Animal Reproduction

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

TAI/AI

Effects of estradiol cypionate dose and body condition score on reproductive performance of *Bos indicus* beef females assigned to a timed-artificial insemination management

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This experiment was designed to evaluate the effects of increasing doses of estradiol cypionate (ECP) and different body condition score (BCS) on reproductive performance of *Bos indicus* beef females assigned to a timed-artificial insemination (TAI) management. In this experiment, 1,673 Nellore cows were blocked by parity and assigned to receive 1) an intravaginal P4 device (1.9 g of P4) and 2.0 mg of estradiol benzoate on day-11, 25 mg (i.m.) of dinoprost tromethamine, 300 IU (i.m.) of equine chorionic gonadotropin, 0.6 mg (i.m.) of estradiol cypionate and CIDR withdrawal on day -2, followed by TAI on day 0 (n = 849; 0.3 ECP) or 2) the same synchronization protocol with 1.0 mg of ECP on day -2 (n = 832; 0.5 ECP). In both treatments, estrus expression was measured between days -2 and 0. Body condition score (BCS) was evaluated on days -11, 31, and 71 of the experiment and the BCS variation (Δ) was also determined between these timepoints. Moreover, transrectal ultrasonography was performed on days 31, 71, and 111 for pregnancy rate determination. All binary data were analyzed using cow as the experimental unit with GLIMMIX, whereas continuous variables were analyzed with the MIXED procedure of SAS. Cows assigned to receive 0.5 ECP had a dominant follicle with a greater diameter than cohorts assigned to receive 0.3 ECP. No treatment effects were observed on estrus expression rate. Moreover, treatment × BCS interactions were observed for pregnancy rates in all time points (days 31, 71, and 111). On the other hand, 0.5 ECP with a low BCS also had a greater P/AI than cohorts assigned to 0.3 ECP. In summary, increasing the dose of ECP benefited the reproductive performance of *Bos indicus* beef cows with a reduced BCS (≤ 2.75), whereas no benefits were seen when the BCS was considered adequate (>2.75).

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TAI/AI

Fixed-time artificial insemination with fresh semen *vs.* frozen in dairy buffalo

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The detection of estrus in dairy buffaloes is a great challenge, as the signs of heat are more discreet compared to the bovine species, which provides a greater probability of failure in the detection of heat. To overcome these limitations, TAI protocols have been used in the field. In this context, this work aims to compare the reproductive efficiency of dairy buffaloes submitted to TAI with fresh semen *vs.* frozen semen. This experiment was carried out in Oliveira/MG, Brazil, latitude 20°41'45' South, and longitude 44°49'37' West, from May to June 2022. Two ejaculates were collected from the same Murrah bull, using the artificial vagina method, the first ejaculate being used for freezing and the ejaculate (collected the next day) used for fresh inseminations, after evaluation following the parameters recommended by the CBRA (3:1-104, 2013). For the field freezer, use a portable Neovet Cryogen HSE® automatic freezing machine (Uberaba, Brazil), with a standardized cooling and freezing curve (curve 1). The semen extender used was Botu-Bov® (Botupharma, Botucatu/SP, Brazil), packaged i.m. 0.5 mL straws (IMV® Technologies, L'Aigle Cedex, France), containing 40x10^6 sptz/mL. For inseminations, 138 Murrah and Mediterranean females, 5.2 years old, 442.7 kg of body weight were used; body condition score = 3.1 (scale 1-5); with 65.4 days postpartum. The females were reared in an extensive system and supplemented with corn silage once a day. The buffaloes were randomly distributed into two groups: fresh semen (n = 69) and frozen semen (n = 69). The two types of semen were evaluated before the beginning of TAIs and presented subjective mean motility of 75.0 and 35.0% for fresh and frozen semen, respectively. For estrus synchronization, the protocol used was: 2.0 mg i.m. of estradiol benzoate (BE, Estrogin®, Biofarm, SP, Brazil) and intravaginal device (PRIMER® 0.5 g of P4, Agener União Saúde Animal, SP, Brazil) in the morning of D0 (8:00 am). On D9 (8:00 am) the P4 devices were removed and 400 IU i.m. eCG (Novormon® 5000 IU, Zoetis, SP, Brazil) + 0.530 mg of PGF 2α Cloprostenol i.m. (Estron®, Agener União Saúde Animal, SP, Brazil) were applied). On D11 (4:00 pm) 25 µg i.m. of GnRH (Sincroforte®, Buserelin Acetate, Ouro Fino Saúde Animal, SP, Brazil) were applied. TAI was performed in the morning of D12 (8:00 am). Pregnancy diagnosis was performed by ultrasonography 32 days after AI. The results were submitted to the z test (TRIOLA, 2013). Thus, the total results obtained for pregnancy rates were 55.1% (38/69) and 46.4% (32/69) for fresh and frozen semen, respectively, with a significant difference of 8.7% ($P < 0.05$). This higher result of the pregnancy rate in favor of fresh semen is probably due to the greater number of intact spermatozoa with a longer half-life in the female reproductive tract, making it possible to fertilize those females that ovulate later. It is concluded that the use of fresh semen in TAI of dairy buffaloes can be an alternative to improve pregnancy rates.

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TAI/AI

GnRH34 with or without estradiol cypionate in timed AI in Bos indicus beef cows

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Two experiments evaluated the effects of GnRH treatment on fertility of suckled Nelore beef cows treated with an estradiol/progesterone (P4)-based protocol for timed artificial insemination (TAI). Experiment 1 was designed to determine the effect of estradiol cypionate (EC) on time of ovulation, in timed AI cows treated with GnRH, 34 h after intravaginal P4 device (IPD) removal (GnRH34). Suckled cows (n=26) were treated with 2 mg estradiol benzoate (EB) and an IPD containing 1.9 g of P4. Eight days later, IPDs were removed, and all cows received 150 µg of d-cloprostenol (prostaglandin F2 alpha analogue), 300 IU of equine chorionic gonadotropin (eCG), and were equally distributed in two treatments: 1) Saline 0.9% (GnRH34), or 2) 0.6 mg i.m. of EC (EC-GnRH34 group). On Day 9 (05:00 PM), all cows received GnRH (10.5 µg of buserelin acetate). No differences were observed on the time of ovulation after IPD removal, and the proportion of cows ovulating between groups (P >0.05). Experiment 2 was designed to determine the effect of GnRH34 associated or not with EC on Day 8 on pregnancy per AI (P/AI) of postpartum beef cows. Cows (n = 981) were treated similarly as in Experiment 1. However, one extra group was included: EC-GnRH48 group, in which cows received EC on Day 8 and the ones that did not show estrus received GnRH at TAI. Therefore, the groups of the experiment were: GnRH34 (n = 322), EC-GnRH34 (n = 335), and EC-GnRH48 (n = 324). A higher proportion of estrus manifestation was observed in cows treated with EC at IPD removal (EC-GnRH34: 69%, and EC-GnRH48: 64.8%) compared to non-treated cows (GnRH34: 45.6%). The P/AI was not different among groups (P = 0.45). However, the P/AI of the cows from group EC-GnRH34 (64.2%) tended to be greater (P = 0.1) in comparison with the cows from group GnRH34 (59.9%). In summary, although the synchronization of ovulation was not different between groups, pregnancy rate in cows treated with EC and GnRH 34 h after IPD removal tended to be greater than cows only treated with GnRH, which may be explained by a shorter proestrus/estrus considering the decreased proportion of cows that demonstrate estrus in GnRH34 group. Moreover, considering that P/AI did not differ between EC-GnRH34 and EC- GnRH48, the administration of EC at IPD removal and GnRH treatment 48h later, only in cows that did not display estrus, represents the best cost-benefit treatment for TAI in South America zebu-based beef operations.

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TAI/AI

Effects of season on pregnancy rate and calving to conception interval in Girolando dairy cattle

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The objective of this study was to compare the pregnancy rates of crossbred dairy cows (*Bos taurus taurus x Bos taurus indicus*) reared in a region with a climate characterized as subtropical in different seasons. The period of service in the stations, the average Temperature and Humidity Index (THI) obtained between pregnant and non-pregnant cows in each of the stations and the pregnancy rate in the stations according to the genetic composition of two different groups were also evaluated. For that, a retrospective study was carried out using data from 735 reproductive services of Girolando crossbred females from different genetic groups from a single commercial dairy farm located in the city of Mar de Espanha/MG (501m of altitude). The seasons were characterized by calculating the THI as proposed by THOM (1959) (Thom, Animal Reproduction Science, 60–61:535–47, 1959). The collection of environmental temperature and humidity data was carried out using the INMET database – Instituto Nacional de Meteorologia, Brazil, with daily averages for temperature and relative humidity being recorded on the day in which the reproductive services occurred between 2014 and 2022 and later grouped into summer (n=150), autumn (n=234), winter (n=228) and spring (n=123) seasons, according to the official calendar. Aiming to evaluate the impact of the seasons on different genetic groups, the data were organized into two distinct groups taking into account the blood grade Bos taurus taurus/ Bos taurus indicus, as follows: Group I composed of data from the genetic groups ½, ¼, 3/8, 3/4, 5/8 (n=287); and Group II concerning the reproductive data of animals belonging to genetic groups 7/8 and 15/16 (n=194). Statistical analysis was performed using the Bioestat 5.3 software. Continuous variables were compared by ANOVA. Pregnancy rates were tested using the non-parametric binomial test. Significant differences were considered for P< 0.05 (5% significance). The lowest THI was observed during the winter (61.5±3.9) while the highest occurred in the summer (68.4±3.6). The pregnancy rate observed in winter (59.9%) was significantly higher than that observed in summer (48.6%). When comparing the average THIs between pregnant and non-pregnant cows within each of the seasons, no significant difference was observed, as well as no difference was observed in the period of service for any season of the year. Animals with a higher Bos taurus genetic composition (7/8 and 15/16) showed a reduction in the pregnancy rate during the summer when compared to animals with a lower Bos taurus composition (30.5% and 62.3%, respectively; p= 0.003), being more sensitive to weather conditions. It is concluded that subtropical climatic conditions during the summer had a negative impact on the pregnancy rate of crossbred bovine females and that this impact occurred more intensely in animals with a higher *Bos taurus* genetic composition.

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TAI/AI

Replacement of intravaginal device by long- action injected progesterone in ovulation synchronization protocol does not alter the follicular dynamics of dairy cows: preliminary results

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The aim of this study was to evaluate the replacement of intravaginal devices (ID) to long-acting injectable progesterone (P4LA) in FTAI protocols in dairy cows. Crossbred cows (Girolando) (n=29) was randomly in four treatments: two groups using ID in protocols of 10 or 11 days (GID-10; n = 7; GID-11, n = 7) and two groups using P4LA, replacing ID, in protocols of 10- and 11-days duration (GP4-10; n = 8; GP4-11, n = 7). Females from ID groups, on a random day of the estrous cycle (D=0), received a new progesterone intravaginal device (1.0 g), associated with the application of 2 mg of estradiol benzoate. The device was removed after eight or nine days and, at the time of its removal, 500 µg of cloprostenol + 300 IU of equine chorionic gonadotropin (eCG) + 1 mg of estradiol cypionate (D8 or D9) were applied. Two days after device removal (D10 or D11), Timed Artificial Insemination (TAI) was performed. The females from P4LA groups received 150 mg of injectable progesterone to replace the ID on D0 + 10 µg of buserelin acetate at the time of TAI, with the other management procedures being similar to those of the ID groups. Ultrasonographic evaluations (Mindray DP10) were performed every 12 hours from D8 or D9, up to 48 hours post-TAI. The evaluated parameters were: application of cloprostenol and ovulation interval (COI - hours); TAI and ovulation interval (TOI - hours); diameter of the dominant follicle (DF - mm) in the application of cloprostenol; DF diameter at the time of TAI (mm); DF growth rate (FGR - mm/day); synchronization rate (SR - %) and ovulation rate (%). The variables according to the treatments were compared using the Bioestat program with 5% significance. No significant difference was observed between treatments for the following variables: COI (GID-10 = 68.4±3.1 h; GID-11 = 54.0±0.0 h; GP4-10 = 59.1±11 .1 h; GP4-11 = 57.4±7.3 h); TOI (GID-10 = 20.0±3.7 h; GID-11 = 6.0±0.0 h; GP4-10 = 22.8±4.8 h; GP4-11 = 14.6±3 .4 h), DF diameter in the application of cloprostenol (GID-10 = 8.2±1.1 mm; GID-11 = 10.3±1.1 mm; GP4-10 = 7.3±1.3 mm; GP4-11 = 7.1±1.7 mm); DF diameter at the time of TAI (GID-10 = 12.5±1.0 mm; GID-11 = 13.4±0.9 mm; GP4-10 = 11.3±1.4 mm; GP4-11 = 13.5±0.6 mm); DF growth rate (GID-10 = 2.1±0.2 mm/day; GID-11 = 1.5±0.6 mm/day; GP4-10 = 1.7±0.6 mm/day; GP4-11 = 2.0±0.7 mm/day) and synchronization rate (GID-10 = 100%; GID-11 = 85.7%; GP4-10 = 100%; GP4-11 = 100%). Regarding the ovulation rate, there was a tendency (p = 0.07) for a lower ovulation rate in the GP4-10 group compared to the other treatments (GID-10 = 85.7%; GID-11 = 85.7%; GP4-10 = 62.5%; GP4-11 = 100%). Under the conditions of the present study, the replacement of ID by P4LA concomitantly with the application of GnRH at the time of TAI did not affect the follicular dynamics or the ovulation rate in dairy cows.

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TAI/AI

Development of strategies to use of liquid sexed semen in TAI of beef cows

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The aim of this study was to develop strategies to optimize the use of liquid sexed semen (LSS) in TAI, kept between 15ºC and 20ºC for up to 48 hours. Four studies were carried out to determine the P/AI with LSS: 1- TAI 48 or 54 hours after P4 withdrawal using LSS or conventional frozen semen (Exp. 1); 2- TAI with LSS for up to 48 hours after the release of semen in the laboratory (TLa Exp. 2a) and the impact of the sexing process on the seminal patterns evaluated by CASA during 84 hours (Exp. 2b); 3- TAI reducing the amount of sperm in the LSS per straw (Exp. 3) and 4- TAI with lower concentration per straw with insemination performed 48 hours after TLab and 48 hours after removal of P4 (Exp. 4). In Exp. 1, there was no interaction treatment*time for P/AI (P=0.27). Also, there was no treatment effect (P=0.09) [Control = 61.6% (167/271) vs 4M LSS = 54.4% (161/296)] and no time effect [48h=54.8% (155/283) vs 54h=60.9% (173/284); (P=0.11)] on the P/AI. In Exp. 2a, there was no interaction (P=0.66) treatment*time of TAI after TLab (24 or 48h) on P/AI. There was no difference (P=0.13) in the P/AI between the Control (63.1%; 125/198), Liquid Conventional 6M (53.2%; 91/171) and LSS 4M (52.8% ;103/195). Still, there was no difference (P=0.84) in P/AI when TAI was performed 24 (54.1%;167/309) or 48 (59.6%; 152/255) hours after semen output from the lab. In Exp. 2b, there was no time*treatment interaction for any of the analyzed variables. There was lower VSL, BCF, LIN, STR and spermatozoa with progressive movement in the LSS compared to conventional liquid semen. However, the LSS showed a higher percentage of cells with an intact membrane (eosin/nigrosi 82.9% vs. 75.3%; P=0.001). There was a reduction in mobile and fast spermatozoa after 72 hours and BCF after 84 hours in both groups. The other analyzed variables showed no time difference. In Exp. 3, there was no difference (P=0.29) for P/AI between the different amounts of sperm in the LSS straw [2M=47.2% (84/178) vs. 3M=47.9% (82/171) vs 4M=56.1% (96/171) vs Control=52.2% (83 /159)]. In Exp. 4, there was no treatment*TLab interaction for P/AI (P=0.18). However, there was a difference between treatments (P=0.01) on P/IA [Control=62.5% (65/104)a vs 2M LSS=50.4% (67/133)ab vs 4M LSS=43.2% (51/118)b]. There was no difference (P=0.37) in P/AI between the different Tlabs [24h=53.8% (64/119) vs 48h=50.4% (119/236)]. It is concluded that the sexing process did not compromise the P/AI compared to conventional semen, when the insemination was performed with LSS. Furthermore, it was possible to inseminate with LSS 48 hours after P4 withdrawal without reducing P/AI. Also, it was possible to reduce the number of spermatozoa in the straw to 2 million and perform insemination with LSS up to 48 hours after TLab without compromising P/AI.

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TAI/AI

GnRH increases the pregnancy rate in cows with low body condition score and that do not express estrus during FTAI protocol

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This study evaluated the GnRH effect on the pregnancy rate in cows with low body condition score (BCS) that do not express estrus during the FTAI protocol. Multiparous Nelore cows (n=1,260) with early postpartum (32.6 ± 8.9; 20 to 50 days), low BCS (2.2 ± 0.4; 1.50 to 2.75; scale 1 to 5) and from a single farm were used in this study. A pre-synchronization with 150mg of injectable progesterone (P4; Sincrogest®, Ourofino, Brazil) was performed 10 days before (D-10) starting a conventional FTAI protocol. Then, on D0 the cows received an intravaginal P4 device (1g, Sincrogest®) and intramuscularly (i.m.) injection of 2mg estradiol benzoate (Sincrodiol®). On D8, the P4 device was removed and performed i.m. application of 0.5mg cloprostenol (Sincrocio®), 1mg of estradiol cypionate (SincroCP®) and 300IU of equine chorionic gonadotropin (Sincro eCG®), in addition to painting the sacrococcygeal region to assess the estrus expression intensity. On D10, the estrus expression was evaluated, and the cows were classified as high estrus (paint completely removed), low estrus (paint partially removed), and non-estrus (paint was not removed). Females with high and low estrus expression received conventional FTAI and those that did not express estrus were randomly divided into GnRH group [n=242; FTAI with i.m. application of 0.010mg of buserelin acetate (Sincroforte®)] and control group (n=249; FTAI without treatment). Artificial inseminations were performed by 3 inseminators with semen from two bulls with known fertility. Pregnancy diagnosis was performed at 40 days. The statistical analysis was performed by logistic regression. Treatment effect, inseminator and bull were considered as fixed variables, and postpartum days and BCS as continuous variables. A P-value ≤ 0.05 was adopted and data were presented as % and M±SD. The overall pregnancy rate was 56.6% (713/1260). Cows with high estrus expression had a pregnancy rate of 68.6% (221/322) and those with low expression 55.7% (249/447). Cows that did not express estrus and received GnRH [54.5% (111/249)] had a pregnancy rate almost 10% higher (P=0.02) than the control group [44.6% (132/242)]. There was no influence of bull (P=0.23), inseminator (P=0.12), and postpartum days (P=0.24). BCS showed influence (P=0.01), but no interaction with treatment was observed (P>0.1). Considering FTAI programs with challenges of early postpartum (32.6 ± 8.9 days) and low BCS (2.2 ± 0.4), the use of GnRH in cows that do not express estrus represents a positive strategy to achieve acceptable pregnancy rates in a FTAI program.

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TAI/AI

Does GnRH treatment at TAI regardless of estrus occurrence increase pregnancy rate in crossbred taurine suckled cows?

