, fixed-time embryo transfer, progesterone.

The objective was to evaluate strategies to improve fertility in dairy cows submitted to timed artificial insemination (TAI) and fixed-time embryo transfer (FTET) on estradiol (E2)/progesterone (P4)-based protocols. The experiment was performed in farms that use TAI (4, n=1,642) and FTET (6, n=1,055). The animals were randomly assigned to receive one of the following treatments: (control) D-11 2mg BE (Estrogin®, Farmavet, SP-Brazil) + CIDR (CIDR®, Zoetis, SP, Brazil); D-4 PGF (Lutalyse®, Zoetis, SP-Brazil); D-2 CIDR + 1 mg ECP (ECP®, Zoetis, SP, Brazil); D0 AI or D7 ET; (2PGF) the same protocol control with two doses of PGF, the first on D-4 and second on D-2, (GnRH) the same protocol 2PGF, with GnRH (Cystorelin®, Merial, SP, Brazil) in D-11. The PROC GLIMMIX was used to evaluate the binomial variables and PROC MIXED to evaluate continuous variables. Was considered significant when $P<0.05$ and trend when $P<0.1$. The protocol with GnRH increased the proportion of cows with a corpus luteum (CL) and progesterone (P4) in D-4 (69%, 2.88±0.28ng/mL) compared to control (52%, 2.50±0.28ng/mL) and 2PGF (51%, 2.53±0.28ng/mL). There was an effect of protocol 2PGF (88%) and trend of GnRH (87%) to increase the proportion of cows with CL in D7, compared to control (84%). In cows with CL on D7, the 2PGF protocol resulted in greater P4 on D7 (2.68±0.2ng/mL) compared to GnRH (2.50±0.2ng/mL) and control (2.55±0,2ng/mL). At 32d to the protocol GnRH resulted in higher P/IA (40%) compared to control (32%) and tended to have higher P/AI compared to 2PGF (35%). At 60d the protocol with GnRH showed higher P/IA (33%) compared to control (26%), the 2PGF group not differs from the others (29%). In cows with CL in D7 the protocol GnRH had higher P/AI at 32 and 60d (44 and 36%) compared to control (35.5 and 30%), the 2PGF group not differs from the others (40 and 33.5%). In FTET, there was no difference between treatments at 32 and 60d (control=44 and 35; 2PGF=43 and 35; GnRH =43 and 39%). In cows with CL on D-4 that had P4>1.0ng/mL at D7 the P4 at D0 with the highest accuracy for pregnancy at 60d was ≤0.13ng/mL. Cows with P4≤0.13ng/mL at D0 showed the best P/IA (44%) and P/TE (41%) in relation to P4>0.13 ng/mL (IA = 36, TE = 25%). In cows with CL at D-4, the protocols GnRH and 2PGF resulted in lower P4 at D0 (GnRH=0.17±0.05; 2PGF=0.19±0.05ng/mL), compared to control (0.35±0,05ng/mL). Higher P4 at D-4 resulted in greater P/AI at 60d, but there was no effect of P4 at D-4 on P/TE. Cows with CL at D7 that displayed estrus had a higher P/IA (35%) and P/TE (39%) at 60d, compared to cows that did not display estrus (IA=25, TE=25%). The use of GnRH D-11 combined to the use of two doses of PGF increased P/AI in cows receiving E2/P4-based protocols, and the mechanism to this result is probably associated with increased P4 concentration during follicle development.

A106 FTAI, FTET and AI

The seasonal anestrus does not reduce ovarian responses in dairy buffaloes submitted to synchronization of ovulation protocols for TAI during nonbreeding season

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Keywords: artificial insemination, breeding season, follicles.

The aim of this study was to compare ovarian follicular and corpus luteum dynamics in dairy buffaloes submitted to timed artificial insemination (TAI) during the breeding and nonbreeding season. Thirty one lactating dairy buffaloes cows raised exclusively on grazing from farm in the Ribeira Valley, State of São Paulo, were used. The females were synchronized by the same TAI protocol during the breeding season (n=16; autumn-winter) or out of the breeding season (n=15, nonbreeding season, spring-summer). On D0, cows received a new intravaginal progesterone (P4) device (1 g of P4; Sincrogest®, Ourofino Agronegócio) associated with 2.0 mg of estradiol benzoate (Sincrodiol®, Ourofino Agronegócio). At this time, females were classified according their body condition score (BCS; scale 1-5). On D9 (PM) cows received i.m. 0.53mg of PGF2α (Cloprostenol, Sincrocio®, Ourofino Agronegócio) and 400 IU of eCG (Novormon®, MSD Animal Health), followed by P4 device withdraw. On D11 (PM) 10 µg of buserelin acetate (GnRH, Sincroforte®, Ourofino Agronegócio) was i.m. administrated. The TAI was performed 16 hours after GnRH treatment (D12; AM). The ultrasound evaluations (Mindray DP2200Vet, China) were performed on D0 and D9 to verify the cyclic status (CL presence). Also ultrasound exams were done on D9, D11 and D12 to verify the size (diameter) and growth of dominant follicle (DF). The corpus luteum (CL) diameter was evaluated on 6, 10 and 14 days after TAI. Pregnancy diagnosis was performed 30 and 45 days after TAI. Continuous variables were presented as mean ± standard error (SEM). The variables were analyzed by *Glimmix* procedure of SAS® and P values <0.10 were considered different. Cows synchronized during the breeding season presented more days in milk (DIM; 103.0±10.9 vs. 51.9±4.68 days; P=0.0002), and higher percentage of CL (81.25 vs. 0.0%; P=0.02) at onset of synchronization protocol. However lower BCS was observed in cows synchronized during the Breeding season than those synchronized during the nonbreeding season (3.1±0.1 vs. 3.7±0.1; P=0.02). There was no differences between the seasons on DF at D11 (11.0±0.8 vs. 13.2±0.5 mm; P=0.12), growth of DF (1.6±0.2 vs. 2.6±0.3 mm/day; P=0.35), CL at D18 (19.8±1.2 vs. 17.4±0.5 mm; P=0.20), CL at D26 (23.1±1.3 vs. 19.3±0.7 mm; P=0.12), on pregnancy at D42 (43.8 vs. 66.7%; P=0.1589) and D57 (43.8 vs. 66.7%; P=0.16). However higher DF on D9 (7.9±0.7 vs. 8.7±0.6 mm; P=0.09), FD on D12 (11.3±0.8 vs. 14.0±0.5 mm; P=0.08), and ovulation rate (68.7 vs. 93.3%; P=0.06) was found at nonbreeding season than at breeding season. The CL on D22 (24.1±1.0 vs. 18.0±0.5 mm; P=0.03) were largest at breeding season. Therefore, it was concluded that seasonal anestrus does not reduce the ovarian responses in lactating dairy buffalo cows submitted to TAI protocols during nonbreeding season, corresponding to the period that females had greater BCS.