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Estradiol cypionate (EC) is an ovulation inducer commonly administered at intravaginal (IVD) progesterone (P4) device removal in TAI protocols. EC promotes estrus manifestation and ovulations that occur between 42 to 96 h after treatment, whereas GnRH treatment does not induce estrus but promotes synchronous ovulations (27-30 h after treatment). Two experiments were carried out to evaluate if GnRH treatment at TAI in all cows, regardless of estrus occurrence (GnRH group), would improve fertility compared to selective GnRH treatment (selectGnRH group), with GnRH treatment only in cows that did not show estrus up to 48 h after IVD removal. First, we determined the ovulation rate and luteal function after GnRH treatment at TAI, and then the effect of GnRH at TAI on conception rate (P/AI) in suckled cows (>40 days postpartum). Procedures were approved by UFRGS Ethics Committee. Data were analyzed by ANOVA and Chi-square. In Experiment 1, suckled multiparous Brangus cows (n = 17) received an intravaginal device (IVD; 1 g progesterone; Sincrogest; Ouro Fino) and 2 mg of estradiol benzoate (E Ric-BE; Agener União; im) on D0. On D8, the IVD was removed, cows were treated with EC (0.5 mg; Cipiotec; Agener União; im), cloprostenol (PGF; 0.5 mg; Estro Agener União; im), eCG (300 IU; Novormon, Zoeti im) and were tail chalk marked. Estrus occurrence was evaluated based on chalk removal on D10 (TAI) and cows were assigned to receive GnRH (25µg; Tec-Relin, Agener União; im) according to the group (n = 7, GnRH; n = 10, selectGnRH). Ovulation rate occurring until 77h did not differ (p = 0,17) between selectGnRH (100%; 10/10) and GnRH (85.7%; 6/7). No difference was observed in corpus luteum area, diameter and circumference between groups (P>0.05). Serum progesterone differed between days 5 (D5) and 13 (D13) after treatment (P<0.0001), but there was no difference (P = 0.2) between selectGnRH (D5 = 2.6±0.5; D13 = 10.7±2.0) and GnRH (D5 = 3.3±0.8; D13 = 13.3±2.4), and no interaction between group and day (P= 0.9). In Experiment 2, crossbred taurine suckled cows (n = 384) had the follicular wave synchronized, as described in Experiment 1, and were randomly allocated into selectGnRH and GnRH groups. There was no difference in P/AI (30 days after TAI) between groups (selectGnRH = 55.6%; GnRH = 54.3%; p = 0.08). There was a pronounced effect of estrus expression on P/IA (Estrus = 61.5%; No estrus = 33.0%; P<0.0001), regardless of group. In conclusion, GnRH administration only in cows that do not show estrus is recommended, considering hormone savings and similar conception rate.

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TAI/AI

Factors that affect the efficiency of TAI protocol with pre-synchronization based on long-acting injectable progesterone, in crossbred dairy cows.

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The reproductive performance of dairy cows influences the profitability of dairy farms, since inefficiency reduces milk production and the number of calves born per year. Fixed-time artificial insemination (TAI) could be used to make reproduction management more efficient. The aim of this study was to analyze the factors that affect the results of TAI protocols with pre-synchronization based on long-acting injectable P4. This study was conducted on a commercial farm located in Uberlândia, Minas Gerais, Brazil, with approximately 1,200 lactating crossbred Girolando cows. Cows with more than 45 days in milk, and presented with a healthy uterine and ovarian, were submitted to the hormonal protocol. The management consists of a gynecologic evaluation of the cows on day - 14 (D-14), administration of 300 mg injectable P4 (Sincrogest, Ouro Fino, Cravinhos, Brazil) on D-10, followed by a second gynecologic evaluation on D0 to determine the return to cyclicity, insertion of an intravaginal device of 1.2g of P4 (Fertilcare Implante 1200, Vallée, Montes Claros, Brazil), in addition to an injection of 2 mg of Estradiol Benzoate (Fertilcare Sincronização, Vallée, Montes Claros, Brazil) and 0.2 mg of gonadorelin. On D7, the cows received 0.53 mg of Cloprostenol Sodium (Ciosin, MSD, Cruzeiro, Brazil), followed by the removal of the intravaginal device on D9 and administration of 1 mg of estradiol cypionate (E.C.P. Zoetis, São Paulo, Brazil), 400 IU of eCG (Folligon, MSD, Cruzeiro, Brazil), and 0.53 mg of Cloprostenol Sodium. On D11, they received 0,2mg of Gonadorelin (Fertagyl, MSD, Cruzeiro, Brazil), and the TAI was performed. All handlings were performed at the same time and hormonal administrations were intramuscular. The analyzed data were collected only from the first service animals (n = 763) that were categorized according to milk production per day greater than or equal to the average of the farm (≥ 37.3 L/day); racial composition was classified as animals with racial composition less than 3/4 Gyr/Holstein (1/2 to 5/8 = < 3/4) and greater than or equal to 3/4 (3/4 to 25/32 ≥ 3/4); the order of lactation was group as primiparous and secondiparous; and multiparous (3 or more lactations); as for somatic cell count (SCC), cows were classified with less than or equal to 200,000 cell/mL of milk or SCC greater than 200,000 cell/mL of milk; the calving season, as well as the moment of TAI, were defined according to the season in which they occurred: spring, summer, autumn and winter. The effects of these factors on the efficiency of the TAI protocol used were analyzed by logistic regression, with the variable responses were pregnancy by TAI at 30 days and 45 days. Cows classified as < 3/4 Gyr/Holstein had a higher ($P<0.05$) conception at 30 days (53.40% vs. 45.63%) and at 45 days (52.72% vs. 42.86%) than cows ≥ 3/4 Gyr/Holstein. Multiparous cows tend to have a higher conception rate at 45 days (52.75%) than primiparous and secondiparous cows (44.75%); the other factors analyzed did not interfere with pregnancy/AI. Thus, it is possible to conclude that the racial composition interferes with the pregnancy rate/AI both at 30 and at 45 days of gestation, as well as the order of lactation tends to interfere at 45 days of gestation when the analyzed protocol is used.

Animal Reproduction

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

TAI/AI

Effects of an injection of trace minerals (Cu, Zn, Se, and Mn) and vitamins (A and E) on reproduction and inflammatory responses of *Bos indicus* beef females synchronized to fixed-time AI

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In experiment 1, the objective was to evaluate the effects of trace minerals (Cu, Zn, Se, and Mn) and vitamins (A and E) injection (TMVI) on reproduction and inflammatory responses of Nellore females. Sixty-eight multiparous were synchronized to a fixed-time artificial insemination (FTAI) protocol (d -11 to d 0; d 0 = insemination). On d -11, cows were assigned to 5 mL/cow of Saline or TMVI solutions (10, 40, 10, and 5 mg/mL of Cu, Zn, Mn, and Se, and 59500 and 50 UI/mL of vitamins A and E, respectively; Kit Adaptador; Biogénesis Bagó, Curitiba, Brazil). Dominant follicle was evaluated on d 0, corpus luteum on d 7 and 14, and pregnancy rate (PR) on d 37. Blood samples were collected (n = 12/treatment), and plasma progesterone concentrations were evaluated on d 7 and 14, serum Cu and Zn on d -11, 0, and 37, and plasma haptoglobin, plasma ceruloplasmin and serum cortisol on d -11, 0, 7, 14 and 37. TMVI vs. Saline-treated cows tended to have greater plasma concentration of progesterone on d 14 (5.27 vs. 7.25 ± 0.73 ng/mL; P = 0.10) but not on d 7 (P = 0.63), and had a greater pregnancy rate on d 37 (36.4 vs. 61.3 ± 8.56%; P = 0.05). TMVI vs Saline-treated cows tended to have lower haptoglobin concentration on d 37 (0.30 vs. 0.36 ± 0.02 mg/mL; P = 0.09), and had lower cortisol concentration throughout the study (2.42 vs. 2.96 ± 0.18 µg/dL; P = 0.05). A second experiment was conducted to increase the number of animals from Exp.1 to evaluate the pregnancy rate. In Exp. 2, 866 multiparous, 341 primiparous, and 263 heifers were synchronized to FTAI protocol from d -11 to d 0. On d -11, females were assigned to Saline or TMVI treatments as in Exp.1. Pregnancy was evaluated on d 30, 70 and 120 and blood samples were collected from multiparous (n = 12/treatment) on d -11, 0 and 30 to evaluate serum Cu and Zn concentrations. Non-pregnant females on d 30 were resynchronized in a new protocol, and non-pregnants on d 70 were exposed to bulls at a rate of 1/40. The TMVI increased PR in heifers with body condition score (BCS) < 5 on d 30 (48.2 vs. 63.1 ± 5.61%; P = 0.05), and tended to increase the PR on d 120 (81.6 vs. 88.3 ± 3.21%; P = 0.10) despite of the BCS. In primiparous, the TMVI tended to increase the PR on d 30 in cows with BCS < 5 (54.1 vs. 67.8 ± 5.19%; P = 0.09). In multiparous, the TMVI tended to increase (P = 0.10) the concentration of Cu on d 0. The administration of TMVI at the beginning of a FTAI protocol reduced the inflammatory responses and improved reproduction parameters mainly in females with low BCS.

Animal Reproduction

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

TAI/AI

Effect of double-timed artificial insemination and reduced PGF dose on reproductive performance of ewes

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Timed artificial insemination (TAI) in ewes still yields variable results, which could be partially determined by asynchronous ovulations. Furthermore, viable economic alternatives are necessary for wider dissemination of the technique. Aiming this, we conducted a study during the transition period to reproductive season (December to February in Rio Grande do Sul state - Brazil). Ewes were maintained in native pasture and had a minimum body condition score of 2.5 (0-5 scale). In Experiment 1, to evaluate whether double TAI would increase pregnancy rate, ewes (n=255) received an intravaginal device (IVDs) containing 60 mg medroxyprogesterone acetate (MAP; Fagron do Brasil Farmacêutica, São Paulo, Brazil), which remained for 7 days. At IVD removal, animals received intramuscularly (IM) 200 IU of eCG (SincroeCG®; Ourofino Saúde Animal, Cravinhos, Brazil) and 250 µg of sodium cloprostenol (PGF; Estron®; Agener União, São Paulo, Brazil). All ewes were inseminated 54 h after IVD removal, together with the IM application of 4 µg of buserelin (GnRH; Sincroforte®; Ourofino Saúde Animal, Cravinhos, Brazil). At TAI, the animals were randomly allocated to two groups: Control (n=127), that received no additional treatment; and 2AI group (n=128), in which ewes received a second TAI 14 h later. AIs were performed by superficial cervical insemination with 100x106 motile spermatozoa from a semen pool from rams with known fertility. Pregnancy diagnosis by transrectal ultrasonography was performed 30 days after AIs. Data were compared by logistic regression. The pregnancy rate was lower (P=0.04) in the Control group (34.6%; 44/127) compared to the 2IA group (46.9%; 60/128), with no significant effect of replicate nor interaction. In Experiment 2, the efficiency of two doses of PGF in estrus synchronization and pregnancy rate after natural mating (NM) was compared, the ewes (n=81) received an IVD for 6 days. At IVD removal, the animals were allocated to two groups: PGF 1 (n=40), treated with 250µg of sodium cloprostenol (PGF; Estron®; Agener União, São Paulo, Brazil); and PGF 0.5 (n=41) treated with 125 µg of PGF IM (0.5 mL), no other treatment was performed. The NM was recorded for 5 days using rams (10% ratio) with grease paint on the brisket. Data were compared by chi-square test. No difference (P>0.05) was observed in the estrus manifestation [65% (26/40) for PGF 1 and 78,0% (32/41) for PGF 0.5], conception rate [69,2% (18/26) for PGF 1 and 68,8% (22/32) for PGF 0.5] and pregnancy rate [45% (18/40) for PFG 1 and 53.7% (22/41) for PGF 0.5]. We concluded that performing double TAI (54h and 68h after IVD removal) increases pregnancy rate, when compared to a single TAI (54h after IVD removal). Furthermore, the dose of 125 µg of cloprostenol is as efficient as 250 µg to promote estrus synchronization without affecting conception and pregnancy rates in ewes, reducing the injected-volume and the protocol cost.

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Animal Reproduction

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

TAI/AI

Reproductive outcomes of 11-month-old Nelore (*Bos indicus*) heifers submitted or not to ovulation induction protocols prior to the timed-AI protocol

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The aim was to study reproductive outcomes of Nelore (*Bos indicus*) heifers submitted or not to ovulation induction protocols prior to the timed-AI (TAI) protocol. On D-47 (D0=beginning of TAI protocol), 583 heifers (11.3±1.7mo old; 272±39.8kg of body weight [BW]) were randomly assigned to 1 of 2 treatments: control (CON;n=284): no treatment prior to D0; or 2 ovulation induction protocols (2IND;n=299): heifers received a new intravaginal progesterone device (P4; 0.5g), kept until D-40, when they received 0.5mg estradiol cypionate (EC). On D-19, 2IND heifers were resubmitted to the same protocol. On D0, all heifers received the same TAI protocol, starting with a new P4 device (0.5g) plus 1.5mg estradiol benzoate (EB). On D7, 0.5mg cloprostenol (PGF), 0.5mg EC and 200IU eCG were given, concomitant with P4 withdrawal and tail chalk for estrus evaluation. On D9, 8.4µg of buserelin acetate (GnRH) was administered, and TAI was performed. Ultrasound was performed on D-47, -19 and 0 for uterine and ovarian evaluations, and on D40 and 70 for pregnancy diagnosis (P/AI) and pregnancy loss (PL) evaluation, respectively. Statistical analyses were done by PROC GLIMMIX of SAS 9.4 (P≤0.05). Ovarian and uterine variables on D-47 were similar between treatments. There was no interaction between treatments and the other variables on reproductive outcomes. On D-19 and 0, presence of corpus luteum (CL) was greater in 2IND group (D-19: 73.9 *vs* 18.3%; D0: 80.3 *vs* 32.4%), as well as uterine diameter (UD; D-19: 14.2±0.1 *vs* 13.4±0.1mm; D0: 14±0.1 *vs* 13.7±0.1mm). Follicle diameter (FD) did not differ on D-19. However, on D0, CON group had greater FD than 2IND (9.9±0.1 *vs* 8.7±0.1mm). Although more heifers from 2IND group had expressed estrus (88 *vs* 77.8%), there was no difference between CON and 2IND on P/AI on D40 (41.5 *vs* 39.5%), and D70 (37.7 *vs* 33.1%) nor on PL (9.3 *vs* 16.1%). Regardless of treatment, presence of CL on D0 resulted in greater expression of estrus (86.4 [332] *vs* 78.5% [251]) and greater P/AI (D40: 43.4 *vs* 36.7%; D70: 37.7 *vs* 32.3%), with no effect on PL. Moreover, P/AI was greater in heifers expressing estrus (D40: 43.2 [484] *vs* 27.3% [99]; D70: 38.2 [484] *vs* 22.2% [99]), with no effect on PL. There was effect of BW tercile (D0: ≤293 [192], 294-335 [194], and ≥336 kg [197]) on P/AI on D40 (32.8^{b} *vs* 42.3^{ab} *vs* 46.2%^{a}) and on D70 (27.6^{b} *vs* 38.7^{a} *vs* 39.6%^{a}), although no differences were detected on expression of estrus or PL. The P/AI on D40 was linearly affected by BW, as the greater the BW the greater the P/AI. Otherwise, there was a quadratic effect of BW on P/AI on D70 and on PL, in which P/AI increased up to ~352kg, and then decreased, due to a greater PL. In conclusion, although induction ovulation protocols increased the percentage of Nelore heifers with CL at the beginning of the TAI protocol, UD and expression of estrus, there was no effect on P/AI and on PL.

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Animal Reproduction

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

TAI/AI

The impact of albendazole sulfoxide and ivermectin (Evol®) administration on day 0 of fixed-time artificial insemination protocol in beef females

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The present study aimed to evaluate the impact of the administration of a parasite control on day 0 (D0) of the fixed-timed artificial insemination (FTAI) protocol on the estrus expression and pregnancy rate in grazing Nelore and crossbred females. A total of 2,120 females [including multiparous (n= 1,597), primiparous (n= 295) and heifer (n= 228)] were homogeneously divided according body condition score (BCS= 2.91±0.01) and cyclicity on D0 (25.6%) into two experimental groups: 1) Control: females received no treatment (n=1,019) and 2) Evol: females received 1mL/40kg of parasite control (Evol®: albendazole sulfoxide + ivermectin; Ourofino, Cravinhos, SP; n=1,101) on the first day of the FTAI protocol. All females included in the study were synchronized for FTAI by inserting a progesterone (P4) device (Sincrogest, Ourofino) and received an injection of 2 mg of benzoate estradiol (Sincrodiol, Ourofino). The P4 device was removed after seven, eight or nine days, and a subset of females (n= 1,342) had the base of their tail painted to evaluate the estrus expression. The females also received injections of 1 mg of estradiol cypionate (Sincrocp, Ourofino), 300 UI of equine chorionic gonadotropin (Sincro eCG, Ourofino) and 0.53 mg of sodium cloprostenol (Sincrocio, Ourofino). FTAI was performed 48 hours after P4 device removal, and the females were inseminated using semen from bulls distributed homogeneously within the groups. The BCS was also recorded at this time. After 30 days, the pregnancy diagnosis was performed by ultrasonography. Data were analyzed using the GLIMMIX procedure of SAS (9.4) and the value P 0.05 was considered for effect. The percentage of females expressing estrus did not differ between groups [Evol: 85.1% (598/703) vs. Control: 83.3% (532/639); P= 0.37] and no interaction was observed between treatment and category (P= 0.60), BCS (P= 0.70), or days with P4 device (P= 0.60). However, Evol treated females at D0 had better BCS at FTAI (P= 0.05) compared to the control group (3.10 ± 0.02 vs. 3.04 ± 0.02) respectively. Additionally, the pregnancy rate was significantly greater (P= 0.001) for the Evol group than the control group [55.9% (615/1,101) vs. 48.9% (498/1,019)]. No interaction between treatment and category (P= 0.32), BCS (P= 0.39), or days with P4 device (P= 0.10) was observed for pregnancy rate. In conclusion, the use of the parasite control Evol® at D0 of the TAI protocol increased the BCS and P/AI of the treated females.

Animal Reproduction

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

TAI/AI

Association between the corpus luteum blood perfusion determined 20 days after timed-AI and false positive rate for pregnancy diagnosis in Nelore cows

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The objective of the study was to evaluate the association between the corpus luteum blood perfusion (CLBP) in cows with an active CL determined by Doppler ultrasonography at day 20 after timed-AI (TAI) and the false positive (FP) results in the confirmatory pregnancy diagnosis. We hypothed that the FP rate is negatively associated with CLBP. For this, a retrospective analysis was performed using data from 313 Nelore cows (276 suckled between 30 and 60 days postpartum, and 37 non-lactating) subjected to a TAI protocol. At the beginning of the protocol (Day-10), animals received the insertion of a progesterone intravaginal device (0.96g, Progestar®, Biogénesis Bagó) and application of 2 mg of estradiol benzoate (EB; i.m, Bioestrogen®, Biogénesis Bagó). On D-2, the devices were removed and 1mg of estradiol cypionate (i.m, Croni-Cip®, Biogénesis Bagó), 300 IU of eCG (i.m, Ecegon®, Biogénesis Bagó) and 0.53mg of cloprostenol sodium (i.m, Croniben®, Biogénesis Bagó) were applied. On Day 0, TAI was performed. On Day 12, all females were submitted to a resynchronization based on P4/E2 protocol, using 1 mg of EB (i.m, Bioestrogen). On D20, all females were evaluated by transrectal Doppler ultrasonography (DP50-Power, Mindray, China; settings: frequency 5,7 M, gain 10, and PRF 1.2k) to estimate the CLBP (subjective evaluation of color signals on a scale of 0-100%, 5 points between degrees). Only animals with CLBP ≤ 25% continued in the resynchronization protocol and were inseminated 48h later. Cows with an active CL (≥30% of CLBP) were submitted to a confirmatory pregnancy diagnosis based on the identification of an embryo with heartbeats on Days 55-60. A Receiver Operating Characteristic (ROC) analysis was performed to determine a cutoff point for classifying the CLBP in low (≤40%; n=62) or high (>40%; n=134). The FP rate was analyzed by the PROC GLIMMIX and PROC LOGISTIC of the SAS software. The pregnancy rate at the Doppler diagnosis and confirmatory diagnosis were, respectively, 62.6% (196/313) and 53% (166/313); therefore, the FP rate was 15.2% (30/196). For non-pregnant females on Day 20, the rate of animals without detection of a CL at Doppler evaluation was 30.8% (36/117), and the rate of females that had CL with BP ≤ 25% was 69.2% (81/117). The ROC curve indicated that the CLBP was a significant (P=0.003) predictor of the FP rate in the confirmatory diagnosis (area under the curve=0.66). When comparing the two classes of CLBP, a greater (P= 0.03) FP rate was observed for the group of cows with low CLBP (27.4%; 17/62) than in the group with high CLBP (9.7%; 13/134). Finally, the logistic regression analysis indicated a negative linear relationship (P=0.004) between the CLBP on Day 20 and the probability of an FP result. In conclusion, cows with a CLBP ≤40% determined by Doppler imaging on Day 20 are more likely to be identified as an FP in the confirmatory diagnosis of pregnancy.