Acknowledgments: Ourofino Agronegócio.

A107 FTAI, FTET and AI

Effect of inhibition of angiotensin converting enzyme (ACE) on relaxing degree of the cervix in goats submitted to fixed-time artificial insemination

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Keywords: enalapril, goat, uterus.

In order to improve the reproductive rates in species of economic interest, the enalapril, an ACE inhibitor, has been used as a tool in the growth of reproductive biotechnologies, after discovering an new peptide involved with reproduction: the presence an new component of the renin-angiotensin system in ovaries of rats, Angiotensin-(1-7) (Costa et al. 2003), whose production is increased by inhibition of ACE. The aim of this study was to evaluate the effect of ACE inhibition on relaxing of the cervix in goats subjected to fixed-time artificial insemination (FTAI). Ninety four goats were undergone to estrus synchronization (D0) with intravaginal sponges (60 mg, MAP) for 12 days (D0-D12) and intramuscular injection of 300 IU of eCG and 75μg of PGF2α on the tenth day of treatment (D10). In D10, the goats were divided into three groups: G1 (n = 34) Control, G2 (n = 30) enalapril maleate in the formulation of vaginal tablets, and G3 (n = 30) were given subcutaneously 3 mL of enalapril maleate suspended in oil (20 mg/mL). There were two inseminations with fresh semen ($50x10^6$ spermatozoa/insemination dose), collected from reproducers of proven fertility, diluted in coconut water, the first being held 36 hours after sponge removal and the second 12 hours after the first insemination. The relaxation of the cervix was measured by the easiness of pipette passage by cervical rings, being classified as contracted (C) when the pipette passed the first three rings, semi-relaxed (SR) when the pipette surpassed until the sixth ring and relaxed (R) when the pipette achieved the uterine lumen. The results were tested with χ^2 (chi-square $p \leq 0.05$) using SAS 9.0. In the first insemination, the best cervix relaxation represented by the largest percentage of intrauterine inseminations, was observed in G2: G1[C=70.59% (24/34), SR=23.53% (8/34) and R=5.88% (2/34)]; G2 [C=50.00% (15/30), SR=26.67% (8/30) and R=23.33% (7/30)]; and G3 [C=70,00% (21/30), SR=23,33% (7/30) and R=6,67% (2/30)]. In the second insemination the degree of relaxation was worse in G3 represented by the lower percentage of intrauterine insemination: G1[C=32.35% (11/34), SR=17,65% (6/34) and R=50,00% (17/34)]; G2 [C=26,67% (8/30), SR=26.67% (8/30) and R=46.66% (14/30)]; and G3 [C=46.67% (14/30), SR=20.00% (6/30) and R=33.33% (10/30)]. In conclusion, this study suggests that enalapril administered via intravaginal can anticipate the relaxation of the cervix of goats subjected to FTAI.

A108 FTAI, FTET and AI

Equine chorionic gonadotropin effect on the occurrence of estrus and pregnancy rates of primiparous and multiparous *Bos indicus* cows submitted to TAI

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Keywords: eCG, fertility, zebu.

The objective of this study was to evaluate the effect of equine chorionic gonadotropin (eCG) treatment on the occurrence of estrus and pregnancy rate in *Bos indicus* primiparous and multiparous cows submitted to timed artificial insemination (TAI). The study enrolled 603 lactating cows (288 primiparous and 315 multiparous; with 30 to 60 days post-partum) with body condition score (BCS) of 2.75±0.01 (1 to 5 scale). On a random day of the estrus cycle (D0) all animals received 2 mg of estradiol benzoate (Sincrodiol®, Ourofino, Brazil) and an intravaginal progesterone device (Sincrogest®, Ourofino, Brazil). On D8 all cows received 150µg of cloprostenol (Sincrocio®, Ourofino, Brazil), 1 mg of estradiol cypionate (ECP®, Pfizer, Brazil) and the progesterone devices were removed. Still on D8, the females were randomly assigned to one of two treatments (Control Group and eCG Group). Animals from eCG group received 300UI of eCG (Folligon, MSD, Brazil) and cows from Control group received no additional treatment. The cows were inseminated at fixed time, 48 hours after progesterone device removal. Pregnancy diagnosis was performed 35 to 45 days after the TAI. Estrus detection was performed using a chalkmarker on the tailhead. All data were analyzed by GLIMMIX procedure of SAS. Regarding the occurrence of estrus, there was no interaction treatment and category (P=0.27), treatment effect [control group 60.3% (190/315) and eCG group 66.0% (190/288); P=0.17] and animal category effect [Primiparous 60.8% (175/288) and multiparous 65.1% (205/315), P=0.26]. However, cows that displayed estrus following the TAI protocol had higher pregnancy rate [Cows that displayed estrus, 41.1% (153/372) and that did not display estrus 24.6% (50/219)]. For pregnancy rate, there was an interaction treatment and animal category (P=0.005). Treatment with eCG increased (P=0.0001) pregnancy rate in both primiparous cows [control group 9.7% (15/154) and Group eCG 41.4% (55/133)] and multiparous cows [Control group 37.5% (60/160) and group eCG 49.7% (77/155)]. However, the difference between treatments was more evident in primiparous cows. Moreover, primiparous cows [24.4% (70/287)] had lower pregnancy rate than multiparous cows [43.5% (137/315); P=0.001]. Thus, despite the administration of eCG in TAI protocols do not interfere in the estrus occurrence, the eCG is essential to increase the pregnancy rate, especially in primiparous cows.

A109 FTAI, FTET and AI

Resynchronization 22 days after the first TAI does not alter the pregnancy loss in non-lactating zebu cows

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Keywords: bovine, estradiol, reproduction.

Preliminary studies have shown that replacement of GnRH by estradiol benzoate (EB) at the beginning of the resynchronization protocol 22 days after the first timed artificial insemination (TAI), with no previous pregnancy diagnosis, determines acceptable conception rates. However, it is still not established whether the administration of 1 mg of EB, 22 days after the first TAI, could increase the pregnancy loss of pregnant females at this treatment. Thus, the present study aimed to evaluate the occurrence of pregnancy loss in non-lactating beef cows submitted to resynchronization 22 days after the first TAI (RE) or submitted only to natural mating (NM) 15 days after the first TAI. Therefore, 351 cows (112 Nellore and 239 crossbred cows) were randomly assigned in two experimental groups: RE (n = 178) and NM (n = 173). All animals were subjected to a same synchronization of ovulation protocol for the first TAI, which consisted of inserting an intra-vaginal progesterone device (P4; DIB, MSD Animal Health) previously used for eight days plus 2 mg of estradiol benzoate (EB; Gonadiol, MSD Animal Health). After eight days, the intravaginal devices were removed and the females received Sodic Cloprostenol (0.265 mg; Ciosin, MSD Animal Health), 1 mg of Estradiol Cypionate (ECP, Zoatis Animal Health) plus 300 IU of eCG (Folligon, MSD Animal Health). The cows received a TAI 48 hours after intravaginal devices removal. The females of the NM group were maintained at a 1:25 bull-cow proportion, from 15 to 90 days after the first TAI. The females from RE group were synchronized again 22 days after the first TAI. The same protocol previously described was used, except by the use of a norgestomet auricular implant (3 mg; Crestar; MSD Animal Health) and the EB dosage at the implant insert (1 mg; Gonadiol, MSD Animal Health). In the latter group the bulls were introduced 10 days after the second TAI. The pregnancy diagnosis was performed by ultrasonography 30 days after each TAI and after the end of the breeding season (120 days after the first TAI). No differences were found on pregnancy rates at first TAI protocol (NM=63.0%; 109/173 vs. RE=68.5%; 122/178; P=0.12) and on pregnancy rates after the end of breeding season (NM=95.4%; 165/173 vs. RE=93.8%; 167/178; P=0.41). The pregnancy rate of the resynchronization was 46.4% (26/56). Also, there was no difference (P=0.40) on the pregnancy loss between 30 and 60 days of resynchronized females (RE=2.5%; 3/122) or only NM (NM=0.9%; 1/109). It was concluded that the use of resynchronization at 22 days after the first TAI, without previously diagnosis, does not alter the pregnancy loss of non-lactating zebu females.

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A110 FTAI, FTET and AI

The use of sorted sperm in dairy cows under different reproductive managements

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Keywords: dairy cows, reproduction, reproductive efficiency.

The aim of this study was to evaluate the reproductive efficiency of dairy cows subjected to synchronization of ovulation protocol for timed artificial insemination (TAI) or artificial insemination after estrus detection (ED+AI) using sex-sorted sperm. A total of 626 crossbreed (Gir x Holstein) and Holstein lactating cows, receiving the 1st to 3rd service, producing 23.8±0.4 Liters of milk/day, and with 77.0±1.7day in milk were used. At onset of the program, cows that had corpus luteum (CL) by ultrasonography exam (U.S.) were randomly assigned to one of two groups (ED+AI or TAI/CL). Females that presented absence of CL at that moment were synchronized and were timed inseminated (TAI/noCL). The females from ED+AI were inseminated 12 h after estrus detection during six consecutive days after 500 mg im of cloprostenol (PGF2, Ciosin®, MSD Animal Health) adminstration plus an adhesive aid to detection of estrus (Estrotec®, Brazil IVP). Cows from TAI groups (CL or noCL) received a synchronization of ovulation protocol. At onset of the treatment cows received an intravaginal progesterone device (P4; DIB®, new or previously used for 8 days, MSD Animal Health) plus 2 mg i.m. of estradiol benzoate (EB; Gonadiol®, MSD Animal Health). At P4 device removal (8 days after insertion), cows received a dose of PGF2α and 1 mg i.m. EB was administered 24 hours later. The TAI was performed 60 hours after P4 device removal. Pregnancy diagnosis was performed 32±3 days after insemination. Statistical analysis was performed by orthogonal contrast using PROC GLIMMIX of SAS. The analyzed contrasts were: 1) Type of reproductive management: ED+AI vs. TAI (CL or noCL), and 2) The presence of CL at onset of synchronization: TAI CL vs. TAI noCL. Cows receiving ED+AI had lower service rate (ED+AI= 45.1%, 101/224; TAI CL= 94.2%, 180/191; and TAI noCL= 97.2%, 205/211; P<0.0001); however, higher conception rate (ED+AI= 31.7%, 32/101; TAI CL= 19.4%, 35/180; and TAI noCL= 23.9%, 49/205; P=0.03] than cows receiving TAI. Still, there was a tendency to enhance the pregnancy rates in cows receiving TAI than cows inseminated after estrus detection (ED+AI=14.3%, 32/224; TAI CL= 18.3%, 35/191; and TAI noCL= 23.2%, 49/211; P=0.07). There was no effect of the presence of CL at onset of the synchronization protocol on conception (P=0.45) or pregnancy (P=0.28) rates. Thus, it is concluded that TAI programs increase the service rate of lactating dairy cows, regardless of the presence of CL at beginning of the treatment. Furthermore, conception rate achieved after TAI using sexed semen, regardless of the presence of CL in the beginning of the synchronization protocol, is lower than that obtained upon detection of estrus in lactating dairy cows.

A111 FTAI, FTET and AI

TAI anticipation in buffaloes submitted to ovulation syncronization during the non breeding season

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Keywords: anestrus, estradiol benzoate, induction.

The timed artificial insemination (TAI) anticipation in buffaloes submitted to ovulation induction with estradiol benzoate (EB) during the non breeding season was evaluated. The hypothesis was that early TAI increases the pregnancy rate of females submitted to ovulation induction with EB. In this study, 204 buffaloes were distributed according to age, number of births, the body condition score and ovarian activity in one of two groups (Group TAI 64h, n=101 and Group TAI 56h, n=103). At a random day of the estrous cycle (D0), in the morning (Group TAI 56h) or afternoon (Group TAI 64h), buffaloes received an intravaginal progesterone device (P4; Sincrogest®, Ourofino Agronegócio, Brazil) and 2mg im of EB (Sincrodiol®, Ourofino Agronegócio). On D9 AM (Group TAI 56h) or PM (Group TAI 64h), females received 530µg im of Cloprostenol sodium (Sincrocio®, Ourofino Agronegócio) and 400IU of eCG (SincroeCG®, Ourofino Agronegócio), followed by P4 device removal. On D10 AM (Group TAI 56h) or PM (Group TAI 64h), buffaloes received 1mg im of EB (Sincrodiol®, Ourofino Agronegócio). The buffaloes of TAI 56h group and TAI 64h group were timed inseminated 56h and 64h after the P4 device removal (D11, PM and D12, AM, respectively). Females were submitted to ultrasonographic evaluation (Mindray DP2200Vet, China) on D0 to assess ovarian activity (presence of corpus luteum) and on D42 for the pregnancy diagnosis. A subset of animals (TAI 64h group, n=48 and TAI 56h group, n=47) was submitted to ultrasonography exam to evaluate the dominant follicle diameter (DF) at the time of TAI. The statistical analysis was performed using the GLIMMIX procedure of SAS®. There was no difference between experimental groups (TAI 64h and TAI 56h) on DF at the time of TAI (12.5 ± 0.4 vs. 12.9 ± 0.3 mm) and on the pregnancy rate [52.5% (53/101) and 49.5% (51/103)], respectively (P>0.05). However, it was found effect of the DF on pregnancy rate (P<0.02), being 8.3% (1/12)^{b} to DF <10.0 mm; 26.1% (6/23)^{b} to DF between 10.1 to 12.0 mm; 68.9% (31/45)^{a} and DF > 12.1 mm. The hypothesis of this study was rejected. It is concluded that the anticipation of TAI in buffaloes submitted to ovulation induction with EB during the non breeding season do not change the fertility of these females. However, it is possible to perform TAI during entire time of the day (AM and PM) in buffaloes synchronized during the non breeding season.