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Animal Reproduction

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

TAI/AI

Combination of estradiol valerate and cypionate as a successful alternative for TAI 48 hours after P4 device withdrawal in Bos indicus cows

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The objective was to evaluate the timing of insemination (48h vs. 54h) and the additional treatment with estradiol cypionate as ovulation inducer in an estradiol valerate plus progesterone-based synchronization of *Bos indicus* cows. In experiment 1, Bos indicus cows (n=434) with body condition score (BCS) of 2.62±0.01 (2.25 to 4.00 and Median=2.75) were used. At the beginning of protocol (D0), cows received a new intravaginal progesterone device (P4) and 5mg of estradiol valerate (EV). Nine days later (D9), all cows received 300IU of eCG and P4 devices were removed. At the same time, cows were distributed into one of two experimental groups (TAI48 or TAI54). The TAI48 and TAI54 group cows were inseminated 48h and 54h after removing the P4 device, respectively. In experiment 2, Bos indicus cows (n=429) with BCS of 2.80±0.01 (2.25 to 4.00 and Median=2.75) were distributed into one of two experimental groups (Control and VE/CE groups). At the beginning of the protocol (D0), all cows received a new P4 device. Cows in the Control group received 2 mg of estradiol benzoate (EB) and cows in the VE/EC group received 5 mg of EV. Nine days later (D9), all cows received 1mg of estradiol cypionate, 300IU of eCG and P4 devices were removed. At same moment, cows in the Control group received 0.265µg of sodium cloprostenol (PGF). In addition, the tail-head of was marked with chalk for evaluate estrus occurrence between removal of the P4 device and insemination. All cows were inseminated 48 hours after the P4 device removal. In both experiments, products from MSD, São Paulo, Brazil were used (EV: Valerol®, P4: Fertilcare® 1200/Crestar IVG® 1g, EB: Fertilcare sincronização®, EC: Fertilcare ovulação®, eCG: Folligon®, PGF: Ciosin®). Ultrasound exams were performed 30 days after TAI to evaluate the cyclicity (D40) and pregnancy per AI (P/AI). Statistical analyses were performed by GLIMMIX procedure of SAS. The experiments were performed in farms with significant nutritional challenges at the end of breeding season. In experiment 1, the P/AI was lower in cows inseminated 48h after removal of the P4 device [TAI48=36.4% (79/217) and TAI54=45.2% (98/217); P=0.07]. In experiment 2, estrus rate [Control = 70.9% (156/220) and VE/CE = 76.6% (160/209); P=0.12], P/AI [Control = 43.2% (95/220) and VE/CE = 45.5% (95/209); P=0.56] were similar between experimental groups. In summary, protocols using estradiol valerate without an exogenous ovulation induction requires adjustments on the timing of the AI from 48 to 54 hours after progesterone device withdrawal. However, the combination of estradiol valerate at the beginning of protocol and estradiol cypionate (D9) to induce ovulation allows the TAI to be performed 48 hours after P4 device withdrawal in Bos indicus cows.

Animal Reproduction

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

TAI/AI

Equine chorionic gonadotropin (eCG) at the time of TAI on the fertility of *Bos indicus* cows submitted to the ovulation synchronization protocol

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The objective was to evaluate the fertility of *Bos indicus* beef cows submitted to ovulation synchronization protocol using eCG at the time of TAI. In the study, multiparous (n=361) and primiparous Nelore cows (n=45) at body condition score of 2.91±0.26 were used. On a random day of the estrous cycle (D0), cows received 2 mg of estradiol benzoate (Sincrodiol®, Ourofino, Brazil) and a progesterone intravaginal device (Sincrogest®, Ourofino, Brazil). Eight days later (D8), 1mg of EC (SincroCP®, Ourofino, Brazil), 300IU of eCG (SincroeCG®, Ourofino, Brazil), 500µg of sodium cloprostenol (Sincrocio®, Ourofino, Brazil) were administered and P4 intravaginal devices were removed. In addition, the sacrococcygeal region was painted for further evaluation of estrus expression. All cows were inseminated 48 hours after the removal of P4 device. At the same time, females were randomly allocated into two experimental groups (Control and eCG). Cows in the Control group (n=208) received no additional treatment and cows in the eCG group received 200UI of eCG (SincroeCG®, Ourofino, Brazil). Pregnancy diagnosis and cyclicity rate were evaluated 30 days after TAI (D40). Statistical analyses were performed by GLIMMIX procedure of SAS. The estrous expression rate (Control = 65.4% and eCG = 68.2; P=0.55), pregnancy rate (Control = 51.4% and eCG = 52.0%; P=0.90) and cyclicity rate (D40; Control = 43.6% and eCG = 34.7%; P=0.15) did not differ between the experimental groups. It is concluded that the use of eCG at the time of TAI does not increase fertility in *Bos indicus* beef cows.

Animal Reproduction

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

TAI/AI

Relationship between prostaglandin application and puerperal uterine recovery in Nelore cows

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We evaluated the effect of prostaglandin on uterine health during the postpartum period in cows submitted to TAI program. Twenty-eight days after calving the beef cows were separated into 3 groups to receive the following treatments: 1) no prostaglandin analog, Control group (n = 173); 2) a single dose of prostanglandin analog (1PG, n = 171), 0.5 mg of Cloprostenol, and 3) double dose of prostaglandin analog (n = 173), 1 mg Cloprostenol (2PG, Ciosin®, MSD, São Paulo, Brazil). All females were submitted to an estradiol- progesterone based TAI protocol, on average 47.5 ± 9.6 (Mean ± SD) days postpartum. On Day 0 of the protocol, endometrial cytobrush samples were collected for analysis of cells polymorphonuclear (PMN) infiltration intensity. Slides for PMN cell counting were prepared and stained (Quick Panoptic®, Laborclin, Pinhais, Brazil). Two hundred cells per slide were counted under an optical microscope (100X magnification), including mononuclear and epithelial cells to calculate the proportion of polymorphonuclear (PMN) cells. Thirty days after insemination, the pregnancy diagnosis was performed. Continuous variables were analyzed by ANOVA (PROC GLIMMIX; SAS Inst. Inc., Cary, NC). Binomial variables were analyzed using the chi-square test. Cows were also divided according to the postpartum period in which the TAI protocol was started, as follows: up to 35 days postpartum (35 DPP, n = 72) and older than 35 days postpartum (>35 DPP, n = 445). No difference (P = 0.77) was observed in the pregnancy by AI (P/AI) among groups (Control = 69.9%; 1PG = 71.34%, and 2PG = 73.4%). The PMN cells ratio also did not differ (P = 0,69) among groups (Control = 3.9 ± 0.7%; 1PG = 4.2 ± 0.6%; and 2PG = 4.9 ± 0.9). Regarding pregnancy rate according to the time of postpartum in which the TAI protocol was started, there was no difference considering the applied treatments. However, considering only early postpartum cows it is possible to observe a numerical difference in the P/AI (Control = 73%; 1PG = 74% and 2PG = 87%), but no statistical difference (P = 0.48). A particularly relevant aspect of this experiment are the unusually high pregnancy rates observed. Therefore, we believe that the assessment of prostaglandin analog, cloprostenol, influence on pospartum uterine recovery warrants further investigation.

Animal Reproduction

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

TAI/AI

Different TAI protocols without the use of estradiol in the fertility of *Bos indicus* cows

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The objective was to evaluate the fertility of suckled *Bos indicus* cows pre-synchronized with progesterone and submitted to the TAI protocol based on GnRH. In experiments 1 (n=109) and 2 (n=389) cows received 150mg of injectable progesterone (P4i) 10 days before (D-10) of the TAI protocol. On D0 cows received a new progesterone intravaginal device (P4) and were allocated in three groups (Control, GnRH/EC and 2GnRH). Cows received 2mg of estradiol benzoate (E Control group) or 20µg of buserelin acetate (GnRH GnRH/EC and 2GnRH). On D8, P4 devices were removed and 300IU of eCG and 500µg of Cloprostenol (PGF) were administered in all cows. In addition, cows received 1mg of estradiol cypionate (EC; Control and GnRH/EC). TAI was performed 48 hours after P4 removal (D10). In this day, cows of the 2GnRH group received 10µg of GnRH. In experiment 3, cows (n=347) were allocated in four groups (Control, GnRH/EC, 2GnRH48 and 2GnRH54). Seven days before of the TAI protocol (D-7), the cows of the GnRH groups (GnRH/EC, 2GnRH48 and 2GnRH54) received a new P4 intravaginal device that remained until D8. On D0, cows of the control group received 2mg of EB and the P4 intravaginal device and cows of the GnRH groups received 200µg of gonadorelin (GnRHg). On D8, all cows received 0.265µg of cloprostenol sodium (PGF) and 300UI of eCG and the P4 devices were removed. In Control and GnRH/EC groups, cows received 1mg of EC. TAI was performed 48h (Control, GnRH/EC and 2GnRH48) or 54h (2GnRH54) after P4 removed. In this day (48h), the cows of 2GnRH48 and 2GnRH54 groups received 100µg of GnRH. In experiments 1 and 2, products from Ourofino, Ribeirão Preto, Brazil (P4i: Sincrogest Injetável, P4:Sincrogest, EB:Sincrodiol, EC:SincroCP, GnRHb:Sincroforte, eCG:SincroeCG, PGF:Sincrocio) and experiment 3, products from MSD, São Paulo, Brazil were used (P4:Crestar 1,0g, EB:Fertilcare sincronização, EC:Fertilcare ovulação, GnRHg:Fertagyl, eCG:Folligon, PGF:Ciosin). Ultrasonographic exams were performed to determine the follicular dynamic and the pregnancy diagnosis (D40). Statistical analysis was performed by SAS. In Exp. 1, the time of ovulation was higher in the 2GnRH group (Control=71b, GnRH/EC=73b and 2GnRH=78a; P=0.002). The ovulation rate on D0 was greater in GnRH groups (Exp. 2 - Control=0%b, GnRH/EC=39%a and 2GnRH=45%a; P=0.001) and (Exp. 3 - Control=2%b, GnRH/EC=79%a, 2GnRHTAI48=76%a, 2GnRHTAI54=72%a; P=0.001). However, the estrus rate was lower in cows that received GnRH in the TAI (Exp. 2 - Control=49%a, GnRH/EC=52%a and 2GnRH=35% P=0.01) and (Exp. 3 - Control=61%a, GnRH/EC=55%ab, 2GnRH48=42%b and 2GnRH54=57%a P=0.08)]. Furthermore, P/IA did not differ between groups in Exp. 2 (Control=36%, GnRH/EC=34% and 2GnRH=26%; P=0.19). However, in Exp. 3, the P/IA was lower in the GnRH/EC group (Control=57%a, GnRH/EC=34%b, 2GnRH48=42%ab and 2GnRH54=39%a P=0.04). In conclusion, GnRH based synchronization protocols are less efficient in synchronizing ovulation in suckled *Bos indicus* cows.

Animal Reproduction

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

TAI/AI

Reducing the period of permanence of the intravaginal progesterone device decreases the conception rate of suckled *Bos indicus* primiparous cows submitted to E2/P4-based TAI protocol

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The objective was to evaluate the fertility of suckled *Bos indicus* primiparous cows submitted to TAI protocol using 7 or 9 days of intravaginal progesterone-releasing (P4). Nelore primiparous cows (n=380) at 2.60±0.01 (2.25 to 3.00 and Median=2.50) were randomly distributed in two experimental groups (7P4 and 9P4 groups). In TAI protocol using P4 for 9 days, the beginning of the treatment (D0) was carried two days before of TAI protocol (D-2). At beginning of the TAI protocol, the animals received 2mg estradiol benzoate IM and insertion of an intravaginal progesterone-releasing device. Seven (7P4 group) or nine (9P4 group) days later, the P4 device was removed, and cows received 1mg of estradiol cypionate, 0.265mg of sodium cloprostenol and 300IU of eCG. Hormones used in the study were of different companies and with proven effectiveness. In addition, the tail- head of was marked with chalk for evaluate estrus expression between the progesterone device withdrawal and artificial insemination. TAI was performed 48h after progesterone device withdrawal. In a subset (n=195) the conception rate by natural-mating (1 bull/25 cows) and total pregnancy rate (TAI + bull) was analyzed. Ultrasound exams were performed 55 days after TAI to evaluate the conception rate. Statistical analyses were performed by GLIMMIX procedure of SAS. Estrus expression rate [7P4=49.5% (93/188) and 9P4=49.0% (94/192); P=0.92] and the conception rate by natural-mating [7P4=7.4% (5/68) and 9P4=2.4% (1/42); P=0.29] were similar between groups. However, conception rate at TAI ([7P4=25.0% (47/188) and 9P4=41.1% (79/192); P=0.01]) and pregnancy rate [7P4= 33.7% (32/95) and 9P4=59.0% (59/100; P=0.01] were greater for the 9P4 group. It is concluded that the greater period of permanence (9 days) of P4 device in E2/P4-based TAI protocol increased the fertility in suckled Bos indicus primiparous cows.

Animal Reproduction

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

TAI/AI

Effect of buserelin acetate administered at the time of insemination on the conception rate of dairy cows inseminated with sexed semen: preliminary results

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The objective of the present study was to determine whether the application of buserelin acetate (GnRH analogue), at the time of insemination, improves the conception rate of dairy cows subjected to Timed Artificial Insemination (TAI) with the use of sexed semen. Therefore, 96 crossbred females were selected on rural properties located in the semi-arid region of Bahia based on physical and clinical conditions. On a random day of the estrous cycle (D0), all animals underwent a gynecological examination with the aid of the ultrasound device and those who were fit received an intravaginal progesterone device (P4) associated with 2 mg of estradiol benzoate intramuscularly (im). On the ninth day, the P4 device was removed and 300 IU of Equine Chorionic Gonadotropin (eCG) + 500 µg of Cloprostenol and 1 mg of estradiol cypionate were applied, all via im. The TAI was performed 48 hours (D11) after the removal of the device using sexed semen, simultaneous with the ultrasound evaluation to measure the diameter of the pre-ovulatory follicle. At the time of the TAI, the females were randomly divided into two groups: Buserelin acetate group (BG; n = 48) in which the animals received 10 µg of buserelin acetate (Sincroforte®, Ourofino, Cravinhos, SP) concomitant with artificial insemination with sexed semen; Control group (CG; n = 48) in which females were inseminated with sexed semen but did not receive a GnRH agonist. The gestational diagnosis was made 30 days after artificial insemination using transrectal ultrasound. The conception rates were compared using the Bioestat program with 5% significance. No improvement (P=0.27) in the conception rate was observed with the use of buserelin acetate (buserelin acetate group = 20.8% x control group = 12.5%). The conception rate was not affected by the pre-ovulatory follicle at the time of the TAI. It is concluded that the application of 10 µg of buserelin acetate at the time of insemination does not improve the conception rate of dairy cows inseminated at a fixed time with sexed semen. However, the number of animals was limited and further studies are needed to determine the effect of the GnRH analogue on the conception rate of dairy cows inseminated with sexed semen.

Animal Reproduction

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

TAI/AI

Biochemical profile and oocyte quality of primiparous *Bos Indicus* cows submitted to ovulation synchronization protocol

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The objective was to evaluate the biochemical profile and quality of oocytes and follicular cells of primiparous *Bos indicus* cows submitted to ovulation synchronization protocol. In experiment 1, primiparous (n=24) and multiparous (n=24) cows with BCS of 2.6±0.03 received 2mg of estradiol benzoate (EB) and a intravaginal progesterone (P4) device that remained for twelve days. Follicular aspirations were performed on day 5 (D5) in all follicles larger than 2mm and on day 12 (D12) in the dominant follicle. Blood samples were collected on D5 and D12 for glucose, cholesterol, NEFA, IGF-1 and insulin concentrations. In experiment 2, primiparous (n=77) and multiparous (n=84) Nelore cows with BCS of 2.89±0.02 received 2mg of EB and a P4 device on day 0 (D0). Eight days later (D8), 500 µg of sodium cloprostenol, 1 mg of estradiol cypionate and 300 IU of eCG were administered and the P4 device was removed. TAI was performed 48 hours after P4 removal (D10). At the same time, blood samples were collected from a subgroup of animals (n=100) for metabolic and hormonal analysis. Pregnancy diagnosis was evaluated 30 days after TAI (D40). Statistical analysis was performed by SAS. In experiment 1, there was no difference between categories in the number of oocytes grade 1, 2, 3 (P=0.83, P=0.23 and P=0.51, respectively), total oocytes recovered (P= 0.14), oocyte quality index (P=0.93) and total viable oocytes (P=0.38). However, the number of follicles at the time of follicular aspiration (P=0.05) and the diameter of the largest follicle on D12 (P=0.04) were higher in multiparous cows and the number of degenerate oocytes was higher in primiparous cows. (P=0.05). On D5, there was no difference between categories in serum concentrations of IGF-1 (P=0.47), insulin (P=0.08), total cholesterol (P=0.47) and NEFA (P=0.77), plasma concentrations of glucose (P=0.55) and IGF-1 (P=0.97) and insulin (P=0.11) concentrations in the follicular fluid. On D12 there was no difference between categories in IGF-1 (P=0.26), total cholesterol (P=0.32), NEFA (P=0.31) and glucose (P=0.93) circulating concentrations. However, serum insulin concentration (P=0.04) was higher in primiparous cows. There was no correlation between serum and follicular fluid insulin concentrations (r=0.17; P=0.31). However, there was a low correlation between serum and follicular fluid for IGF-1 concentrations (r=0.47; P=0.002). No differences in the quantification of transcripts were observed between categories. In Experiment 2, there was no difference in serum concentrations of NEFA (P=0.12) and insulin (P=0.16) and pregnancy rate between categories [60% (30/50) primiparous; 60% (30/50) multiparous]. However, IGF1 (P=0.0001), total cholesterol (P=0.005) and glucose (P=0.01) concentrations were greater in primiparous cows. It was concluded that lower fertility of *Bos indicus* primiparous cows in recent postpartum is not related to follicular cells, oocyte quality and changes in the metabolic profile evaluated.

Animal Reproduction

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

TAI/AI

Effect of pre-synchronization with injectable progesterone (P4i) and breeding method (natural mating *vs.* FTAI) on the pregnancy rate of Brangus cows

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This study evaluated the effect of pre-synchronization with injectable progesterone (P4i), as well as the breeding method [Natural Mating (NM) vs. Fixed-Timed Artificial Insemination (FTAI)] on the pregnancy rate of Brangus cows. Brangus cows (n=969), secundiparous (SEC; n=307) and multiparous (MUL; n=662) were used. At D-10, cows were randomized according to body condition score (BCS=2.72±0.01) and allocated into four treatments using 2x2 factorial design: 1) NoP4i_NM (n=226): cows without P4i and bred by NM; 2) P4i_NM (n=245): cows with P4i (150 mg) and bred by NM; 3) NoP4i_FTAI (n=239): cows without P4i and synchronized for FTAI; and 4) P4i_FTAI (n=259): cows with P4i (150 mg) and synchronized for FTAI. At D0, cows of the FTAI groups received an intravaginal P4 device and 2 mg of EB. At the same time, clean-up bulls enter the herd on a proportion of 4% (1:25). Nine days later (D9), concomitant with P4 device removal, animals of the FTAI groups received 1 mg of EC, 0.150 mg of PGF2α, and 400 IU of eCG. All cows were painted with chalk on their tailheads, and the removal of chalk on D11 was used as an indicator of estrus. Also, bulls were removed from the herd. FTAI was performed 48 hours after removing the P4 device. Three days post-FTAI, bulls were reintroduced to the herd. Cows were evaluated by ultrasound 30 days after the FTAI to confirm the pregnancy of all the groups. Statistical analyses were performed using GLIMMIX in SAS 9.4. The variables chosen for this study were: Pre-synchronization, breeding method, parity, estrus expression rate at D11, and pregnancy rate at D30. No interaction P4i*breeding method was observed for any of the variables analyzed (P›0.05). The breeding method significantly influenced the estrus expression rate. Cows bred with FTAI had a greater estrus expression rate [FTAI=50.2% (250/498) vs. NM=5.1% (24/471); P‹0.001]. The treatment with P4i had no effect on estrus detection [P4i=27.6% (139/504) vs. NoP4i=29.0% (135/465); P=0.41]. Parity also had a significant effect on estrus expression rate; multiparous cows had a greater estrus expression rate [MUL=31.6% (209/662) vs. SEC=21.2% (65/307); P=0.02]. Regarding the pregnancy rate at D30, cows bred with FTAI had a greater pregnancy rate [FTAI=33.5% (167/498) vs. NM=7.9% (37/471); P‹0.001]. Multiparous cows tend to have a greater pregnancy rate [MUL=22.2% (147/662) vs. SEC=18.6% (57/307); P=0.07]. There was also an interaction Pre-synchronization*Parity for pregnancy rate. The treatment with P4i was effective mainly in secundiparous cows [MUL_P4i=21.1% (74/350)^{A}; MUL_NoP4i=23.4% (73/312)^{A}; SEC_P4i=23.4% (36/154)^{A}; SEC_NoP4i=13.7% (21/153)^{B}; P=0.03)]. In conclusion, cows that received FTAI had a higher pregnancy rate at 30 days of the breeding season when compared to cows submitted to NM. The pre-synchronization with P4i presented a positive effect on pregnancy rate in secundiparours cows; however, no effect of the treatment was observed in multiparous cows.

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TAI/AI

Use of GnRH associated with estradiol cypionate in crossbred dairy cows subjected to TAI protocols

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This study aimed to evaluate the effect of GnRH injection associated with estradiol cypionate (EC) on ovulation and pregnancy per AI (P/AI) in crossbred dairy cows subjected to estradiol-progesterone (E2-P4) based protocols. In Experiment 1, 30 Girolando (*B. indicus* × *B. taurus*) cows were treated as follows: D0, 2 mg EB + intravaginal P4 device - IVPD (1.0 g); D7, 500 µg PGF (Cloprostenol Sodic); D8, IVPD removal + 1 mg EC + 500 µg PGF. On Day 9, 34 h after IVPD removal, ultrasound (US, Mindray®, M5 VET® with a linear probe of 5 MHZ) evaluations of the ovaries were performed in all cows to measure the diameter of largest follicle (DLF). Therefore, cows were homogeneously distributed into two groups, according to the DLF, and were treated with: 1) 2.5 mL of NaCl 0.9% (CTL, n=15) i.m.; and 2) 10.5 mcg of GnRH analog (bureselin acetate - Gonaxal®, Biogenesis Bagó, Curitiba, Brazil; GnRH34, n=15) i.m. Further US evaluations were performed at 12 h intervals from IVPD removal to ovulation. Ovulation was defined as the disappearance (from one US scanning session to the next) of a previously identified follicle ≥ 8 mm in diameter. In Experiment 2, Girolando dairy cows (n=137) were treated as in Experiment 1, and were randomly distributed into two groups: CTL group (n = 66), and GNRH34 group (n = 71). All cows were inseminated with semen from two sires 48 h after IVPD removal. Body condition score (BCS) was evaluated, and cows were classified as Low BCS (BCS ≤ 2.5), Adequate (2,5 < BCS ≥ 3,5), and High (BCS > 3,5). Continuous variables were analyzed using the analysis of variance (PROC GLIMMIX; SAS Institute. Inc., Cary, NC). Binomial variables (ovulation rate and P/AI) were analyzed using the chi-square test. In experiment 1, cows treated with GNRH34 ovulated earlier (60.4 ± 2.44 h; P = 0.03) in comparison with the CTL group (72.85 ± 4.42 h) cows. Cows from the GnRH34 group ovulated more synchronously (P = 0.006; from 54 to 66 h after IVPD removal) than cows from the CTL group (100% x 60%). No differences were observed between groups for the DLF (CTL, 14.95 ± 1.60 × GNRH34, 13.81 ± 1.26; P = 0.1), and for ovulation rate (CTL, 93.3% x GnRH34, 100%; P = 0.29). In Experiment 2, P/AI did not differ (P = 0.63) between the CTL (26/66, 39%) and GNRH34 (31/71, 43%) groups. No effect of BCS (P = 0.6) or group × BCS interaction (P = 0.34) on P/AI was observed. Similarly, no effect of sire (P = 0.46) or sire × group (P = 0.2) on P/AI was observed. The preliminary results of this study indicate that although GnRH34 induced more synchronous ovulation, no effect on the P/AI in crossbred dairy cows was observed. Further studies, including a larger number of animals, must be performed to properly evaluate the effect of GnRH34 on fertility in dairy cows.

Animal Reproduction

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

TAI/AI

Impact of fetal programming by two weaning strategies on the development and reproductive performance of Nelore heifers

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We aimed to compare the fetal programming effects of early (EW; 150 days) or conventional (CW; 240 days) weaning of the previous offspring in primiparous (Pri) and pluriparous (Plu) Nelore cows on the development and reproductive performance of the female calves. For this experiment, 55 Nelore calves were used: 30 from CW (15 from Pri and 15 from Plu) and 25 from EW (10 from Pri and 15 from Plu). Calves had access to creep-feeding (20.5% of CP and 70.8% of TDN) provided 0.5% of body weight (BW) from 90 days of age until weaning (150 days). After weaning, calves were maintained on *Brachiaria brizantha* cv. Marandu pasture and received a supplement (26.3% of CP and 70.4% of TDN) provided at 1% of BW until 16 months (mo). From 12 to 16 mo, all heifers were evaluated every 28±3 days for BW, body condition score (BC scale 1 to 9), average daily gain (ADG), carcass characteristics (longissimus muscle area [LMA], backfat thickness [BF], and rump fat thickness [RFT]), reproductive tract score (RT scale 1 to 5), endometrium thickness and pregnancy rate. At 5 and 14 mo, a subgroup of 22 heifers (n= 10 for EW and 12 for CW) was selected for liver biopsy to evaluate the gene expression of IGF-I, IGFBP and GHR. At 14 mo, heifers were submitted to a P4/E2-based protocol for three timed-artificial inseminations (TAI) within 44 days (Days 0, 22, and 44, respectively). Data were analyzed by ANOVA or GLIMMIX procedures of SAS. Any interaction between dam parity, weaning strategy, or time was observed for BW, BCS, ADG, LMA, RTS, endometrium thickness, or pregnancy rate (P>0.1). As expected, a significant effect (P<0.05) of time indicated an increase in BW, BCS, LMA, BF, and RTF over time. Despite not observing a dam parity by time interaction, heifers from Plu cows were heavier from 13 to 15 mo than heifers from Pri cows (P=0.04). BCS was also affected by the dam's parity (P=0.01); heifers from Plu cows presented a higher BCS than from Pri cows over time. For carcass characteristics, a dam parity by weaning interaction (P=0.04) in RTF indicated that EW-Pri heifers presented a lower fat deposition than EW-Plu and CW heifers. For IGF-I, only a 1.4-fold greater (P=0.01) expression at 3 mo than at 14 mo was observed, regardless of the weaning strategy or parity. For IGFBP a dam parity x time interaction was observed (P=0.05), heifers from Plu cows presented a 1.8-fold greater expression at 14 mo than at 3 mo. Expression of GHR was not affected by the factors evaluated (P>0.1). The RTS (overall mean: 2.9±0.1), endometrium thickness (overall mean: 10.2±0.2 mm), and pregnancy rates (overall for three TAIs: 76%) were not affected by the weaning strategy or the dam parity order (P>0.1). In conclusion, the body growth, hepatic gene expression related to IGF-I system, and reproductive performance of Nelore heifers are not affected by the anticipation of weaning time to 150 days during fetal programming.

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Animal Reproduction

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

TAI/AI

The treatment with injectable mineral supplementation improves the estrus and pregnancy rates at FTAI in dairy heifers

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The present study evaluated the effect of intramuscular treatment with injectable mineral (inorganic phosphorus, organic phosphorus, selenium, magnesium, copper and potassium) on the cyclicity rate [presence of a corpus luteum (CL) at the beginning of the synchronization protocol), estrus detection (presence of estrus between P4 device removal and artificial insemination) and conception rate (P/AI) of Holstein and Girolando heifers submitted to the fixed-time artificial insemination (FTAI). A total of 1,146 heifers were used, 571 in the Control Group and 575 in the Treatment Group. Heifers were treated with 10 mL i.m. of Fosfosal® (Virbac Brasil, São Paulo, SP, Brasil) 30 days before (D-30) and at the beginning (D0) of the synchronization protocol for FTAI. Control group received no treatment. Ultrasound examinations were performed on D-30 and D0 to assess the presence of CL. Synchronization protocol based on progesterone plus estrogen (P4+E2) was used. Heifers received an intravaginal P4 device (PRIMER®; Agener União), together with the administration of 2 mg i.m. of estradiol benzoate (RIC BE®; Agener União) and 0.530 mg i.m. of sodium cloprostenol (Estron®; Agener União) on D0. The device was removed seven days later (D7) and the FTAI was performed on D9 (48h after P4 device removal). At the time of device removal, heifers were painted with chalk on tailheads, and removal of chalk on the day of FTAI was used as an indication of estrus. The P/AI was evaluated by ultrasonography 30 days after insemination. There was no effect of the mineral supplement on the cyclicity rate [Control = 23.7% (91/384) vs Treated = 21.7% (83/382); P = 0.38]. However, the mineral supplementation increased the estrus rate [Control = 48.1% (205/427) vs Treated = 90.9% (400/440); P = 0.0001] and the pregnancy rate at FTAI [Control = 38.5% (220/571) vs Treated = 45.4% (261/575); P = 0.01]. There was interaction Treatment*Body condition score (BC scale from 1 to 5) for P/IA (P = 0.01). The treatment with intramuscular minerals increased P/AI only in heifers that presented high BCS (≥3.50). Furthermore, there was an interaction Treatment*Estrus (P = 0.07). The treatment with intramuscular minerals increased the P/AI only in heifers that did not show estrus. These results suggest that intramuscular mineral supplementation with Fosfosal increases the estrus detection rate and the pregnancy rate at FTAI, contributing to increase the fertility of dairy heifers. The positive effects of the treatment were more evident in heifers that did not show estrus and in heifers with higher BCS.

Animal Reproduction

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

TAI/AI

Exposure to injectable progesterone previous to ovulation synchronization protocol increases the conception rate in high production Bos taurus dairy cows

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The objective was to evaluate the effect of exposure to injectable progesterone previous to timed artificial insemination (TAI) protocol on conception rate of high production Bos taurus dairy cows. In the study, 490 Holstein and crossbred (Holstein X Jersey) dairy cows at 58.2±0.3 days in milk, body condition score of 2.78±0.01 (scale of 1 - 5) and milk production of 33.1±0.4 L/d were used. Seven days before TAI protocol (D-7), cows were divided into two experimental groups (Control and P4i groups). In this moment, cows of P4i group received 300mg of injectable progesterone (Sincrogest Injetável®, Ouro Fino, Brazil). On day 0 (D0), all cows (Control and P4i groups) received 2mg of estradiol benzoate, 25µg de lecirelina and an intravaginal device containing 1g of progesterone. After seven days (D7), cows received 500µg of Cloprostenol. On day 8 (D8), the progesterone device was removed and cows received 500µg of Cloprostenol and 1mg of estradiol cypionate. All cows were inseminated 48 hours after the removal of progesterone device. Ultrasound exams were performed on D-7 to evaluate the presence of CL, D40 and D70 to evaluate the conception rate at 30 and 60 days and pregnancy losses from 30 to 60 days after TAI. Statistical analyses were performed by GLIMMIX procedure of SAS. There was tendency of interaction effects between treatment and presence of CL on D-7 in the conception rate at 30 days after TAI (P=0.10) and in the P/AI at 60 days after TAI (P=0.07). The P/AI at 30 and 60 days after TAI was greater (P=0.05) in the cows of P4i group [30 days: Control - 52.1% (122/234)b and P4i - 56,2% (144/256)a; and 60 days: Control - 49.6% (115/232)b and P4i - 53.5% (136/254)a]. In addition, in the subgroup of cows without CL on D-7 the P/AI at 30 and 60 days after TAI was greater in cows of the P4i group [30 days: Control-NoCL - 32.7% (17/52)b and P4i-NoCL - 50.7% (35/69)a; P=0.04; and 60 days: Control.-NoCL 31.2% (15/48)b and P4i-NoCL 50.0% (33/66)a; P=0.05]. However, the pregnancy losses were similar between groups [Control - 4.2% (5/120) and P4i 4.2% (6/142); P= 0.99]. In conclusion, exposure to injectable progesterone previous to TAI protocol increases the conception rate at 30 and 60 days after TAI in high production *Bos taurus* dairy cows, especially in anestrous cows. Support: Ouro Fino Saúde Animal, FAPEMIG, Santo Antonio da Estiva Farm, Cachoeira Farm, Terra Garcia Group and Prenhez Consultoria.

Animal Reproduction

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

TAI/AI

Factors that affect pregnancy rate and pregnancy loss in two-year-old primiparous Nelore cows synchronized for TAI

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The objective of the present study was to evaluate factors that affect the pregnancy rate (P/AI) and pregnancy loss (PL) in 2 years Nelore primiparous cows submitted to TAI. A total of 3,250 Nelore (Bos indicus) primiparous heifers from a commercial farm (Agropecuária Jacarezinho, Brazil) were used. On day 0 (D0), intravaginal P4 device (CIDR®; Zoetis, Guarulhos, SP-Brazil) and 2mg of EB (Gonadiol®, Zoetis), i.m were administrated. On D9, cows received 16.8 mg (2.5mL) of dinoprost tromethamine (Lutalyse®; Zoetis), 300 IU of eCG (Novormon®; Zoetis) and 0.5 mg of EC (ECP®; Zoetis) i.m. The primiparous had the P4 device removed on D9, and TAI was performed 48h later (D11). Cows were painted with chalk on tailheads, and removal of chalk at TAI was used as an indication of estrus. Pregnancy diagnoses were evaluated by ultrasound (Mindray® DP-2200Vet) after 30 days (PD) and at the end of the breeding season (120 days after AI). Statistical analyses were performed using GLIMMIX of SAS 9.4. Receiver operating Characteristic (ROC) curve analysis (MedCalc®) was used to select optimal cut-off points for the variables analyzed. The body weight (BW) on D0 influenced the P/AI (Quadratic effect; P=0.04). The BW cut-off points calculated by ROC curve was 339kg [≤339kg = 37.6% (n=1,357) vs. >339kg = 44.9% (n=1,699); P<0.001]. The primiparous age on D0 impacted the P/AI (Linear effect; P=0.06). The age cut-off points calculated by ROC curve was 25.7 months [≤25.7m = 39.8% (n=1.933) vs. >25.7m = 43.2% (n=1,307); P=0.008]. The P/AI was influenced by BCS on D0 (Linear; P<0.001). The BCS cut-off points calculated by ROC curve was 2.25 months [≤2.25 =33.0% (n=695) vs. >2.25 = 43.8% (n=2,446); P<0.0001]. Primiparous that presented estrus had greater P/IA [Estrus= 43.5% (978/2249) vs. noEstrus= 34.5% (484/1402); P=0.001]. The pregnancy loss (PL) was not influenced by the BW and BCS. The PL was influenced by age (Quadratic effect; P=0.04). The age cut-off points calculated by ROC curve was 26.2 months [≤26.2m = 11.7% (n=1,322) vs. >26.2m = 6.8% (n=1,147); P<0.001]. The presence of estrus presents a tendency to decrease PL [Estrus=9.8% (59/484) vs. noEstrus=12.2% (96/978); P=0.08]. In conclusion, BW, age, and presence of estrus impact the efficiency of P/AI. Furthermore, the PL was influenced by age in 2 years old Nelore primiparous.

Animal Reproduction

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

TAI/AI

Injectable progesterone and prostaglandin F2α treatments on the fertility of postpartum beef cows

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This study aimed to evaluate the effect of injectable progesterone (iP4) and prostaglandin F2α (PGF2α) previously to the timed AI (TAI) protocol on the pregnancy per AI (P/AI) in postpartum *Bos indicus* beef cows. Nelore postpartum cows: 455 primiparous precocious (24 months), 775 primiparous conventional (36 months), and 830 multiparous, from a single farm were enrolled in the study. On day 22 ± 2 (M ± SD) postpartum cows were equally distributed to receive the following treatments: 1) Control group (CG, n = 520); 2) 150 mg of iP4 i.m. (Sincrogest®, Ourofino, Brazil; iP4, n = 521); 3) 0.526 mg of cloprostenol i.m. (Sincrocio®, Ourofino, Brazil; PGF, n = 513); and 4) 150 mg of iP4 plus 0.526 mg of PGF i.m. (iP4PG, n = 506). A FTAI protocol started after 10 days from the distribution of groups and application of the respective hormones. Cows were subjected to an estradiol-progesterone based TAI protocol, with intravaginal device containing 1 g progesterone, and 2 mg estradiol benzoate im on Day 0; 8 days later, the intravaginal device was removed, and they were given 0.25 mg of cloprostenol sodium, and 300 IU of eCG, and 48 hours later, on day 30.4 ± 3.2 postpartum and were inseminated by two technicians. Semen from six bulls was used for TAI and equally distributed among the treatments. Treatment, category, bull, and inseminator were considered as fixed effects on the model. Logistic regression was used to analyze the effects of these factors on P/AI. All analyses were performed using Minitab software. No effects of the inseminator (P = 0.15) or category (P = 0.47) on P/AI were observed. However, treatment (P = 0.05) and bull (P = 0.05) were effects on P/AI. No interaction between treatment and any other fixed effects was observed (P > 0.05). The iP4PGF group had a greater (P = 0.05) conception/artificial insemination rate (70%) than the CG (62.5%), iP4 (63.5%), and PGF (64.9%) groups. In conclusion, although more studies are needed to understand how iP4 and PGF affect the re-establishment of postpartum uterine function, the association between iP4 and PGF increases pregnancy at first TAI in postpartum beef cows.