A112 FTAI, FTET and AI

Effect of supplementation of progesterone after AI in corpus luteum formation and conception rate of dairy cows

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Keywords: *Bos taurus*, fertility, hormone.

In order to evaluate the effect of supplementation of progesterone (P4) in the formation of the corpus luteum (CL; Exp 1), the pregnancy to AI (Exp 2) and ET (Exp 3) experiments were carried out. In Exp 1, 42 Holstein cows were divided into two groups: CtrlAI (n=21) and SP4AI (n=21; intravaginal P4 device with 1.9g, CIDR, Zoetis) 3 d after AI. The fixed-time artificial insemination (FTAI) protocol used consisted of placing a CIDR and 2mg estradiol benzoate (EB; Gonadiol, MSD, im, D -10). On D -3, 25mg dinoprost (Lutalyse, Zoetis, im) and D -2, 1mg estradiol cypionate (ECP, Zoetis, im) with CIDR removal. On D 3, CIDR was inserted in group SP4AI and removed after 17 d (D 20). Blood samples were collected on D 3 (before CIDR insertion in group SP4AI), 4, 7, 11, 14, 17, 30 and 31 for plasma concentration of P4 by RIA. Ultrasound scans were performed at 4, 7, 11, 14 and 20 after FTAI to calculate the CL volume. In Exp 2, 668 Holstein cows and crossbreds were divided into the same groups of Exp 1 and subjected to the following FTAI protocol: CIDR insertion and 2mg of EB or 0.1mg Gonadorelin (Fertagyl, MSD, im), D -10. On D -3, 25mg of dinoprost (Lutalyse). On D -1.5, CIDR removal, 1mg EB and Lutalyse. AI occurred on D 0. CIDR after AI remained in the cows for 14 d (D 3 to 17). Pregnancy diagnoses occurred at 31 and 90 d after AI. In Exp 3, 360 Holstein cows were divided into three groups: CtrlET (n=132); SP4ET4 (n=119), CIDR was placed 4 d before ET and removed on the day of ET, and SP4ET14 (n=109), CIDR was placed 4 d before ET and removed 14 d after insertion. The recipients received Lutalyse 10 d before ET and estrus was observed. In vitro-produced embryos were transferred to cows on Day 7, 8 or 9 after estrus. Statistical analysis was performed using the GLIMMIX procedure of SAS ($P<0.05$). In Exp 1 there was Trt x Day interaction related to greater plasma concetration of P4 due to supplementation (Day 4: 0.9±0.2 vs. 2.2±0.2; and Day 7: 2.7±0.2 vs 3.6±0.2 ng/mL). In relation to CL, there was no Trt x Day interaction or treatment effect, with only effect of day. In Exp 2, there was no effect of supplementation of P4 on conception rates at 31 (32.2 vs. 31.5% [CrtlAI and SP4AI]) or 90 (26.6 vs. 24.6%) days after AI, or embryonic loss (16.4 vs. 18.0%). In Exp 3, there was no effect of day of the estrous cycle of the recipient or Trt x Day interaction. However, the treatment decreased the pregnancy per ET, both in cows supplemented for 4 or 14 d at Day 31 (39.6 vs. 21.0 vs. 14.7% [CtrlET, SP4ET4 and SP4ET14]) or Day 90 (26.7 vs. 11.0 vs. 10.0%), with no effect on embryonic loss (31.0 vs. 43.9 vs. 22.2%). Therefore, supplementation with a P4 device on Day 3 after FTAI does not seem to impair the development or luteal function, but also does not increase conception in lactating cows submitted to FTAI. However, supplementation with P4 decreased pregnancy per ET in embryo recipients.

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A113 FTAI, FTET and AI

The reduction of proestrus period changes the occurrence of estrus, but does not affect follicular development and luteal function in Nelore cows synchronized for TAI

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Keywords: bovine, estradiol, progesterone.

The aim of the present study was to evaluate the effects of exogenous estradiol supplementation and the reduction of proestrous period (48 vs. 24 hours) in zebu cows. Lactating cycling (i.e. with a CL) pluriparous cows (n=47) with a body condition score (BCS) 3.68±0.04 were used. Cows were synchronized using an intravaginal progesterone (P4) device containing 0.6 g of P4 (Cronipress Monodose M-24®, Biogênesis-Bagó, Paraná, Brasil) plus 2 mg of estradiol benzoate (Sincrodiol®, Ouro Fino Agronegócio). Eight days later, cows received 0.25 mg of cloprostenol (PGF2α, Sincrocio®, Ouro Fino Agronegócio) and were randomly assigned into one of three groups. The Control (n=15) and ECP (n=17) groups had the P4 devices removed at the time of PGF2α administration, whereas the short proestrous period treated cows (SHORT; n=15) had the P4 devices removed 24 hours later. In addition, ECP treated cows received 1mg de ECP (ECP®, Zoetis Brasil) at the P4 removal. Cows from all groups also received an Estrotec® to evaluate the estrus occurrence and 10µg i.m. of buserelin acetate (GnRH, Sincroforte®, Ouro Fino Agronegócio) was administrated 48 hours later. Thus, control and ECP treated cows had a 48 hours proestrus and the SHORT treated cows had 24 hours of proestrus. Plasma progesterone concentration was measured seven days after GnRH injection (ng/mL). Ultrasound exams were performed: 1) at the onset of the protocol (cyclicity); 2) on the eighth day of treatment, i.e., at time of P4 device removal of control group (dominant follicle, FD, at withdrawal); 3) at TAI (FD at TAI and vascularization of DF) and 4) seven days after TAI (ovulation rate, area and vascularization of CL). Data were analyzed using Proc GLIMMIX of SAS and the results described as mean ± SEM. Short proestrus treated cows had lower (P=0.004) occurrence of estrus (0.0%^{b}) than females from control (33.3%^{a}) and ECP group (52.9%^{a}). There was no effect of treatment (control, ECP and SHORT, respectively) in the any of the other variables: 1) diameter (mm) of DF on eighth day of the treatment (12.0±0.8, 12.1±0.8 and 11.9±0.8, P=0.97), 2) DF at TAI (14.3±0.7, 14.2±0.7, 14.8±0.7, P=0.82), 3) vascularization (%) of DF at TAI (36.1±4.2, 36.8±3.8 and 35.0±4.1, P=0.95), 4) ovulation rate (100.0%, 94.1% and 86.7%, P=0.78), 5) area (mm^2) of CL (2.9±0.2, 2.9±0.2 and 2.7±0.2 P=0.56), 6) vascularization (%) of CL (63.5±3.3, 65.7±3.1 and 60.4±3.4%, P=0.53) and P4 concentration (4.9±0.4, 4.3±0.4 and 4.2±0.4 ng/ml, P=0.34). It is concluded that the estradiol supplementation or the reduction of proestrus period did not affect the follicular development and the luteal function of cyclic zebu cows synchronized to TAI.