Animal Reproduction

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

TAI/AI

Uncovering metabolic markers for early sexual precocity and feed efficiency in young Nelore heifers and bulls

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The aim of this study was to identify metabolites associated with early sexual precocity and feed efficiency in Nelore heifers and bulls. The experiment involved 66 heifers (Age= 11.1±0.07-month BW=260.9±1.33 kg; BCS=2.84±0.02) and 128 bulls (Age=11.1±0.14-month BW=340.9±3.94 kg, BCS=3.09±0.02) from two commercial farms. At the beginning of the experimental period (Day -70), heifers and bulls were randomly assigned to pens equipped with automated feeding systems (Intergado, MG, Brazil) that recorded individual feed intake and BW to calculate residual feed intake (RFI). Following a 14-day adaptation period, the feed efficiency test lasted 52 days. On day 0, the heifers were synchronized with a P4/E2-based FTAI protocol, and pregnancy was determined on day 40 via US. Heifers that were pregnant were classified as precocious (n= 35) and non-pregnant as non-precocious (n= 31). Also, heifers were classified as low RFI (good feed efficiency; -1.56±0.08 kg DM/d, n=33) or high RFI (1.41±0.04 kg DM/d, n= 33). On day 0, semen was collected from bulls through electroejaculation and were classified as non-precocious (n= 97) if they did not produce sperms cells or produced less than $50x10^6$ cells, and precocious (n= 31) if they produced more than 50×10^6 sperm cells with >10% motility. The bulls were also classified as low RFI (-0.62±0.03 kg DM/d, n=64) or high RFI (0.56±0.02 kg DM/d, n= 64). In both experiments, blood samples were collected on day -70 for targeted metabolomics LC-MS/MS analysis. Biomarker analyses based on receiver operating characteristic (ROC) curves and PLS-DA were conducted using MetaboAnalyst 5.0. The results showed significant differences in metabolite profiles between sexually precocious and non-precocious heifers (ROC-AUC=0.87; P 0.05). Pathway analysis revealed significant effects on methionine metabolism (MM), glutathione metabolism (GM), carnitine synthesis, glycine and serine metabolism (GSM), glutamate metabolism (GTM), alanine metabolism, and arginine and proline metabolism. Moreover, significant differences in metabolite profiles between low and high feed efficiency in heifers were observed (ROC-AUC=0.81; P 0.05), with pathway analysis indicating an effect on GSM MM, GM, betaine metabolism, urea cycle, and GTM. Metabolite profiling of bulls revealed significant differences in sexual precocity (ROC- AUC=0.96; P 0.05), with pathway analyses indicating an effect on the oxidation of branched-chain fatty acids, β-oxidation of very long chain fatty acids, purine metabolism (PM), ammonia recycling, methyl histidine metabolism and beta-alanine metabolism. Additionally, significant differences in metabolite profiles between low and high feed efficiency in bulls were observed (ROC-AUC=0.80; P 0.05), with pathway analysis indicating an effect on GSM, PM and MM. In conclusion, metabolomics analyses accurately identified differences in sexual precocity and feed efficiency in both Nelore heifers and bulls.

Animal Reproduction

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

TAI/AI

Progesterone dose on intravaginal device affects pregnancy rate after TAI in high BCS *Bos taurus* suckled beef cows

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Intravaginal progesterone devices (IVD) for single (1g P4) or multiple uses (≥1g P4) are widely used in hormonal protocols for TAI in cattle. This study aimed to test the hypothesis that Bos taurus suckled beef cows with high body condition score (BCS) need greater progesterone dose on IVD to obtain higher pregnancy rate. In experiment 1, 35 suckled beef cows with BCS ≥3.5 (1-5 scale; 1= very thin and 5= severely over-conditioned), maintained exclusively on grazing conditions were submitted to the estrus synchronization protocol. On D0, cows received 2 mg of estradiol benzoate (E Agener União, São Paulo, Brazil) and 0.52 mg of cloprostenol (PGF; Estron, Agener União, São Paulo, Brazil) i.m., and an IVD containing 0.5g (n=20; IVD0.5g; Agener União, São Paulo, Brazil) or 1g P4 (n=15; IVD1g; Agener União, São Paulo, Brazil). On D8, the IVD was removed and PGF and 1 mg of estradiol cypionate (EC; Agener União, São Paulo, Brazil) were i.m. administered. On the same day, the animals were marked with ink in the sacral-caudal region, follicles were measured by transrectal ultrasonography, and blood samples were collected from the coccygeal vein for progesterone analyses. In experiment 2, pregnancy rate was evaluated in IVD0.5 (n=152) and IVD1g (n=156) groups, using the same hormonal protocols for both groups, except for the IVD P4 dose. The hormonal protocol was the same from experiment 1, except that PGF was not administered on D0 (only on D8) and 300IU eCG was administered on D8. The study was conducted in four different farms (four replicates) with suckled Bos taurus beef cows (n=308) with BCS ≥3.5. Semen from a single bull was used for all the cows and TAI was performed by the same technician, in each farm. Pregnancy diagnosis was performed 30 days after TAI by transrectal ultrasonography. Shapiro-Wilk, ANOVA and logistic regression tests were used (p 0.05 considered as significant) to analyze data and the results for follicular diameter and P4 concentration are presented as mean ± SEM. In experiment 1, average follicular diameter on D8 was greater for IVD0.5g compared to IVD1g (p=0.03), being observed 9.54±0.49 and 7.94±0.51mm, respectively. Progesterone concentration on D8 did not differ (p=0.36) between IVD0.5 (4.25±0.84 ng/mL) and IVD1g (5.37±1.15 ng/mL). Pregnancy rate 30 days after TAI was lower for IVD0.5g compared to IVD1.0g, being observed 46.7% (71/152) and 58.9% (92/156), respectively. No significant effect of replicate was observed (p=0.12) nor treatment*replicate interaction (p=0.74). Overall, regardless of treatment, the pregnancy rate was 52.9% (163/308). The fact that IVD0.5 group had greater follicular diameter on D8 suggests that *Bos taurus* cows with high BCS require higher progesterone concentration in IVD to promote an adequate dominant follicle turnover. It is concluded that *Bos taurus* suckled beef cows with high BCS need greater progesterone dose on IVD to obtain higher pregnancy rates.

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Animal Reproduction

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

TAI/AI

Lecirelin as an ovulation inducer in *Bos taurus* beef heifers timed artificial insemination protocol

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These experiments were designed to evaluate the effect of GnRH as an ovulation inducer, 34 h after progesterone (P4) intravaginal device (IVD) removal, in a timed artificial insemination (TAI) protocol in Bos taurus beef heifers. In Experiment 1, Aberdeen Angus, Hereford and crossbreed cycling, 24 months-old females (n=17) under grazing conditions on native pasture were submitted to a TAI hormonal protocol. On D0 the animals received 2 mg of estradiol benzoate (E Bioestrogen®, Biogénesis Bagó, Curitiba, Brazil) IM and an IVD containing 1g P4 (Repro Neo®, GlobalGen, Jaboticabal, Brazil). On D8, on IVD removal, animals received 0.15 mg of d-cloprostenol (Croniben®, Biogénesis Bagó, Curitiba, Brazil) IM and 1 mg of estradiol cypionate (Croni-CIP®, Biogénesis Bagó, Curitiba, Brazil) IM and were paint-marked on the sacro-caudal region. Thirty-four hours after IVD removal, the animals were allocated into two groups: GnRH (n=10) which received 0.05 mg of lecirelin (TEC-Relin®, Agener União, São Paulo, Brazil) IM; and Control (n=7) with no additional treatment. All animals were artificially inseminated on D10, 48 h after IVD removal. Follicular dynamics were evaluated on D8 (IVD removal), D9 (34h after IVD removal) and D10 (48 h after IVD removal) by transrectal ultrasonography. Progesterone serum concentration was measured 7 and 12 days after TAI. In Experiment 2, 390 Bos taurus beef heifers, under the same features and conditions from Experiment 1, from four different farms, received the previously described hormonal protocol to evaluate the pregnancy rate. Pregnancy diagnosis was performed 30 days after TAI, by transrectal ultrasonography. Shapiro-Wilk, ANOVA, Tukey and logistic regression tests were used to evaluate data with results presented as mean±SEM. The preovulatory follicle diameter (Control=13.11±0.41mm; GnRH=12.25±0.47mm; p>0.05), follicular growth rate (Treatment, p=0.63; Moment, p=0.88; Treatment*Moment, p=0.91), ovulation rate (100% for both groups), estrus manifestation (Control=100%; GnRH=90%; p>0.05) and P4 concentration were similar between groups. Pregnancy rate (Control=50.26%; GnRH=50.24%), as well as pregnancy rate according to estrus manifestation (treatment, p=0.96; estrus, p=0.9; treatment*estrus, p=0.96) were also similar between groups. Thus, the use of GnRH as an ovulation inducer, administered 34h after the IVD removal, did not affect follicular growth, luteal function and pregnancy rate in Bos taurus beef heifers.

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Animal Reproduction

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

TAI/AI

Estrous expression and conception rate of *Bos indicus* heifers submitted to E2/P4-based TAI protocol using intravaginal progesterone-releasing device for 7 or 8 days

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The objective was to evaluate the fertility of Bos indicus heifers submitted to ovulation synchronization protocol using 7 or 8 days of intravaginal progesterone-releasing (P4). Previously to the study, the heifers were submitted to the puberty induction protocol with progesterone. Nelore heifers (n=457) at BCS of 2.97±0.01 (2.50 to 3.50 and Median=3.00) were randomly distributed in two experimental groups (7P4 and 8P4 groups). In TAI protocol using P4 for 8 days, the beginning of the treatment (D0) was carried one days before of TAI protocol (D-1). At beginning of the TAI protocol, the animals received 2 mg estradiol benzoate IM (Fertilcare sincronização® - MSD, São Paulo, Brazil), 0,265mg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil) and insertion of an intravaginal progesterone-releasing device (Crestar IVG 0.588® - Monodose - MSD, São Paulo, Brazil). Seven (7P4 group) or eight (8P4 group) days later, the P4 device was removed, and cows received 0.5mg of estradiol cypionate (Fertilcare ovulação® - MSD, São Paulo, Brazil), 0.265mg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil) and 300 IU of eCG (Folligon®, MSD, São Paulo, Brazil). In addition, the tail-head of was marked with chalk for evaluate estrus expression between removal of the progesterone device and insemination. All heifers were inseminated 48 hours after the progesterone device withdrawal. Ultrasound exams were performed 30 days after TAI to evaluate the cyclicity and conception rates. Statistical analyses were performed by GLIMMIX procedure of SAS. The estrous expression rate [7P4=70.2% (158/225) and 8P4=77.2% (179/232); P=0.10], pregnancy rate [7P4=45.8% (103/225) and 8P4=43.5% (101/232); P=0.88] and cyclicity rate at the time pregnancy diagnosis [7P4=33.6% (41/122) and 8P4= 43.5% (57/131); P=0.10] were similar between experimental groups. In conclusion, the period of permanence of intravaginal progesterone-releasing device for 7 or 8 days showed similar estrous rate, cyclicity at the time pregnancy diagnosis and pregnancy rates in *Bos indicus* heifers submitted to E2/P4- based TAI protocol.

Animal Reproduction

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

TAI/AI

Recent experimental and field results indicate key factors in improving fertility to timed-artificial insemination programs in lactating dairy cows

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Experimental and field results available to us during the last decade provided 8,602 timed- artificial inseminations (TAI) in lactating Holstein cows in commercial dairy herds in Brazil. The aim was to identify factors impacting pregnancy per AI (P/AI) and pregnancy loss (PL). All protocols had a P4 implant and two PGF injections (24 h apart). Experiments compared strategies to initiate the protocol (estradiol benzoate [EB], GnRH or both), and 2 experiments compared different fertility programs (Presynch + TAI protocol). The GLIMMIX procedure of SAS 9.4 was used for analyses (P≤0.05). All results are from experimental data, otherwise indicated. Results from all experiments demonstrated that: **1)** Primiparous had greater P/AI than multiparous cows at both first (49.8 vs 38.2%) and later services (43.4 vs 35.2%); **2)** Healthy cows during the pospartum period had greater P/AI than cows with 1 or ≥2 diseases (52.4a, 43.7b, 27.9%c; respectively; n=798); **3)** Cows with CL on d0 and at the time of PGF treatment had greater P/AI (44.0 vs 38.8%, and 44.9 vs 33.2%, respectively; n=3,482); **4)** Ovulation after d0 improved overall P/AI (46.1 vs 39.3%), increasing fertility of cows with CL (46.5 vs 41.6%), and especially those without CL on d0 (45.1 vs 25.5%; n=3,482); **5)** Expression of estrus near TAI was associated to greater P/AI (47.7 vs 29.7%) and reduced PL (12.4 vs 19.3%; n=3,263). The comparison from 5 randomized studies demonstrated that protocols initiated with GnRH vs EB increased percentage of cows with CL at PGF (85.6 vs 55.6%; n=3,323) and increased P/AI by 8.5 percentage points (39.8 vs 31.3; range: 4.5 to 10.2; relative increase of 27.2%; n=4,923). A study showed that a fertility program developed by our team, called Double E-Synch (final ovulation of Presynch and TAI protocol induced by E2 cypionate), had similar P/AI as the Double-Ovsynch (52.5 vs 48.4%; n=800). Thus, high fertility was achieved in high-producing dairy cows in commercial Brazilian dairies using optimized protocols. To further demonstrate this idea, 4 herds were switched from conventional TAI protocols to Double E-Synch and all had increased P/AI (57.9 vs 43.9%, 46.4 vs 30.1%, 40.0 vs 33.6%, and 57.1 vs 40.9%; n=2,301). Interestingly, when milk production was divided into quartiles, there was no effect of milk yield on P/AI (40.6, 44.4, 42.8, and 40.9%; n=3,251). The results established important factors associated with fertility in Brazilian dairy herds (e.g. parity, BCS, health, ovarian dynamics and expression of estrus during TAI protocols, and protocol optimizations). It was shown that fertility programs such as Double-Ovsynch or Double E-Synch substantially improve fertility and promote outstanding P/AI in well-managed herds. We expect the results will help dairy operations to understand and control factors associated with fertility and to implement optimized reproductive managements and TAI programs, achieving high reproductive performance with high milk production.

Animal Reproduction

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

TAI/AI

Variation of body condition score and reproductive performance in beef cows in natural mating or submitted to fixed-time artificial insemination (FTAI)

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The objective of this work was to evaluate the effects of body condition score (BCS) variation in postpartum moments on the reproductive performance of beef cows submitted to FTAI and passed with bulls or only with bulls. Station data between 2018/2019 from Vitória farm, Fama/MG were analyzed. Cows were divided into two groups: TAI + Bull (n = 526) and Bull (n = 255). Cows 30 days postpartum were considered suitable for reproduction. The females underwent rectal palpation and ultrasonography with an M5 device (Mindray, Brazil), a linear transducer at a frequency of 7.5 MHz. In the TAI + Bull group, the protocol used was three handlings, lasting ten days, and products from MSD Animal Health, Brazil. Only one TAI was performed and 15 days later all cows were placed with Nellore bulls, at the ratio of one bull to 52 cows, remaining until the end of the season. Cows in the Bull group were placed with bulls on the same FTAI day, at the ratio of one bull to 25 cows. BCS measurements (1 to 5) were performed at the stages: at calving, beginning of the breeding season and pregnancy diagnosis 30 days after TAI and/or mating. Pregnancy diagnosis by transrectal ultrasonography was performed 30 days after insemination or possible mating and confirmed by the same method between 45 and 60 days after the end of the season. Data were analyzed using the Statistical Analysis System 9.4 program (SAS Institute Inc., 2004), considering the comparisons: BCS at delivery; difference between BCS calving-early season; and difference between BCS early season-30 days post-insemination. Pregnancy and cyclicity binary data were analyzed using the Chi-square test within the GLIMMIX procedure (PROC GLIMMIX of SAS). In all statistical analyses, the significance level was $P \leq 0.05$. The results indicate an influence of nutrition on the reproductive performance of beef cows, which can be evidenced by the variation in the BCS. Animals with better BCS at parturition (>4.0) had a higher cyclicity rate and fewer days to become pregnant. When evaluating the difference in BCS from calving to the beginning of the reproductive season, a significant effect was obtained on the pregnancy rate, where animals restricted to the loss of up to 0.5 units of BCS had better pregnancy rates, the same happened with animals who gained 0.5 BCS units during the season. When analyzing the difference between the BCS at calving and the beginning of the reproductive season, it was noticed that the use of TAI was efficient in reducing the time until the establishment of pregnancy, compared to natural mating. When evaluating the time until pregnancy establishment according to the BCS at the beginning of the season, there was no significant difference. It is concluded that the variations in BCS interfere in the reproductive performance of beef cows, as well as the use of TAI can, in some cases, attenuate these effects.

Animal Reproduction

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

TAI/AI

The influence of genetic evaluation for daughter pregnancy rate on fertility in dairy cattle submitted to artificial insemination

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The aim was to evaluate the influence of the estimated genetic value for daughter pregnancy rate (DPR) on fertility of Holsteins heifers and lactating cows. Retrospective data from 1,284 heifers and 2,819 lactating cows were collected from the same dairy herd between 2018 and 2023. The DPR value for each dam was based on the genetic evaluation of their fathers, and the range of DPR values was from −3.8 to 5.7. Then, dams were categorized in 3 DPR classes: High (H:≥1; n=1,542), Intermediary (I:>-0.5 1; n=1,567), or Low (L:≤-0.5; n=1,415). The association between DPR classes and reproductive performance, such as overall pregnancy per artificial insemination (P/AI), as well as in 1st service and resynchronizations, and pregnancy loss (PL) were assessed for heifers and cows. The type of service (AI in estrus or timed-AI [TAI]) and semen (conventional or sexed) were also considered in the analyses. Moreover, in cows, data were evaluated within parities (1st, 2nd, and 3rd or greater lactations). Statistical analyses were done by PROC GLIMMIX of SAS 9.4 (P≤0.05). In heifers, both the overall and 1st service P/AI were greater in H than L group, without difference for I group (53.4a[260/487] vs 41.4b[109/263] vs 50.6%ab[270/534]; (55a[132/240] vs 39.4b[43/109] vs 52.9%ab[127/240]; respectively). There was no effect of DPR class on P/AI in resynchronization, neither interaction with type of service. There was an interaction effect between semen and DPR, in which, only in heifers inseminated with sexed semen, H group had greater P/AI than L group, but did not differ from I group (54.9a[214/390] vs 36.7b[72/196] vs 47.9%ab[191/399]). In cows, the overall P/AI was greater in H than I and L groups (45.6a[426/934] vs 38.2b[349/914] vs 31.4%b[305/971]). In 1st service, P/AI did not differ between H and I group (51.5a[197/390] vs 43.6%ab[146/335] vs 38.1b[104/273]), but it was different in further services (42.1a[229/544] vs 35.1b[203/698] vs 28.8%b[201/698]). In addition, an interaction effect was found between DPR and parity. Primiparous from H group had similar P/AI than I, but greater than L (54.2a[172/317] vs 43.4%ab[180/414] vs 30.9b[125/404]), whereas in 2nd lactation, H group had greater P/AI than the other groups (45.6a[126/276] vs 36b[101/280] vs 28.4%b[78/274]). However, in multiparous the DPR did not affect P/AI (37.5[128/341] vs 30.9[68/220] vs 34.8%[102/293]). There was no interaction between DPR and semen or type of service. In both types, the H group had greater P/AI than the L group. However, only in TAI the I group differed from H group (AI in estrus: 46,5a[131/282] vs 30,4b[93/306] vs 41,4%ab[103/249]); TAI: 45,2a[295/652] vs 31,9%b[212/665] vs 37,0b[246/665], respectively). The PL for heifers and cows was not affected by DPR classes. In conclusion, the selection of dams with higher DPR values can improve reproductive efficiency of dairy herds.

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Animal Reproduction

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

TAI/AI

How fast can beef cattle become pregnant in a herd? An observational and preliminary experimental study of ReBreed21, an early resynchronization program

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Early pregnancy in the breeding season (BS) leads to many benefits for beef production. The aim was to compare two resynchronization programs in the same farm during two consecutives BS. In BS1, 23 d after 1st timed-artificial insemination (TAI; D23), all females received an intravaginal progesterone device (IPD; 1 g for heifers; 2 g for cows) and 1 (heifers) or 1.5 mg (cows) estradiol benzoate. On D30, ultrasound was performed for pregnancy per AI (P/AI). Nonpregnant females received 0.53 mg cloprostenol sodium, 200 (heifers) or 300 IU (cows) eCG, and 0.5 (heifers) or 1 mg (cows) estradiol cypionate (EC). TAI was performed on D32. In BS2, the ReBreed21 started 12 d after 1st TAI (D12), with insertion of a 1 g IPD, kept until D19, when they received 200 (heifers) or 300 IU (cows) eCG and 0.5 mg EC for heifers. At this moment, cows (body condition score = 4.1 ± 0.04) were randomly assigned to two groups: EC0.5 (0.5 mg EC) or EC1.0 (1 mg EC). On D21, P/AI was determined based on corpus luteum blood flow evaluated by Color Doppler ultrasound, and nonpregnant females received 8.4 µg buserelin acetate, along with TAI. In both BS, four TAI were performed, and pregnancy diagnosis was at 30, 60 and 90 d after TAI. Statistical analyses were done by Student's T or chi-square test of SAS 9.4 ($P \leq 0.05$) only to compare the EC doses. In BS1 (n = 218) and BS2 (n = 222), the proportion was: heifers 45.0 *vs* 37.4%, primiparous was 30.7 *vs* 18.0%, and multiparous was 24.3 *vs* 44.6%, respectively. Overall pregnancy in BS1 was 70% (91/130) and, 85.8% (115/134) in BS2, with a shorter length for BS2 (95 vs 64d). The mean number of services per female was similar (1.6 ± 0.1) between BS. P/AI 30 d after TAI, in BS1 and BS2 was: heifers (35.7 [35/98] and 31.3% [26/83]), primiparous (43.3 [29/67] and 70.0% [28/40]) and multiparous cows (50.9 [27/53] and 61.6% [61/99]). Expression of estrus was 79.4% (173/218) in BS1 and 73.9% (164/222) in BS2. Also, in females expressing estrus, P/AI was 43.5% in BS1 and 55.2% in BS2, while in females that did not express estrus P/AI was 35.6% and 45.8%, respectively. The preliminary results comparing EC doses in cows during ReBreed21 did not show effect on P/AI (EC0.5 = 59.7% [34/57] *vs* EC1.0 = 68.3% [41/60]; $P = 0.33$) or false-positive pregnancy diagnosis in cows (21.4% [9/42] *vs* 9.5% [4/43]; $P = 0.13$), although the number of cows was too small. There was no effect of sire or AI technician. In conclusion, the ReBreed21 program is a strategy to reduce BS duration without affecting overall pregnancy. More research is needed to determine if treatment with 1.0 mg EC will hasten luteolysis in nonpregnant cows, thereby reducing false-positives at the early pregnancy diagnosis or increase P/AI. Nevertheless, apparently the ReBreed21 can increase the number of opportunities for cows to become pregnant during a shortened BS.

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Animal Reproduction

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

TAI/AI

17-day reinsemination program in sheep: is it possible?

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To reduce costs and optimize hormonal protocols for artificial insemination, this experiment sought to evaluate the possibility of two inseminations in consecutive cycles in ewe-lambs. For this purpose, a total of 62 ewe-lambs (Ile de France and Texel crossbreed) from a commercial sheep farm in southern Brazil in November, during the non-reproductive season in the southern hemisphere, were used. The animals were around 1 year old, with median body condition score of 3.5 (scale of 1-5) in all experimental treatments, and mean body weight of 54.7 ± 1.01 kg. The first timed artificial insemination (TAI) was conducted with intravaginal devices (IVDs), containing 60 mg medroxyprogesterone acetate (MA Fagron do Brasil Farmacêutica, São Paulo, Brazil), for 14 days and 300 IU eCG (Novormon®, Zoetis, Campinas, Brazil) was administered intramuscularly (IM) at IVD removal. All animals were submitted to superficial cervical insemination, 54 h after, using a dose of 130 x 10^6 motile spermatozoa, obtained from one ram of known fertility, diluted in 100 µL of skimmed milk. Ewe-lambs inseminated on D0 were allocated to three groups: control group (CG; n=18), ReSynch17 group (ReSynch17; n=22) and Reinsemination group (ReIns n=22). Females of the CG were kept with ram from D7 to D20. Animals in the ReSynch17 group received IVD on D7 (post first TAI) until D15. Both treatment groups, ReSynch17 and ReIns, received 300 IU eCG IM on D15, and a second TAI was performed 54 h afterwards (D17), as described for the first TAI. Teaser rams with chest paint markings were used for estrus identification. Pregnancy was diagnosed by transrectal ultrasonography on D27 and D60 after the first TAI. Follicle was measured on D15 and compared by ANOVA (P 0.05). Pregnancy rate after the first TAI was 43.5% (27/62). Pregnancy rates for the CG, ReSynch17 and ReIns groups were 27.7% (5/18), 50.0% (11/22) and 50.0% (11/22), respectively (P=0.58). Thus, a total of 35 ewe-lambs remained non-pregnant on the second cycle after first TAI. Pregnancy rate after the second service (NM or FTAI) in the CG group was 35.3% (4/13), whereas in the ReSynch17 group was 9.1% (1/11) and in ReIns group was 0% (0/11) (P=0.01). Cumulative pregnancy rate was 50.0% (9/18), 54.5% (12/22) and 50.0% (11/22), respectively. Mean follicular diameters on D15 in the ReSynch17 and ReIns groups were 2.6 ± 0.24 and 4.33 ± 0.25 mm (P=0.0007), respectively. In the ReSynch17 and ReIns groups, teaser rams marked 6 out of the 21 ewes (3 from each group) between D15 and D17, with no females being marked between D7 and D15. Thus, although pregnancy rates were extremely low in the ReSynch17 and ReIns groups, estrus manifestation (D15-D17) and follicular size on D15 suggest that reinsemination may be possible after adjusting the time of TAI. However, to test this hypothesis, further studies with more animals during the breeding season are required.

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Animal Reproduction

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

TAI/AI

Factors that affect conception rate and pregnancy loss in dairy cattle

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This study aimed to evaluate the main conditions related to parturition and milk production that affect conception rates (CR) and pregnancy losses (PL). An observational and retrospective study was conducted through data mining referring to the period from 2016 to 2019, at Fazendas Reunidas ACP and Filhos located in the municipality of Carmo do Rio Claro, MG, where the animals are kept in an intensive system (Free-Stall). The tabulated crude data consists of reproduction and production information in a spreadsheet where each line corresponds to a reproductive service, and columns contain information on parity order (PO), body condition score (BCS), month and year of insemination and information on diseases in that lactation: retained placenta (RP) or occurrence of any disease during lactation (DL). The data were adjusted for analysis, keeping information up to the 3rd PO and the 5th service of each cow. For BCS, two categories were created: 1: 3; and 2: ≥3. Pregnancy diagnosis was performed at 30 (PD30) and confirmed 60 days postpartum (DPP). In this first analysis, 3201 observations were included and the statistical model to explain the CR and PL included the effects of PO, BCS, RP, DL, MONTH, and YEAR, performed in Proc Glimmix adjusted for binomial data (SAS University Edition, SAS Institute). The conception rate at 30 days was affected by BCS at calving, being higher ($p<0.004$) in cows with a BCS greater than or equal to 3 (39.1±1.3 vs. 33.1±1.1). Cows that did not have any disease during lactation also had a higher ($p<0.02$) CR (37.0±1.0 vs. 33.1±1.3). Pregnancy losses between 30 and 60 days were affected only by parity order, being higher ($p<0.07$, approximation) in cows with 2 or more lactations compared with primiparous (22.0±2.8 vs. 15.7±1.2). Milk production and somatic cell count (SCC) data obtained on the day of service were adjusted in the same way as the previous models, and information on SCC above 1 million cells was removed. Binary logistic regression (Proc Logistic) models for PD30 and PL considering milk production and SCC were adjusted. The linear model between milk production and CR approached statistical significance ($p<0.06$), demonstrating a variation of 0.008% in the probability of conception for each kg of milk produced. The linear model also adjusted for PL ($p<0.001$), and for each kg of milk produced, a variation of 0.026% in the probability of pregnancy loss was observed. SCC did not show a direct relationship with conception rate ($p=0.14$) or pregnancy loss ($p=0.69$). In milk production systems, animal performance can be monitored by indicators, helping to adjust herd management. The results showed that lower BCS at calving and occurrence of subsequent disease decreased CR, but did not change later pregnancy loss. Therefore, milk production and greater parity order increased the chance of PL.

Animal Reproduction

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

TAI/AI

Different doses of hCG administered at the time of artificial insemination on fertility of *Bos indicus* cows submitted to E2/P4-based TAI protocol

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The objective was to evaluate the administration of different doses of hCG at the time of TAI in Bos indicus cows that did not displayed estrus during the ovulation synchronization protocol in the state of Roraima/Brazil in the rainy season. On a random day of the estrous cycle (D0), suckled Nelore cows (n=651) at BCS 2.64±0.02 received 2mg of estradiol benzoate (Fertilcare Sincronização®, MSD, Brazil) and an intravaginal progesterone device (Fertilcare 1200®, MSD, São Paulo, Brazil). Eight days later (D8) 1mg of estradiol cypionate (Fertilcare ovulação® - MSD, São Paulo, Brazil), 400IU of eCG (Folligon®, MSD, São Paulo, Brazil), 500µg of sodium cloprostenol (Ciosin®, MSD, São Paulo, Brazil) were administered concurrently to P4 devices withdrawal. In addition, the tail head of was marked with chalk for evaluate estrus expression between D8 and D10. Estrus detection and TAI was performed 54h (D10 PM) after the removal of progesterone device. On day D10 (D10 PM) the cows that did not remain marked were considered in estrus and excluded from the experiment. Cows that remained with the marking on the tail head were considered non- estrus and, simultaneously, were randomly distributed into four experimental groups (Control, hCG500, hCG800 and hCG1000 groups). Cows in the control group (n=158) received no additional treatment. Cows in the hCG500 (n=162), hCG800 (n=165) and hCG1000 (n=168) groups received 500UI, 800UI and 1000UI of hCG (Chorulon 5000UI® - MSD, São Paulo, Brazil) respectively. Ultrasound exams were performed on 30 days after TAI (D40) to evaluate the pregnancy rate. Statistical analyses were performed by GLIMMIX procedure of SAS. The pregnancy rate tended to be greater in cows of the hCG1000 group [Control=31.7% (50/158)^{b}, hCG500=31.5% (51/162)^{b}, hCG800=31.5% (52/165)^{b} and hCG1000=41.0% (68/166)^{a}; P=0.07] and did not differ between the experimental groups on the natural mating pregnancy rate [Control=11.1% (12/108)^{b}, hCG500=17.1% (19/111)^{b}, hCG800=18.6% (21/113)^{b} and hCG1000=15.3% (15/98)^{a}; P=0.45]. However, at the end of the breeding season, the pregnancy rate was greater in animals that received 1000UI of hCG compared to the control group [Control=39.2% (62/158)^{b}, hCG500=43.2% (70/162)^{ab}, hCG800=44.2% (73/165)^{ab} and hCG1000=50.0% (83/166) ^{a}; P=0.05]. In conclusion, the administration of 1000UI of hCG at the time of TAI in cows that did not display estrus increases the pregnancy rate of suckled *Bos indicus* cows submitted to ovulation synchronization protocol. Although the hypothesis was confirmed further studies are required to confirm these conclusions and recommendations.

Animal Reproduction

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

TAI/AI

The replacement of estrogen by GnRH in the timed artificial insemination protocol does not affect pregnancy rate in *Bos taurus* cows with adequate BCS

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The most commonly used timed artificial insemination (TAI) protocols are based on estradiol esters and progesterone. Considering the existing restrictions on estrogen use worldwide, it is necessary to explore alternatives that do not negatively affect the technique's efficiency. Therefore, the effect of replacing estrogen by GnRH on ovarian response, estrus and pregnancy rates was evaluated in 251 multiparous suckling Hereford and Angus cows (minimum 45 days postpartum). Ten days before the protocol's start (D-10), cows received 150 mg (1 ml) of injectable progesterone (P4) (Sincrogest®, Ourofino) intramuscularly (IM). On day zero (D0), all animals received a progesterone-releasing intravaginal device (IVD) (1g P4, Sincrogest®, Ourofino) and were allocated to Control, GnRH/CE or GnRH/GnRH groups. On D0, cows in the Control group received 2 mg of estradiol benzoate (2 ml, IM, Sincrodiol®, Ourofino), while animals in the GnRH/GnRH and GnRH/CE groups received 20 mcg of Buserelin Acetate (5 ml, IM, Sincroforte®, Ourofino). Upon removal of the IVD (D7), all cows received 0.526 mg of sodium Cloprostenol (2 ml, IM, Sincrocio®, Ourofino), 300 IU of eCG (1.5 ml, IM, SincroeCG®, Ourofino), and tail paint for estrus evaluation. At the same time, animals in the Control and GnRH/CE groups received 1 mg of Estradiol Cypionate (1 ml, IM, SincroCP®, Ourofino). At TAI (D9), animals in the GnRH/GnRH group received 10 mcg of Buserelin Acetate (2.5 ml, IM, Sincroforte®, Ourofino). Data were analyzed using JMP software. In a subgroup of cows (n=251; BCS 3.8±0.1; scale 1-5), no difference was observed between Control (n=85), GnRH/CE (n=82), and GnRH/GnRH (n=84) groups in the percentage of cows with CL at D-10 (58.8, 73.2, and 70.2%), follicular diameter at D0 (6.4±0.2, 6.7±0.3, and 6.5±0.2), percentage of CL at D0 (75.3, 82.9, and 78.6%), dominant follicle diameter at D7 (7.3±0.2, 7.6±0.3, and 7.6±0.3), and percentage of CL at D7 (87.1, 80.5, and 81.0). The CL rate on D11 was lower in the control group (62.4%) compared to GnRH/CE (75.6%) and GnRH/GnRH (69.0%). Considering all cows (n=455; BCS ≥ 3), no difference was observed in the estrus manifestation until TAI (D9; 64.0, 56.5 and 61.3%) and pregnancy rate (56.9, 53.1 and 53.6%) for the Control (n=153), GnRH/CE (n=147), and GnRH/GnRH (n=155) groups, respectively. In conclusion, estradiol esters can be replaced by GnRH in TAI protocols in taurine cows with adequate BCS, without affecting estrus manifestation and pregnancy rate.

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Animal Reproduction

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

TAI/AI

The bovine appeasing substance (BAS) increased performance without changing the reproductive efficiency of confined heifers (Angus x Nellore) submitted to FTAI

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During the FTAI protocol, the application of exogenous hormones and animal restraint entail substantial stress, which in turn causes the females to lose weight during the protocol, which may negatively impact reproductive performance. The objective of this work was to evaluate the effect of the bovine appeasing substance (SAB) on the reproductive efficiency of super precocious heifers in the confinement system. For this purpose, 201 heifers (½ Angus X ½ Nelore) were used, with an average age of 10 months old, average weight of 295 kg and average ECC of 3.44 (on a scale of 1 to 5). The animals were kept in a feedlot system throughout the experimental period, divided into 2 groups: control group and SAB group, which received water or a bovine appeasing substance, respectively, in the nape of the neck. The animals were submitted to a three-management FTAI protocol based on progesterone and estrogens, and were submitted to gynecological evaluation, evaluation of body condition and weight gain and blood collection during the corral management. Thirty days after AI, all animals underwent a pregnancy diagnosis and animals identified as pregnant underwent a second diagnosis, sixty days after AI. The data obtained were submitted to statistical analysis using the MIXED and GLIMMIX procedures of SAS, adopting $P<0.05$ for significance and $P<0.10$ for trend. A reduction in cortisol concentrations was observed between D0 and D10 in both groups, so that it was not possible to characterize stress in heifers. The estrus intensity was lower in the SAB group, but the preovulatory follicle diameter and serum E2 concentrations, as well as the pregnancy rate and pregnancy loss did not vary between groups. On the other hand, the weight gain of the SAB group was higher between D0 and DG 30. It is concluded that the bovine appeasing substance analogue was able to cause appeasement in the behavior of heifers during the FTAI program, however it provided greater weight gain. weight, without altering the reproductive performance of heifers crossed in the feedlot system submitted to FTAI.

Animal Reproduction

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

TAI/AI

Effect of cow temperature on fertility of lactating dairy cows submitted to fixed time artificial insemination

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The aim of this study was to evaluate the effect of cow temperature (CT) on the fertility of lactating dairy cows submitted to fixed-time artificial insemination (FTAI). Outcomes of 11,925 inseminations, from 63 commercial farms, were evaluated. Cow temperature was measured transrectally at TAI, using a digital thermometer. The ovaries were evaluated by ultrasound at the beginning of the protocol (d-11; n=11497), prostaglandin treatment (d-4; n=7,510), time of insemination (d0; n=6,653) and 7 days after FTAI (d7; n=8,589) to assess the presence of corpus luteum (CL), ovulatory follicle diameter, double ovulation rate and ovulation to the protocol. Pregnancy diagnosis was performed by ultrasonography 32 and 60 days after FTAI. Blood samples were collected on d0 (n=2,532) and d7 (n=6,378) to determine circulating P4 concentration. Data were analyzed by SAS PROC GLIMMIX (SAS / STAT® 9.2), receiver operating characteristic (ROC), and curves were generated to determine the point of greatest sensitivity and specificity in which CT impacts pregnancy. The CT that resulted in the greatest combined sensitivity (69.3%) and specificity (41.4%) was <39.1°C (P<0.01), this indicates that (69.3%) of the pregnant cows from the 32d pregnancy diagnosis had a temperature <39.1°C. Cows with temperature <39.1°C had higher (P<0.01) pregnancy per insemination (P/AI) at d32 (37.6% [2605/7005] vs. 24.6% [1206/4920]), at d60 (32.3% [2253/7005] vs. 19.6% [964/4920]) and lower pregnancy loss between 32 and 60 days (14.2% [352/2605] vs. 20.7% [242/1206]) compared with CT ≥39.1°C. Cows with temperature ≥39.1°C had smaller ovulatory follicle diameter (15.15 ± 0.05mm vs. 14.76 ± 0.05mm; <0.01), had reduced estrus expression (84.0% [4405/5452] vs. 80.1% [3646/4504]; P<0.01), lower presence of CL on days d-11 (68.8% [4538/6598] vs. 65.0% [3185/4899]; P<0.01) and d-4 (65.5% [2349/3587] vs. 60.3% [2366/3923]; P<0.01), lower ovulation to the protocol (88.8% [3999/4501] vs. 84.5 [3456/4088]; P<0.01) and increased double ovulation (11.5% [239/2086] vs. 23.2% [288/1239]; P≤0.01) compared with CT <39.1°C. Analyzing all cows, the P4 concentration on d0 (0.17 ± 0.01 vs. 0.25 ± 0.01 ng/mL; P<0.01) and d7 (2.60 ± 0.03 vs. 2.81 ± 0.03 ng/mL; P<0.01) were higher for animals with CT ≥39.1°C, however, evaluating only the animals that ovulated to the protocol, separating cows with double ovulation (3.98 ± 0.15 vs. 4.2 ± 0.12 ng/mL; P=0.24) or single ovulation (3.13 ± 0.04 vs. 3.1 ± 0.06ng;/mL; P=0.50) there was no effect of BT on P4 concentration on d7. It is concluded physiological factors associated with higher fertility in TAI protocols such as CL at the beginning of the protocol, ovulatory follicle diameter, estrus expression, and ovulation to the protocol were reduced in cows with temperature ≥39.1°C, and these factors help to understand the reduction P/AI and increased pregnancy losses.