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A114 FTAI, FTET and AI

Validation of a method for estrus detection in Nelore females submitted FTAI protocol

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Keywords: bovine, conception rate, estrus.

The study was conducted with the objective to evaluate the efficiency of the chalk marker use as a practical and economical method of estrus detection in Nelore females submitted to the fixed-time artificial insemination (FTAI) program. For that, 410 multiparous Nelore cows with average of postpartum interval of 64.58 ± 18.42 days and body condition score of 3.19±0.42 (1-5 scale) were used,and synchronized with the following protocol: at a random day of the estrous cycle called Day 0 (D0), the animals received an intravaginal progesterone device (P4, PRIMER®, Tecnopec, São Paulo, Brazil) associated to 2.0mg of estradiol benzoate (RIC-BE®, Tecnopec) intramuscular (im.). On D8, the P4 intravaginal device were removed and 300IU eCG (Novormon®, MSD Saúde Animal, São Paulo, Brazil) im., 150µg of d-cloprostenol (Prolise®, Tecnopec) im. and 1.0mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) im were administrated. At this time, the animals were marked with chalk maker (RAIDEX, Walmur, Porto Alegre, Brazil) between the sacral tuberosity and the insertion of the tail in a delimited area of 150 cm². The animals remained in the same lot, allowing accept mounting by the females expressed estrus. On D10, the animals were characterized into three groups according to the estrus expression checked by removing chalk maker paint: Group 1- No estrus expression, presence and intensity of color ink (n = 85); Group 2- Intermediate estrus expression, loss of the color intensity of the ink (n = 84) and Group 3- Estrus expression, complete removal of the ink (n = 241). Then, proceeded to measure the diameter of the ovulatory follicle (DFOL) by transrectal ultrasonography using a 5.0 MHz linear transducer (Mindray, DP2200vet, São Paulo, Brazil) and FTAI was performed in all animals. Pregnancy diagnosis was performed by ultrasonography 45 days after FTAI. Data were analyzed by SPSS (version 19). In this study there was an overall mean of DFOL of 11.81 ± 3.48 mm. There was a difference (P=0.0007) among groups 1, 2 and 3 on the DFOL, which showed average of 10.00 ± 3, 03; 11.24 ± 3.46 and 12.64 ± 3.36 mm, respectively. The overall conception rate was 44.90% (184/410). Groups 1, 2 and 3 had conception rates, respectively, 29.40% (25/85); 33.30% (28/84) and 54.40% (131/241). In comparing the pregnancy rates between groups 1 and 2 was not significant difference; however, group 3 had higher conception rate (P=0.0002) to the other groups. The results of this experiment suggest that estrus detection with the use of the chalk marker is a method of easy applicability, inexpensive and can be used efficiently to assist in the animal identification with high probability of having greater follicular diameter and conception rates.

A115 FTAI, FTET and AI

Effect of single-use or re-used intravaginal progesterone devices on the pregnancy rate of lactating *Bos indicus* cows submitted to TAI

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Keywords: fertility, sincronization, zebu.

The aim of the present study was to evaluate single-use and reused intravaginal progesterone devices on pregnancy rate of lactating *Bos indicus* cows. The hypothesis of the study was that the number of previous use of intravaginal progesterone device or device with single use does not interfere in the pregnancy rate of lactating Nelore cows. In the experiment, 712 lactating *Bos indicus* cows were used with body condition score of 3.18±0.01 (1 to 5 scale). On a random day of the estrous cycle (D0) cows received 2mg of BE (Gonadiol®, MSD, Brazil) and were randomly assigned to one of four treatments according to the type of the progesterone device: new DIB group (MSD, Brazil, n = 178), DIB 1x group (previously used for 8 days, n = 184), DIB 2x group (previously used for 16 days, n = 169) or single use group (new device with single use; Cronipres single dose, Biogenesis Bago, Brazil). On D8, all animals received 397.5µg of sodic Cloprostenol (Ciosin®, MSD, Brazil), 300IU of eCG (Folligon®, MSD, Brazil), 1mg of estradiol Cipionate (ECP®, Pfizer, Brazil) and intravaginal progesterone withdraw. Cows were inseminated at fixed time, 48 hours after progesterone device removal. The pregnancy diagnosis was performed 55 days after timed artificial insemination (TAI). The data were analyzed by GLIMMIX procedure of SAS. In these study, there was no difference in pregnancy rate between treatments [new DIB – 56.2% (100/178), DIB 1x – 64.1% (118/184), DIB 2x – 59.2% (100/169) and Single use – 60.8% (110/181); P=0.52]. Therefore, lactating *Bos indicus* cows synchronized with new, reused (previously used for 8 or 16 days) or single use progesterone device had similar pregnancy rates in TAI programs.

A116 FTAI, FTET and AI

Effect of heat stress and repeat breeding on P/AI of high-producing Holstein cows

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Keywords: heat stress, repeat breeder, reproductive efficiency.

This study was conducted on three commercial dairy farms in Carambeí and Céu Azul – PR cities during summer and winter. Heifers (average age of 17.0±0.2 mo and 0.4±0.2 previous services), peak lactation cows (PL; 2.0±0.1 lactations, 103.7±3.2 DIM, average milk production of 39.6±0.6 L/day and 0.9±0.1 previous services) and repeat breeders (RB; 2.1±0.1 lactations, 345.2±10.5 DIM, average milk production of 26.9±0.7 L/day and 5.8±0.2 previous services) started a protocol to synchronize the follicular wave emergence and ovulation for timed artificial insemination (TAI). Thus, 179 heifers, 198 PL and 233 RB cows were treated with a progesterone-releasing intravaginal device (Sincrogest®, OuroFino, Brazil) plus 2 mg estradiol benzoate (Sincrodiol®, OuroFino) on random days of the estrous cycle (D0). On D7, 530 µg sodium cloprostenol (Sincrocio®, OuroFino) was administered. On D8, the progesterone device was removed and 1 mg estradiol cypionate (E.C.P®, Pfizer, Brazil) was administered. After 48 hours, all animals were treated with 100 µg gonadorelin (Fertagyl®, MSD, Brazil) and TAI was performed. Same batch of a single Holstein bull was used for all TAI. Pregnancy diagnosis was performed 35 days after TAI by ultrasonography. Data were analyzed by logistic regression using the PROC GLIMMIX from SAS. No interaction was found between category (heifeir, PL and RB) and season of the year (summer and winter). Overall P/AI of Holstein heifers (43.0%[a]) was greater than PL (25.3%[b]), and both were greater than RB (16.7%[c]; P<0.0001) Holstein cows. Regardeless of category, summer heat stress reduced the overall P/AI compared to winter period (21.8% vs 30.8%; P=0.05). When categories were analyzed per season, the following P/AI was observed: heifers during the summer = 37.2% (29/78), heifers during the winter = 49.3% (35/71), PL during the summer = 21.6% (19/88), PL during the winter = 28.2% (31/110), RB during the summer = 11.8% (14/119), and RB during the winter = 21.9% (25/114). In the present study it was observed that RB cows had lesser P/AI compared to heifers and PL cows treated with the same protocol for TAI and using the same batch of semen. This result confirms data found in previous large retrospective studies. Also, the negative impact of heat stress on P/AI was observed in the three categories of Holstein bovine female analyzed herein.

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A117 FTAI, FTET and AI

Anti-neospora caninum antibodies occurrence in embryo recipient cows in South Western Amazon

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Keywords: bovine, embryo, neosporosis.

An animal reproduction disease, neosporosis is widespread in the national herd and to date it has not been diagnosed or reported in Acre State. We used 235 crossbred cows (*Bos taurus* x *Bos indicus*) as embryo recipients, subjected to identical synchronization of ovulation protocol for fixed-time embryo transfer (FTET). On the 16th day after the beginning of the protocol, an *in vitro* produced embryo was transferred to each recipient and, at the same time it was collected from each recipient a blood sample by venous puncture of the coccygeal vein in tubes with vacuum without anticoagulant. The detection of antibodies against *Nespora caninum* was performed by indirect immunofluorescence test and reagent animals were considered with titer ≥ 200. The diagnosis of pregnancy and reassessments of recipients for pregnancy or abortion confirmation were performed on the 25th and 55th days after FTET, respectively, both by ultrasonography (Aloka SSD 550, Aloka, Japan). The immunoassay was performed at the Biological Institute of São Paulo and statistical analysis used was the Chi-square test at a significance level of 5%. Serum-reactivity to *Neospora caninum* was diagnosed in 143 (60.85%) cows. There were 158 (67.23%) recipient cows with CL at D16. Out of the embryo transferred cows, 54 (34.18%) became pregnant and pregnancy loss was 20.37% (11/54). Five (45.45%) cows that had embryo/fetal loss had an indirect immunofluorescence reaction with antibody titers above 400, and one recipient with a 3200 title, which was considered high and suggestive of active infection. The high antibody titers may be associated with a latent infection reactivation, which may have occurred in cows with antibody titer equal to 3200. However the statistical analysis, showed independent variables, i.e., abortion cases were not associated with seropositivity of recipients (P>0.05). This is a pioneering report on the occurrence of cow serum reagent to neosporosis in Acre and can serve as a warning to authorities involved in the animal health.

A118 FTAI, FTET and AI

Effect of numbers of use of a CIDR with 1.9 g or 1.38 g of P4 on pregnancy rate in lactating Nelore cows submitted to a TAI protocol

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Keywords: CIDR, pregnancy, progesterone.

The aim of this study was to evaluate the effect from numbers of use of a CIDR with 1.9 g of P4 (CIDR®-BR, Zoetis, Sao Paulo, Brazil) or 1.38 g of P4 (CIDR®-US; Zoetis, New Jersey, USA) on pregnancy rate in lactating Nelore cows submitted to a synchronization protocol with a new (1st use) or a device that had been previously used for 9 (2nd use), 18 (3rd use) or 27 d (4th use). A total of 1,612 Nelore cows with 30 to 50 d postpartum and BCS of 3.02±0.01 (1 to 5 scale) were divided into 15 handling groups. The cows were randomly assigned within handling group to either receive a CIDR®-BR or a CIDR®-US (1st use, 5 groups: BR=317, US=220; 2nd use, 2 groups: BR=134, US=131; 3rd use, 4 groups: BR=209, US=201 and 4th use, 4 groups: BR=211, US=189) and 2 mg im of estradiol benzoate (2.0 mL, Estrogin®, Farmavet, Sao Paulo, Brazil) on D0, 12.5 mg im of dinoprost tromethamine (2.5 mL, Lutalyse®, Zoetis) on D7, 0.5 mg im of estradiol cypionate (0,25 mL, ECP®, Zoetis) + 300 IU of eCG (1.5 mL, Folligon®, MSD Animal Health, São Paulo, Brazil) and CIDR® withdrawal on D9. All cows were bred by AI 48 h after CIDR® withdrawal (D11) and the pregnancy diagnosis were performed by ultrasonography on D41. The PROC GLIMMIX from SAS® 9.2 was used to analyze pregnancy rate, were included in the model the effects of AI techinitian, semen and type of device, significance was set when $P<0.05$. There was no effect of type of device ($P>0.10$) when cows were synchronized with a 1st (BR: 56.8%; 180/317 vs. US: 63.6%; 140/220), 2nd (BR: 55.2%; 74/134 vs. US: 56.5%; 74/131) and 3rd use device (BR: 51.2%; 107/209 vs. US: 51.2%; 103/201); however, cows synchronized with a 4th use device had the pregnancy rate greater ($P<0.05$) for CIDR®-BR (55.5%; 117/211) in comparison with CIDR®-US (41.8%; 79/189). The possible reason for the lower pregnancy with the fourth use from an intravaginal device that contains 1.38 g of P4 could be the lower P4 available during the follicle development.

A119 FTAI, FTET and AI

Hormone supplementation after artificial insemination increases summer pregnancy rate in dairy cows

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Keywords: bovine, GnRH, hCG.