Animal Reproduction

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

TAI/AI

Reproductive performance of 12- to 16-month-old *Bos indicus* heifers submitted to programs for induction of cyclicity and nutritional management

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The aim was to evaluate strategies for induction of ovulation prior to timed-AI (TAI) in prepubertal Nelore heifers. On D-24, 2300 heifers (12.2±1.2mo old; 230.1±30.7kg of body weight [BW]) managed in a confinement system were randomly assigned to ovulation induction protocols: P4+E2 (n=1085): heifers received an intravaginal progesterone (P4) device (0.5g), kept until D-12, when they received 0.5mg estradiol cypionate (EC); or P4i (n=1175): heifers received only 150mg of long-acting injectable P4 im on D-24. All heifers received the same TAI protocol that started on D0 with insertion of a P4 device (0.5g) and 1.5mg estradiol benzoate. On D7, 0.5mg cloprostenol sodium, 0.5mg EC and 200IU eCG were given, concomitant with P4 device withdrawal and tail chalk for estrus evaluation. On D9, 8.4µg buserelin acetate was given, and TAI was performed. Ultrasound was done on D0 for uterine and ovarian evaluations and, on D40 and 121 for diagnosis of pregnancy (P/AI) and pregnancy loss (PL). Pregnant heifers after D40 were moved to pasture and were assigned to either receive a supplementation with DDGS (1% of BW of dry matter) or with protein (0.2%) added to mineral salt. Non-pregnant heifers were resubmitted to the same TAI protocol (2nd and 3rd TAI). Statistical analyses were done by PROC GLIMMIX of SAS 9.4 ($P \leq 0.05$). On D0, presence of corpus luteum (CL) and expression of estrus was greater in P4+E2 than in P4i group (59.3 [1085] *vs* 16.1% [1175]; 66.9 [1085] *vs* 59.1% [1175], respectively), as well as P/AI (47.0 *vs*41.2%). Presence of CL on D40 in non-pregnant heifers and P/AI in further TAI did not differ between groups (20.6 [574] *vs* 21.4% [691]; 24.1 [802] *vs* 29.3% [940], respectively). In addition, considering all TAI, PL was not affected by treatments. Regardless of treatment, presence of CL on D0 resulted in greater expression of estrus (70.0 [833] *vs* 58.7% [1427]) and P/AI (54.9 [833] *vs*37.7% [1427]). Also, P/AI was greater in heifers expressing estrus (49.9 [1421] *vs* 34.1% [839]). There was no effect of age on D0 on P/AI. However, there was an effect of BW on D0 (evaluated as terciles: 238 [596], 238-262 [585], and ≥262 [587]) on P/AI (38.0^{a} *vs* 48.0^{b} *vs* $46.1\%^{b}$, respectively). On D121, there was an effect of age terciles (≤12.4, 12.4-13.6, and ≥ 13.6 mo) and BW (D0) on PL considering all TAIs (17.9^{c} [390] *vs* 6.9^{a} [432] *vs* $11.6\%^{b}$ [455]; 14.4^{b} [312] *vs* 13.1^{ab} [389] *vs*$9.6\%^{a}$[424]). Moreover, there was no effect of nutritional supplementation treatment on PL on D121 (11.4 [663] *vs*12.8% [656], respectively). In conclusion, the P4+E2 ovulation induction protocol increased the number of heifers with CL at the beginning of the TAI protocol, resulting in greater expression of estrus and P/AI. Moreover, although no effect of age was observed, heavier heifers had greater P/AI, regardless of treatment. Supplementation strategies in pasture for pregnant heifers did not differ for PL.

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Animal Reproduction

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

TAI/AI

Impacts of using different estradiol esters on ovulation synchronization protocols in Nelore heifers

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We aimed in this study to evaluate the effects of treatment with different estradiol esters in synchronization protocols on the estrous expression, moment of ovulation, and modulation of the uterine environment in Nelore heifers with a small dominant follicle (DF). Nelore heifers (N=41) were submitted to a pre-synchronization protocol starting on D-7 by the application of two injectable doses of 0.53 mg sodium cloprostenol (Cioprostin®, Boehringer Ingelheim) in a 12-hour-interval, and 0.01 mg of buserelin acetate (Prorelinn®, Boehringer Ingelheim) on D-5. On D0, heifers were injected with 1 mg of estradiol benzoate (Sincrodiol®, Ouro Fino Saúde Animal) and received an intravaginal P4 device (Sincrogest®, Ouro Fino Saúde Animal). On D7, the P4 device was removed, 0.53 mg of sodium cloprostenol IM (Cioprostin®) was injected, and a heat-detection patch (Boviflag; ABS Pecplan) was attached to the tailhead. On the same day, the animals were randomized in groups according to a 2x2 factorial design: Control (C, N=8, no treatment), estradiol cypionate (0.5 mg EC on D7, SincroCP®, Ourofino; N=9), 17β-estradiol (1 mg E2 on D9, 17 Beta®, Botupharma; N=8) and estradiol cypionate + 17β-estradiol (0.5 mg EC on D7 + 1 mg E2 on D9, N=8). Still, on D7, heifers were submitted to transrectal ultrasonography (US) in B-mode and Doppler for evaluation of the ovaries. Animals with DF 11 mm and inactive corpus luteum (blood perfusion <30%) were excluded from the experiment. On D9, a uterine cytological sample was collected 12h after treatment with E2 to evaluate the transcript expression of receptors for E2 (ESR1), oxytocin (OXTR), and P4 (PGR). Between D7-D12, the heifers were assessed daily for endometrial thickness and follicular development by US. Evaluations of endometrial thickness and estrus expression were performed every 12h and follicular development every 6h, both from D9. Variables were evaluated by ANOVA using the MIXED procedure of SAS software. No significant difference was observed in the DF between treatment groups (P>0.1). The moment and rate of ovulation did not differ between treatment groups (P>0.1). Regarding the onset of estrus detection, EC-treated females tended to show signs of estrus earlier (18.7±3.3 vs. 23.3±1.9, P=0.09). For endometrial thickness, animals treated with EC had greater endometrial thickness on D9 (P=0.01) and D10 (P=0.02), while animals treated with E2 had lower endometrial thickness on D10 (P=0.05). Regarding the maximum thickness of the endometrium, animals treated with EC tended to have the thickest endometrium (P=0.08). The abundance of transcripts for ESR1, OXTR, and PGR did not differ between treatment groups (P>0.1). In conclusion, EC administration at the time of P4 device removal modulates the uterine thickness and anticipates estrous behavior; whereas, the E2 treatment does not alter the periovulatory ovarian and uterine characteristics in Nelore heifers with small DF.

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Animal Reproduction

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

TAI/AI

Effects of different strategies to induce puberty before TAI on pregnancy establishment and losses in precocious Nelore heifers

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We aimed to study the reproductive tract development, pregnancy per AI (P/AI), and pregnancy losses (PL) in Nelore heifers submitted to different puberty induction protocols using injectable progesterone (iP4; Sincrogest, Ourofino Saúde Animal) before timed-AI (TAI). Nelore heifers, between 10 and 17 months of age, born at the onset (August to October, n=574), middle (November and December, n=558), or end (January and February, n=588) of the birth season were used in a randomized block design (block=birth period). For each block, heifers were randomly split to receive one of three treatments: control (without puberty induction before the TAI; n=576); 1P4 (150 mg of iP4 at D−12 [D0= beginning of TAI protocol]; n=571); and 2P4 (150 mg of iP4 on D−24 and D−12; n=573). Heifers were submitted to an E2/P4-based protocol and TAI was performed at D9. Body weight (BW) and body condition score (BCS) were recorded at D−24 and D31. Heifers had the uterus and ovaries assessed on D−24, D−12, and D0 and were attributed scores of 1 (less developed) to 5 (more developed) for both structures. On D7 and D9, a subgroup (n=505) had ovaries scanned to measure the dominant follicle (DF) by B-mode transrectal ultrasound. A first pregnancy diagnosis (PD1) was performed 22 days after TAI using color- Doppler ultrasound and heifers with an inactive CL (blood perfusion ≤25%) were considered not pregnant. On days 30-35 and on days 59-61 after TAI, confirmatory pregnancy diagnoses were performed to access, respectively, the embryo (PD2) and fetus (PD3) with heartbeats by ultrasound. Data were analyzed by PROC MIXED and GLIMMIX (SAS). The BW and BCS did not differ among treatments at any time (P>0.1). On D−12, the uterus and ovarian scores were greater (P<0.05) in the 2P4 group than in the control and 1P4 groups; whereas on D0, the uterine score progressive increased according to the number of iP4 treatments, and the ovarian score was greater in 1P4 and 2P4 groups than in controls. On D7, the DF diameter (mm) was greater (P<0.05) in the 2P4i [8.8±0.2] and 1P4i [8.4±0.2] than in controls [7.9±0.1]. On D9, DF was larger (P<0.05) in 2P4i [10.5±0.2] than in the controls [9.6±0.2], and intermediate in the 1P4i group [10.1±0.2]. The P/AI at PD1, PD2, and PD3 was greater (P<0.05) for heifers born at the onset of the birth season than at the end. A treatment effect (P<0.05) was observed on P/AI at PD1 (Control: 31%^{B}; 1P4: 32%^{AB}; and 2P4: 41%^{A}), PD2 (Control: 27%^{B}; 1P4: 29%^{AB}; and 2P4: 35%^{A}) and at PD3 (Control: 24%^{B}; 1P4: 25%^{AB}; and 2iP4: 31%^{A}). The PL did not differ (P>0.05) among treatments, but PL between PD2 and PD3 was affected by the birth period (Onset: 3%^{B}; Middle: 7%^{AB}; and End: 13%^{A}). In conclusion, administering iP4 two moments before the TAI improves uterine and ovarian development, resulting in a larger DF and greater P/AI in precocious heifers. Heifers born at the end of birth season have greater PL between 30 and 60 days.

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Animal Reproduction

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

TAI/AI

Strategies to lyse a young corpus luteum using cloprostenol sodium in *Bos indicus* cows

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The aim was to evaluate the luteolytic effect of cloprostenol sodium (PGF) treatments in a 6- or 7-day-old CL. On d-9, 109 Nelore (Bos indicus) cows (2.8±0.1 body condition score) underwent a synchronization protocol, receiving 8.4µg buserelin acetate on d0 to induce ovulation and formation of a new CL. Ovulation was confirmed on d6 and only cows with a CL (n=88) were randomly assigned to PGF treatments: D (control): a conventional dose (0.53mg) on d7 (n=30); 2xD: a double dose (1.06mg) on d7 (n=29); or D+D: one dose (0.53mg) on d6 followed by the same dose on d7 (n=29). Ultrasound was performed using B- mode and Doppler from d6 to 11 for ovarian evaluations (CL and dominant follicle [DF] , CL blood flow and ovulation). Blood samples were collected from d6 to 10, to analyze circulating progesterone (P4) concentrations. The CL volume was calculated by the formula: V=4/3×π×R3. Luteolysis was considered when P4 concentration was ≤0.5ng/mL, or <0.6ng/mL in addition to a reduction in CL volume (>80% of the original), without blood flow, expression of estrus and/or ovulation. Statistical analyses were performed by PROC GLIMMIX of SAS 9.4 (P≤0.05). In all groups, the CL volume reduced until d11 in response to PGF treatments, but with a greater decrease in D+D than D and 2xD (91a vs 73b vs 80b% reduction from the initial volume). P4 was lower in D+D than D and 2xD on d7 (0.8±0.1b vs 3.4±0.3a vs 3.1±0.3ang/mL) and d8 (0.5±0.1b vs 0.8±0.1a vs 0.8±0.1ang/mL). Moreover, P4 was lower in D+D than D group, but did not differ from 2xD group on d9 (0.36±0.1b vs 0.53±0.1a vs 0.48±0.1ab) and d10 (0.33±0.1b vs 0.69±0.1a vs 0.39±0.1ab). Luteolysis was greater on d8 in D+D than D and 2xD (48.3a [14/29] vs 26.7b [8/30] vs 20.7b% [6/29]), while on d9, it was greater in D+D than in D, but without difference from 2xD (82.8a [24/29] vs 53.3b [16/30] vs 58.6ab% [17/29]). Nevertheless, on d10, luteolysis was similar in D+D and 2xD, and both were greater than D (96.9a [28/29] vs 96.9a [28/29] vs 63.3b% [19/30]). Some cows from D (7), D+D (1) and 2xD (1) had an initial decrease in P4 concentration and CL volume after treatment, followed by an increase in P4 (concomitant or not with increase in CL volume), and were considered as partial luteolysis. Treatments did not affect expression of estrus, although more D+D cows had ovulated than D, without difference between both and 2xD (68.9a [20/29] vs 30%b [9/30] vs 37.9%ab [11/29]). In conclusion, treating Nelore cows with a conventional dose of cloprostenol on day 7 of the estrous cycle was not efficient in inducing complete luteolysis in several cows up to 72h from treatment. In contrast, treatment with a double dose on day 7 or using 2 doses, 24h apart, starting on day 6, resulted in greater incidence of luteolysis within 72h. Further studies are needed to confirm if the double dose is as efficient as 2 doses in consecutive days.

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Animal Reproduction

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

TAI/AI

Influence of the timing of exit from feedlot relative to the insemination on the pregnancy per AI and gestation loss of precocious Nelore heifers

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This study evaluated the pregnancy per TAI (P/TAI) and pregnancy loss (PL) in Nelore heifers, aged 12 to 16 months, subject to different moments of exit from the feedlot to pasture relative to the TAI (5 vs. 30 days after TAI). A total of 1,257 heifers from two farms located in the states of GO (Farm 1; n=324) and MT (Farm 2; n=933) were used. Heifers were kept on feedlot after weaning (210 to 240 days of age) and only females that reached the minimum weight of 280kg were considered suitable to participate of the breeding. All heifers received 150mg of injectable P4 before the initial of the TAI protocol (Farm 1 = 8d and Farm 2 = 24d before). The TAI protocol began (D-9) with the insertion of an intravaginal P4 device and an intramuscular (IM) application of 2mg of EB. On D-2, the P4 device was removed, and 500µg of PGF2alfa, 1mg of EC, and 200IU of eCG were injected IM. Two days later, TAI was performed (D0). Five days after TAI (D5), half of the heifers (G5; n=647) were randomly transferred from the feedlot to pasture where they received a protein/energy supplementation of 0.3g per kg of live weight. The other half remained in the feedlot until 30 days after TAI (G30; n=610). On D30, pregnancy diagnosis (PD30) was performed. Immediately after PD30, the G30 group heifers were also transferred from the feedlot to pasture. At this time, the females from G5 and G30 were regrouped in the same pastures, according to the pregnancy status. On D60, a second exam (PD60) was conducted to assess the occurrence of pregnancy loss in heifers that had been diagnosed as pregnant on PD30. In addition, the average daily gain (ADG) of the heifers was evaluated between the intervals of D-9 to D30 (ADG_-9_30) and D30 to D60 (ADG_30_60). Variables were evaluated using the MIXED and GLIMMIX procedure of SAS 9.4. There was an interaction (P=0.06) between treatment and farm on the PD30, which only in the farm 1 the G5 group had a lower P/TAI [G5=40.9% (67/164) vs. G30=53.1% (85/160)]. There was no difference between treatments on PD30 in the Farm 2 [G5=52.6% (254/483) vs. G30=52.4% (236/450)]. Similar interaction (P<0.001) was observed ADG_-9_30 (Farm 1: 0.028g/d in G5 and 0.250g/d in G30; Farm 2: 0.164g/d in G5 and 0.634g/d in G30). No interaction between treatment and farm was observed on the other response variables analyzed (P>0,1). There was an effect of treatment (P<0.001) on ADG_30_60 (G5=0.204g/d vs. G30=-0.130g/d), however no difference was found on PL (G5=7,3% vs. G30=8,0%; P=0,57) and also in P/TAI following the 2nd (G5=45,6% vs. G30=42,2%; P=0,30) or 3rd (G5=32,6% vs. G30=34,9%; P=0,95) TAI. Furthermore, there was no effect on cumulative 30 days P/TAI (G5=81.8% vs. G30=82.0%; P=0.98). In conclusion, the influence of the timing of exit from the feedlot to pasture depends on the farm, specially on the pregnancy after the 1st TAI, however, these different moments did not affect the subsequent reproductive performance of precocious Nelore heifers.

Animal Reproduction

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

TAI/AI

Evaluation of two synchronization protocols in guinea pigs (*cavia porcellus*)

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Guinea pig production in Latin America has lately increased, however, their reproductive behavior and physiology are still barely known. Therefore, the aim of this study was to evaluate the characteristics of the female reproductive tract, vaginal epithelial cells according to cycle stage and the efficiency of two synchronization protocols in guinea pigs. Three studies were performed. Exp 1: measurements of ovaries, oviducts, uterine horns and body, cervix and vaginal canal were taken of 56 guinea pigs of 8, 9, 10 and 12 weeks. Exp 2: in 69 guinea pigs the vaginal membrane was observed, and cytology was obtained to count and classify vaginal epithelium cells independent of cycle stage. Exp 3: 30 female guinea pigs were randomly assigned either to Protocol 1 (D0: 0,002 mg/kg of GnRH, D4: 0,02 mg/kg PGF2α, D6: 0,002 mg/kg GnRH) or Protocol 2 (D0: 0,002 mg/kg of GnRH + 0,22 mg/kg of P4, D4: 0,02 mg/kg of PGF2α, D6:0,002 mg/kg of GnRH). All hormones were applied intramuscularly (IM). 12h after the last application of GnRH vaginal membrane was observed, vaginal epithelium cells were obtained and for mating a proven male per every four females was applied for a period of 24h. Ultrasonographic exam was used for pregnancy diagnosis. Descriptive statistics, ANOVA variance analysis and Chi-square test (X2) were performed. Results in Exp 1 demonstrated age and weight influence over measurements of ovaries, uterine horns, cervix, and vaginal canal, mentioned structures were greater ($p \leq 0.05$) in guinea pigs of 12 weeks. On the other hand, these measurements in relation to their body weight were greater ($p \leq 0.05$) in most guinea pig structures of 8 weeks. On Exp 2 the absence of the vaginal membrane was related with higher ($p < 0.001$) number of superficial cells (68,22±12,62%) which indicated estrous. Guinea pigs with vaginal membranes showed higher frequency of intermediate cells (40,83±28,95%), followed by parabasal and basal cells. In Exp 3: both protocols showed high presence of the vaginal membrane (93.33%) with parabasal cells 47.85±21.22% and 41.23±24.22% respectively. There was relation ($p < 0.001$) between the vaginal membrane, cell type and protocol application. Protocol 1 obtained the highest count of superficial cells (19,23±20,66%) at 12 h after the last application of GnRH. Pregnancy rate had no difference ($p > 0.05$) between treatments. In conclusion, age and weight of guinea pigs influence reproductive development, females aged over 12 weeks have larger reproductive tract, which may be beneficial for production. Vaginal membrane is related to the type of vaginal epithelial cells which are indicators of estrous in the guinea pigs. Although both protocols of synchronization weren't efficient to present estrus after 12 hours, they had effect over the type of epithelial cells and vaginal membrane presence. These findings provide insights into the study of reproductive cycles in guinea pigs and may be used in further studies.