The maintenance of gestation in the cow after artificial insemination (AI) depends upon an adequate embryo development in order to favor the maternal recognition of pregnancy (MRP). The optimization of ovarian luteal function increases circulating progesterone (P4) and contributes to prepare the uterus to receive and nurse the growing conceptus. Therefore, it was devised a hormone strategy to optimize MRP by means of a supplementation based on the combined effects of a GnRH analogue and hCG, given respectively 5 (D5) and 12 days (D12) after AI. The rationale behind the treatment relies on the fact that GnRH on D5 is capable to induce endogenous secretion of LH, ovulate the first wave dominant follicle and generate an accessory corpus luteum (CL), which secretes additional P4 during the MRP. In its turn, hCG on D12 may prevent the presence of a growing DF during the MRP and retard or inhibit $PGF_{2\alpha}$ secretion. As a result, luteolysis is delayed, conceptus may grow more during the MRP and pregnancy maintenance is favored. In order to evaluate that protocol, 292 Holstein dairy cows of Embrapa Pecuária Sudeste were artificially insemination after estrus detection during a two-year long period. Cows were divided into CONT (n=164) - without additional treatment and GnRH/hCG (n=128) - received IM 250 μg of gonadorrelin (GnRH) on D5 and 2500 IU of hCG on D12. Pregnancy diagnosis was performed 28 days after AI through transrectal ultrasound (MindRay Vet 3300, 5MHz probe). Pregnacy rate (PR) was analyzed through the Chi-square test (χ^2) using the proc FREQ of SAS statistical package. The PR of cows treated in the autumn/winter was not different between treatments and was 47.9% (34/71) for GnRH/hCG and 47.0% for CONT (47/100). Similarly, overall PR was not statistically different (χ^2=2.05) and was 49.4% (81/164) and 57.8% (74/128) respectively for CONT and GnRH/hCG. However, hormonal treatment resulted in higher PR when the AIs were performed during spring/summer. Indeed, spring/summer PR of GnRH/ hCG was higher (70.2%; 40/57) than ($P<0.10$; χ^2=3.69) PR achieved by CONT (53.1%; 34/64). There was no significant effect of bull or technician on PR in any comparison assessed. In conclusion, the GnRH/hCG combination given after AI increases PR in dairy cows inseminated during spring and summer seasons. It is speculated that luteotrophic action of GnRH/hCG mitigates the effects of environmental stress during early stages of conceptus development.

A120 FTAI, FTET and AI

Timed artificial insemination programs during summer in lactating dairy cows: comparison of the 5-d Cosynch protocol with an estrogen/progesterone-based protocol

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Keywords: 5-d Cosynch, dairy cow fertility, E2/P4 protocol.

The objective of this study was to compare a GnRH-based to an estrogen (E2)/progesterone (P4)-based protocol for estrous cycle synchronization and timed artificial insemination (TAI), both designed for synchronization of ovulation and to reduce the period from follicular emergence until ovulation. A total of 1,190 lactating Holstein cows, primiparous (n=685) and multiparous (n=505), yielding 26.5±0.30kg of milk/d were randomly assigned to receive one of the following programs: 5-d Cosynch protocol [D -8: CIDR® (Zoetis, São Paulo, Brazil) + GnRH (100mg im gonadorelin, 1.0mL Fertagyl®, MSD Animal Health, São Paulo, Brazil), D -3: CIDR removal + $PGF_{2\alpha}$ (25mg im dinoprost tromethamine, 5.0mL Lutalyse®, Zoetis), D -2: $PGF_{2\alpha}$, D 0: TAI + GnRH]; or E2/P4 protocol [D -10: CIDR + EB (2mg im estradiol benzoate, 2.0mL Estrogin®, Farmavet, São Paulo, Brazil), D-3: $PGF_{2\alpha}$, D-2: CIDR removal + ECP (1mg im estradiol cypionate, 0.5mL ECP®, Zoetis), D 0: TAI]. Rectal temperature (RT) and circulating P4 concentration were measured on the D –3, –2, 0 and D 7. The estrous cycle was considered to be synchronized when P4≥1.0ng/mL on D 7, in cows that had luteolysis (P4≤0.4ng/mL on D 0). Cows were classified based on the number of times (0, 1, or 2+) they were detected with hyperthermia (RT≥39.1ºC). Pregnancy success (P/AI) was determined on D 32 and D 60 after TAI. The binomial variables were analyzed using the PROC GLIMMIX, and the continuous variables using the PROC MIXED of SAS. The cows in the 5-d Cosynch protocol increased (P<0.01) P4 at the time of $PGF_{2}\alpha$ (2.66±0.13 vs. 1.66±0.13ng/mL). A greater (P<0.01) number of cows in the E2/P4protocol was detected in estrus (62.8%) compared to cows in the 5-d Cosynch protocol (43.4%). Occurrence of estrus improved (P<0.01) P/AI in both treatments. Cows in the 5-d Cosynch protocol had greater (P=0.02) percentage of estrous cycle synchronized (78.2%), compared with cows in the E2/P4 protocol (70.7%). On D60, the E2/P4 protocol tended (P=0.07) to improve P/AI (20.7% vs. 16.7%) and reduced (*P*=0.05) pregnancy loss from 32 to 60d (11.0% vs. 19.6%), compared with 5-d Cosynch protocol. In cows with their estrous cycle synchronized, the E2/P4 protocol had greater (P=0.03) P/AI (25.6% vs. 17.7%) on 60d and lower (P=0.01) pregnancy loss (6.7% vs. 21.7%) compared with cows in the 5-d Cosynch protocol. Follicle diameter affected (P=0.04) pregnancy loss only in the cows in the 5-d Cosynch protocol, with smaller follicles resulting in greater pregnancy loss. P/AI at d60 was different (P=0.01) between protocols in the cows with two or more measurements of heat stress (5-d Cosynch=12.2% vs. E2/P4=22.8%). In conclusion, the 5-d Cosynch protocol produced better estrous cycle synchronization than the E2/P4, however it was observed better P/AI in the E2/P4 protocol.

A121 FTAI, FTET and AI

Follicular development in cyclic Nelore cows treated with intravaginal progesterone device used three times or with single-use (single dose).

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Keywords: beef cows, follicular dynamics, intravaginal devices.

Fixed-time artificial insemination programs (FTAI) present significant cost savings when implants of progesterone (P4) are reused (Motlomelo et al., 2002, Small Rum. Res., 45, 45-49; Barufi et al., 2002, Rev. Bras. Reprod. Anim., 26, 226-229; Almeida et al., 2006, Braz. J. Vet. Res. Anim. Sci., 43, 456-465). However, when reused, they provide lower plasma P4 concentrations than those observed in the first use. Lower P4 can change the frequency of LH pulses and follicle development pattern with reflections at the wave synchronization, the scattering and ovulation rate and fertility, especially in cows without corpus luteum, which are exclusively dependent of P4 derived from the device. Furthermore, care and cleaning of storage devices are not always observed and often do not guarantee product protection or for who will handle it. To improve the management efficiency and devices safety handling, there is a tendency to use single-use implants. The present study evaluated the differences in the follicular development of cycling cows treated with two different intravaginal P4 devices. We used: (A) 10 cows receiving device with 1 g of P4 (Cronipres of three uses®) and (B) 10 cows were given a device containing 0.558 g of P4 (Cronipres Mono Dose® M-24). Thus, we performed ovarian ultrasound examinations, every 24 h, from the insertion of the P4 device until its removal, on Day 8. After removal of the P4 device, all cows were examined by ultrasonography every 12 hours for 4 days to determine the size of the follicle at the time of FTAI, ovulation rate and timing of ovulation. We used a completely randomized design with repeated measures. Ovulation data were analyzed by logistic regression using PROC LOGISTIC and follicular development data using PROC MIXED (SAS, version 9.3). The ovulation rate was 80% for both groups (P>0.05). There was a tendency to anticipate ovulation (P=0.07) in cows from group B. A cow from group B anticipated ovulation in about 36 h. Additionally, group B cows also had a tendency (P=0.06) to present a larger diameter of dominant follicles at FTAI. Therefore, from these results, we conclude that cycling cows treated with single-dose P4 device, present pattern of follicular development similar to that of cows treated with devices containing 1 g of P4, however, there is a tendency to anticipate ovulation and to present a bigger preovulatory follicle at the time of FTAI.