Animal Reproduction

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

TAI/AI

The bovine appeasing substance (BAS) increased reproductive performance of Nelore heifers submitted to FTAI

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The objective of the present study was to evaluate the effect of bovine appeasing substance (BAS) on dominant follicle diameter and conception rate after fixed-time artificial insemination (FTAI) in Nelores heifers. The experiment was conducted at the State University of Mato Grosso do Sul, at the University Unit of Aquidauana - MS. Twenty heifers managed under grazing, with an average age of 25 months and an average body condition score of 2.9 were used. They were divided into two homogeneous groups, Control (n = 10), with an average initial body weight of 378,2±25,9 kg and BAS (n = 10), with an average initial body weight of 384,5±29,6 kg on D-24, the day when the cyclicity induction protocol was initiated. Heifers received 150 mg of injectable progesterone (P4 - Sincrogest®, OuroFino Saúde Animal – Anhanguera – SP - Brasil) intramuscularly (IM) 24 days before the start of the FTAI protocol. After 12 days (D-12), 150 mg of estradiol cypionate - ECP (Cipion®, GlobalGen - Jaboticabal - SP - Brasil) was administered IM. On D0, 2 mg of estradiol benzoate (Syncrogen®, GlobalGen - Jaboticabal - SP - Brasil) were administered IM and the intravaginal P4 device (ReproOne®, GlobalGen – São Paulo - SP - Brasil) was inserted. On D8, the P4 device was removed and 2 mg of Cloprostenol sodium (Inducio®), GlobalGen - Jaboticabal - SP - Brasil 0.5 mg of ECP (GlobalGen - Jaboticabal - SP - Brasil) and 300 IU of Equine chorionic gonadotropin IM (eCGen®, GlobalGen – Yanzhou, Ningbo - China) were applied IM. Artificial insemination was performed 48 h later with semen from a single Nelore bull. The administration of BAS was performed on days D-12 and D0, topically applied to the nuchal skin area, so that the treated heifers were under the action of BAS during all managements since its first application until AI. During the experimental period, ovarian evaluations were performed via transrectal ultrasonography on D -24, D -12, D0 and D9, when MDFD (mean dominant follicular diameter) was measured. The conception rate of the control and BAS group was evaluated 30 days after AI by transrectal ultrasonography. MDFD was analyzed in an entirely randomized design and means were compared by T Student test at 5% probability. Conception rate was analyzed by Qui square at 5% probability. No difference between groups was observed on D0 regarding the presence and diameter of corpus luteum. MDFD was similar between groups (P>0.05) at D -24 (14.52±4,83 x 15.30±5,03), D-12 (13.63±3,45 x 14.53±1,70) and D0 (11.66±2,34 x 12.19±5,27) between control and BAS respectively. At D9, day of FTAI, MDFD was higher (P<0.05) in the BAS group (14,03±2,41^{a} x 12,35±1,95^{b}). The conception rate was higher in BAS, 90% (9/10), than in the control group, 50% (5/10). We conclude that the application of two doses of BAS with an interval of 12 days can improve the reproductive performance of Nelore heifers submitted to FTAI.

Animal Reproduction

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

TAI/AI

Cyclicity induction protocol with Sincrogest injetavel (injectable P4) 24 days before day 0 of the TAI protocol with or without estradiol cypionate treatment 12 days apart in 10-13 months Nelore heifers

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The aim of the study was to assess the effectiveness of the cyclicity induction protocol in heifers using 150 mg of injectable progesterone (iP4; Sincrogest injetavel, Ourofino Saude Animal, Brazil) 24 days (D-24) and 1mg of estradiol cypionate 12 days (D-12) prior to synchronization protocol for fixed-time artificial insemination (FTAI). A total of 638 Nelore (Bos indicus) heifers (aging 10 to 13 months, weight BW=297.5±1.1 kg, body condition score BCS=3.42±0.02) from a commercial farm (Agropecuária Jacarezinho, Brazil) were used. All heifers received 150mg of iP4 24 days before D0 of the FTAI protocol. Twelve days before the begging of FTAI, heifers were randomly divided into two groups: 1) Control (n=319): no treatment; 2) EC (n= 319): received on D-12 1mg of estradiol cypionate (EC; SincroCP®, Ourofino). On D0, an intravaginal P4 device (Sincrogest®, Ourofino), 2 mg of estradiol benzoate (E Sincrodiol®, Ourofino) and 0.53mg of sodic cloprostenol (PGF; Sincrocio®, Ourofino) were administrated. Also, gynecological examination was performed by ultrasound (Mindray® DP-2200Vet) for analyze the presence of corpus luteum (CL). On D7, heifers received 0.5 mg of estradiol cypionate (EC; SincroCP®, Ourofino), 0.53mg of sodic cloprostenol (PGF; Sincrocio®, Ourofino), 200 IU of eCG (SincroeCG® Ourofino), concomitant with P4 device removal. TAI was performed 48h later (D9) in both groups. Pregnancy diagnoses were evaluated by ultrasound after 30 and 60 days of insemination. Statistical analyses were performed using GLIMMIX of SAS 9.4. There was no interaction (P=0.9) estrus*treatment (average 88.5%). The presence of CL on D0 was higher (P=0.001) in the EC group (62.1%; 198/319) than in the Control group (50.8%; 162/319). In addition, there was an interaction (P= 0.026) treatment*weight of the heifer in the CL rate at D0. Heifers weighing below 281.4 presented a positive effect of EC treatment [EC=60.1% (95/158) vs. Control=40.6% (69/170)]. However, no EC effect was observed in heifers weighing over 281.4 [EC=63.9% (103/161) vs. Control=62.4% (93/149)]. No interaction treatment*weight (P=0.35) were observed of P/IA at 30 days. Treatment with EC increased (P=0.05) the P/AI at 30 days [53.3% (170/319) vs. 47.0% (150/319)] and 60 days [47.0% (150/319) vs. 40.4% (129/319)]. The P/AI was affected by the presence of CL at the beginning of the protocol [CL=54.2% (195/360); NoCL=44.9% (125/278); P=0.002]. Pregnancy loss did not differ (P=0.22) between treatments [EC=10.0% (17/170) vs. Control=12.7% (19/150)]. In conclusion, the treatment with EC on D-12 can improve the cyclicity rate and the pregnancy outcomes in heifers undergoing fixed-time artificial insemination protocol.

Animal Reproduction

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

TAI/AI

Productive and reproductive efficiency of primiparous Nelore cows carryout offspring of different genetic groups

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The use of Angus sires on zebu cattle by timed artificial insemination (TAI) has been one of the strategies performed to optimize cow/calf operations. However, there is concern that the greater development of F1 Angus calves may lead to greater stress on their mothers, which could decrease their reproductive performance. Therefore, the reproductive and productive efficiency of Nellore primiparous females nursing progeny from two genetic groups [Angus x Nelore (F1 Angus) and Nelore x Nelore (Nelore)] was evaluated. For this, 2,354 heifers from four commercial farms located in the states of MT (n=1), MS (n=2), and GO (n=1) were used. The heifers were subjected to the same hormonal protocol and, randomly, inseminated with Angus (n=1,169) or Nelore (n=1,185) breeders. To evaluate reproductive efficiency, pregnancy rates were analyzed by TAI at 30 (DG30) and 150 days (DG150) after TAI. To primiparous was to evaluate the pregnancy rates after the first, second, and third TAI plus natural mating with Nellore bulls, and the final pregnancy at the end of the breeding season (BS). For the TAI, semen from Nellore and Angus sires was randomly used, blocked by the breeding group and service phase. Only cows that had a calf at weaning were considered for further evaluations The interval from calving to conception (d; CCI) was also evaluated. For productive traits, adjusted weaning weight at 210 days, cow weight at weaning, and weaning rate (WR; calf weight divided by her mother weight at weaning) were analyzed. All information was analyzed using the Glimmix procedure of SAS 9.4, with P values < 0.05 considered significant. There was no effect of sire breed on pregnancy rate at 30 days (P>0.05), as well as on gestational loss between 30 and 150 days of gestation (P>0.05) in heifers. In the evaluation of these dams as primiparous, there was no effect of calf breed on pregnancy rates following the first TAI (F1 Angus=50.8% vs. Nelore=52%; P=0.66), as well as for pregnancy at the end of BS (F1 Angus=80.1% vs. Nelore=81.7%; P=0.3). However, there was an influence of calf gender on pregnancy rate at the first TAI (Male=45.3% vs. Female=57.2%; P=0.001), pregnancy at end of BS (Male=78.1% vs. Female=83.4%; P=0.04), and CCI, where cows carrying out male calves had approximately 9 days of delay (P=0.04) when compared to cows carrying out female calves. Calf breed and gender impacted the weaning weight (F1 Angus=231.6kg vs. Nelore 200.7kg; P≤0.001; Male = 223.1kg vs. Female = 212.7kg; P≤0.001), as well as the WR (F1 Angus=53.4% vs. Nelore 48.4%; P=0.02; Male=53.9% vs. Female=48.6%; P=0.006). Therefore, it is concluded that the calf breed does not influence the reproductive efficiency of primiparous Nelore subjected to successive TAI synchronization protocols plus natural mating through BS, but calf gender has an impact on both reproductive and productive efficiency. The breed of calves influences weaning weight and WR, where F1 Angus calves weaned heavier, and their mothers had a higher WR.

Animal Reproduction

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

TAI/AI

Effect of early PGF2α administration and CL presence on pregnancy rates in resynchronization protocols after TAI (Resynch 22) in Nelore cows

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The experiment evaluated the outcomes of early administration of PGF2α (PGF), as well as the presence of CL on the diameter of the dominant follicle (DF) on D29 and on the pregnancy rate (P/IA) in Nelore (Bos indicus) cows submitted to the resynchronization protocol (Resynch 22) started 22 days (D22; unknown pregnancy status) after previous time artificial insemination (TAI). Nelore cows from a commercial farm (Agropecuária Jacarezinho, Brazil) were submitted to TAI (D0). On D22, they received a second use intravaginal P4 device (CIDR®, Zoetis) and 2 mg of estradiol benzoate (Gonadiol®, Zoetis). On D30 (Experiment 1) or on D29 (Experiment 2) pregnancy diagnosis was evaluated by US (Mindray® DP-2200Vet). Cows identified as non-pregnant at first TAI (Exp 1=917 and Exp 2=485) had their ovaries scanned to measure the presence of CL (Exp 1 and 2) and the diameter of the largest follicle (LF; Exp 2). In Exp 1, 55.9% (513/917) of the non-pregnant cows presented CL and 44.1% (404/917) presented no CL in the ovaries. In Experiment 2, the non-pregnant cows were assigned according to the to one of the following groups (2 x 2 factorial design): 1) noCL and noPGF (n=137); 2) noCL and PGF (n=140); 3) CL and noPGF (n=100) and; 4) CL and PGF(n=108). Animals in PGF groups received 16.8mg (2,5 mL) of dinoprost tromethamine (Lutalyse®; Zoetis) on D29. On D30, all animals had the P4 device removed, and TAI was performed 48h later (D32). Cows were painted with chalk on tailheads, and removal of chalk on the day of TAI was used as an indication of estrus. The pregnancy diagnosis was evaluated by ultrasound 30 days after the second TAI. Statistical analyses were performed using GLIMMIX of SAS 9.4. In Experiment 1, cows with CL at the moment of P4 device removal presented lower (P=0.01) P/IA (52.2%; 268/513) than cows without CL (59.9%; 242/404). In Experiment 2, the diameter of the LF was higher in cows without CL (CL=8.56±0.18 vs. noCL=10.86±0.16mm; P=0.0001). There was no interaction presence of CL*PGF treatment on estrus rate (P=0.97; average of 71.1%). Estrus rate was similar (P=0.32) between cows treated or not with PGF on D29 [noPGF=70.1% (164/234) vs PGF=74.1% (183/247)]. However, the presence of CL decreases (P=0.0004) the estrus rate [CL=64.3% (133/207) vs noCL=77,9% (215/276)]. There was no interaction presence of CL*PGF treatment (P=0.41) on P/IA. The P/AI was higher (P=0.0001) in groups without CL [CL=44.4% (92/208) vs. noCL=63.8% (176/277)]. A tendency for a greater P/IA (P=0.07) was observed in PGF groups [PGF=58.7% (145/247) vs noPGF=52.1% (123/236)]. These results suggest that the presence of CL at the end of the resynchronization protocol (Resynch 22) decreased the estrus rate and the P/IA in Nelore cows. However, no effect of PGF treatment 24 hours before the P4 device removal was observed.

Animal Reproduction

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

TAI/AI

Removal of estradiol treatments from synchronization protocols decrease endometrial thickness and circulating estradiol concentration in *Bos indicus* cows

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This study aimed evaluate the effects of removing estradiol [E2; at the beginning (E2D0) or at end (E2D8) of a FTAI protocol] for synchronization of follicular growth and ovulation in *Bos indicus* cows. A total of 50 suckled cows from a commercial farm in Brazil were used. On day 0 (D0), cows received an intravaginal P4 device (P4D; Primer®, BR) and were randomized according to presence of CL (18%; P=0.54) and BCS (2.9±0.03; P=0.41) to either one of the treatments (2x2): 1) EB/EC: 2mg of estradiol benzoate (EB; RICBE®, BR) on D0 and 1mg of estradiol cypionate (EC; Cipiotec®, BR) on D8 (n=14); 2) EB/GnRH: 2mg of EB on D0 and 25µg of lecirelin (GnRH; TecRelin®, BR) on D10 (n=12); 3) GnRH/EC: 50µg of GnRH on D0 and 1mg of EC on D8 (n=11) and 4) GnRH/GnRH: 50µg of GnRH on D0 and 25µg of GnRH on D10 (n=13), i.m. Cows received 0.530mg of PGF (0,482mg; Estron®, BR) and eCG (300IU; Novormon®, BR) i.m, concomitant with P4D removal (D8). On D8, cows were painted with chalk on their tailheads, and removal of chalk on day 10 (D10) was used as an indication of estrus. The ovaries and endometrium were examined by ultrasonography (US; DP10Vet, Mindray®), every 12h, from D8 until ovulation (96h). Blood was collected (n=20) during every US examination and E2 concentrations were determined (ELISA). The following variables were evaluated: largest follicle diameter (LFD), CL presence at D8 (CLD8), endometrial thickness diameter (ETD), circulating E2 concentration (CE2), estrus detection rate (EDR), ovulation rate (OVR) and time of ovulation. Statistical analyses were performed using PROC GLIMMIX of SAS© 9.4. No interaction (P>0,05) between E2D0*E2D8 was observed for the variables and data was presented by main effects. Cows treated with GnRH on D0 presented greater LFD on D8 (GnRH=13.0±0.6^{a} *vs* EB=10.3±0.6 ^{b} mm; P=0.003) and D10 (GnRH=15.1±0.5^{a} *vs* EB=12.8±0.5 ^{b} mm; P=0.004). No effect was observed in the CLD8 [GnRH= 20.8% (5/24) *vs* EB=11.5% (3/26); P=0.29]. Cows treated with EB presented greater ETD on D8 and D10 [D8) EB=9.7±0.2^{a} *vs* GnRH=9.3±0.2^{b} mm and D10) EB=11.6±0.2^{a} *vs* GnRH=10.4±0.3^{b} mm; P=0.02]. Cows treated with EC presented greater ETD on D8 and D10 [D8) EC=10.4±0.2^{a} *vs* GnRH=9.8±0.2^{b} and D10) EC=11.2±0.2^{a} *vs* GnRH=10.9±0.2^{b}; P=0.03]. Also, cows treated with EC presented higher CE2 on D9 (EC=10.0±2.7^{a} *vs* GnRH=5.7±1.5^{b} pg/mL; P=0.05). Furthermore, the EDR was greater in cows that received EC on D8 [EC=84% (21/25) *vs* GnRH=28% (7/25); P=0.0008]. No differences were observed to OVR [EC=88% (22/25) *vs* GnRH=76% (19/25); P=0.31]. However, cows that received GnRH on D10 ovulated later (GnRH=85.9±2.4 *vs* EC=77.4±3.3 h; P=0.05). The removal of E2 at the beginning of protocol induced a greater LFD (D8/D10), although, with no effect to CLP. Cows treated with GnRH (D0/D10) presented a decrease of ETD and CE2 when compared to cows treated with E2 (D0/D8). Also, the removal of E2 at the end of protocol as an ovulation inducer, reduced the EDR and delayed the time of ovulation.

Animal Reproduction

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

TAI/AI

Factors associated with pregnancy loss in *Bos indicus* Nelore beef cattle

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Currently, the pregnancy loss (PL) in Bos indicus Nelore cattle is a hot topic. Unfortunately, there are very few prospective or retrospective studies which evaluated the factors or mechanisms associated with PL in this genetic group. We investigated several factors that may influence PL of Nelore beef cattle submitted to TAI. Data were retrieved from 2017 to 2023, and a total of 41,528 pregnancies from 36 herds were available for the analysis. Five classes of animals were established: young heifers with age between 11 to 16 mo (YH, n=4,571), heifers with age between 16 to 36 mo (H, n=3,373), young primiparous with age between 20 to 26 mo (YP, n=958), primiparous (P, n=4,177), and multiparous cows (M, n=28,085). All farms had a minimum of 100 pregnancies, and the females received the 1st, 2nd or 3rd TAI of the breeding season. The TAI protocol was E2/P4-based with 7, 8 or 9 d of P4 implant. Statistical analysis was carefully considered due to possible confounding effects (e.g. number of each class of animal within a farm, number of farms and class of animals a sire was used, etc.). The PL was assessed between the pregnancy diagnosis on d ~30 and the final preg check of the breeding season. The experimental approach did not allow testing or inferring the mechanisms, or the biological effects of the factors evaluated on PL. Thus, most of the data will be presented with a descriptive purpose, and the goal was to use this large dataset to help direct future manipulative studies and increase the technician's attention for this hot topic. The PL was above 10% on YH (11.7%) and YP (13.3%), and H had 6.9% of PL. The P had 67% greater PL than M cows (8 vs. 4.8%). One result we highlight is the fluctuation on PL among farms within classes of animals: YH (3.7 to 16.7%), H (2.5 to 16.7%), YP (7.7 to 14.8%), P (3.6 to 17.7%), and M (2.1 to 7.3%). The high variation also was observed among sires within classes of animals: YH (0.3 to 24.2%), H (1.4 to 19.6%), YP (3.2 to 22.0%), P (2.1 to 20.0%), and M (0.0 to 9.9%). We evaluated the PL according to days postpartum (DPP) at TAI in M (the number of observations for YP and P were not powerful enough). After performing a logistic regression for the probability of PL according to DPP, there was no linear or quadratic effect. Moreover, after dividing the M in quartiles (≤45, 46 to 65, 66 to 73, and ≥74 DPP), the PL was very similar among them (5.1, 3.6, 5.9 and 5.9%). The study will stimulate publication in this topic, and we hope the results from this large dataset would increase the interest of technicians, farmers and the academy to improve the measurement and evaluation of PL in commercial beef cattle operations, and to perform more studies to deeply understand some of the fluctuations in PL and the mechanisms associated with it in Nelore beef cattle. Acknowledgements: we are extremely thankful for the many, many farms and technicians that collaborated providing data for the analysis performed in this study.