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A122 FTAI, FTET and AI

Uterine diameter and induction of ovarian cyclicity in prepubertal Nelore heifers treated with progesterone

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Keywords: *Bos indicus*, heifers, uterus.

The present study aimed to evaluate the effect of progesterone (P4) treatment on the uterine diameter and pregnancy rates of heifers receiving fixed-time artificial insemination (FTAI). A total of 99 Nelore heifers between 22 and 24 months with body condition score between 3.0 and 3.5, in the region of Congoinhas, State of Parana were used. The heifers were submitted to ultrasound examinations of the uterus to measure uterine diameter, after obtaining three transversal evaluations and calculating the average among measurements. The cyclicity was evaluated prior to the hormonal treatment by the presence of the corpus luteum in two ovarian ultrasound examinations, with an interval of ten days. The heifers were separated into two groups according to the presence of the corpus luteum (pubertal heifers, n=36 and prepubertal heifers, n=63). The prepubertal heifers were treated with intravaginal progesterone devices previously used for 32 days (CIDR®, Pfizer, Brazil). Ten days later the devices were removed and 1 mg of estradiol benzoate (EB, Estrogin®, Farmavet, Brazil) was administered im. Seven days after the EB administration a new ultrasound examination was performed to measure the uterus. The animals received an intravaginal P4 device previously used for 24 days and 2 mg of estradiol benzoate im. On the seventh day 12.5 mg of Dinaprost im (Lutalyse®, Pfizer, Brazil) was administered and on the ninth day the device was removed and 400 IU of eCG were administered im (Novormon®, MSD, Brazil), and 0.25 mg of estradiol cypionate (ECP®, Pfizer, Brazil) im. Forty-eight hours later, the heifers were inseminated with semen from the same bull. The pregnancy diagnosis was performed 35 days after FTAI protocol, by means of ultrasound exam. The results were submitted to analysis of variance ($P<0.05$). The uterine diameter was different ($P<0.05$) between the the times before and after treatment with P4 (10.0 ± 1.8 vs 12.5 ± 1.4 mm) only in prepubertal heifers (pubertal heifers: 12.0 ± 1.1 vs 12.5 ± 1.3 mm). The induction of cyclicity occurred in 71% (44/62) of the prepubertal heifers. The conception rate after FTAI was similar between prepubertal and pubertal heifers (61.9% vs 55.5%, respectively; $P>0.05$). In conclusion the treatment of prepubertal heifers with P4 was effective in inducing cyclicity, increase uterine diameter and provide adequate pregnancy rates compatible with pubertal heifers.

A123 FTAI, FTET and AI

Effect of the utilization of diferents intravaginal progesterone device in the fertility of dairy cows

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Keywords: CIDR, progesterone, TAI.

The objective of this study was to evaluate the fertility of dairy cows submitted to timed artificial insemination (TAI) protocols using intravaginal device for three times with different progesterone concentration (P4 1.38g x 1.9g). A total of 1,073 lactating Holstein cows (695 multiparous and 378 primiparous) were used in five different farms, with the average of 173±4.47 days in lactation, 2.42±0.05 inseminations, 32.1±0.31 kg of milk/day and 3.0±0.02 of body condition score, in the scale of 1–5. All cows received the following TAI protocol: D0 – 2mg of EB im (2.0mL of Estrogin®, Farmavet, SP, Brazil) + intravaginal P4 device (CIDR®, Zoetis, SP, Brazil); D7 – 25mg of PGF im (5.0mL de Lutalyse®, Zoetis); D8 – removal of the P4 device + 1mg of ECP im (0.5mL, ECP®, Zoetis); D10 – TAI. On D0 the cows were randomly assigned to receive CIDR A (1.38g) or B (1.9g), new [1st use], previously used for 8 days [2nd use] or previously used for 16 days [3rd use]. Blood samples were collected on D8 of the protocol to evaluate the P4 concentration. The pregnancy diagnostic was performed by ultrasound 30 days after insemination. Binomial variables were analyzed using PROC GLIMMIX and the continuous by PROC MIXED. Significant differences were considered when $P \leq 0.05$ and tendency when $P > 0.05$ and $P < 0.10$. The P4 concentration with the CIDR of 1st use tended (P=0.09) to be higher for CIDR A (1.64±0.10 ng/mL) compared to CIDR B (1.49±0.10 ng/mL). There was no difference (P=0.36) on the P4 concentration between the CIDR A and B of 2nd use (A=1.07±0.7 ng/mL vs. B=1.13±0.7 ng/mL). In the 3rd use, the CIDR A tended (P=0.10) to present lower P4 concentration (0.95±0.13 ng/mL), when compared to CIDR B (1.12±0.13 ng/mL). There was no effect (P=0.68) on P/AI between CIDR A and B of 1st use [A=25.2% (48/190) vs. B=27.1% (51/188)] and of 2nd use [A=25.8% (48/182) vs. B=24.8% (46/182); P=0.82]. Effects (P=0.02) were observed between the type of CIDR on P/AI with CIDR of 3rd use [A=21.6% (37/171) vs. B=33.1% (53/160)]. Cows with P4 concentration on the D8 lower than 0.5 ng/mL [10.6% (114/1073)] had lower (P=0.01) P/AI [16.6% (19/114)] compared with ≥0.5 ng/mL [27.4% (264/959)]. There was no effect (P=0.99) on the distribution of cows with P4<0.5 ng/mL on D8 by the type of CIDR (A=1% vs. B=1%) of 1st use. There were differences on the distribution of cows with P4<0.5 ng/mL at D8 between the type of CIDR when used for the 2nd (A=12% vs. B=3%; P<0.01) and 3rd time (A=32% vs. B=16%; P<0.01). Cows with temperature ≥39.1ºC at the moment of AI had lower (P=0.04) P/AI [21.3% (36/169)], compared to cows with temperature <39.1ºC [29.8% (117/393)]. Both intravaginal P4 devices had similar pregnancy results when used for two times, however, when used for more than 16 days, the CIDR with 1.38g of P4 resulted in lower P/AI.