Reproductive efficiency Nellore and crossbred cows submitted to administration of melengestrol acetate (MGA) after FTAI protocols

Eficiência reprodutiva de vacas Nelore e cruzadas submetidas a administração de acetato de melengestrol (MGA) após protocolos de IATF

Eficiencia reproductiva de vacas Nellore e cruzadas sometidas a la administración de acetato de melengestrol (MGA) después de los protocolos de IATF

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Abstract
This study aimed to evaluate the influence of medroxyprogesterone acetate (MGA) supplementation between the 13th and 20th day after Fixed-Time-Artificial Insemination (FTAI), on the conception and gestation rates of Nellore and crossbred cows (Nellore x Angus), pluriparous and lactating. For the study, 349 Nellore cows and 270 crossbred cows were selected, distributed into 1) group G1 (n=176): Nellore cows submitted to the FTAI protocol, without MGA supplementation; 2) group G2 (n=173): Nellore cows submitted to the FTAI protocol, with supplementation for 7 days of MGA® (0.28g/AU/day) from the 13th post-FTAI; 3) group G3 (n=119): crossbred cows: FTAI protocol, without MGA supplementation; 4) group G4 (n=151): crossbred cows: FTAI protocol followed by MGA supplementation for 7 days (0.28g/AU/day), from the 13th to the 20th day after FTAI. For statistical analysis, the procedures MIXED and GLIMMIX from SAS were used. G3 had higher rates of conception (CR=85.7%) and pregnancy (PR=95.8%) at the end of the breeding season (P<0.05), without the interference of supplementation with MGA (P>0.05). G1 females had a lower conception rate (TC=76.7%) (P<0.05), with the use of MGA® (CR-MGA=59.5%), but similar rates of pregnancy (PR=90.3%; PR-MGA=92.5%) to the G2, at the
end of the breeding season (P>0.05). When G2 and G4 were compared, we found that crossbred females supplemented had higher rates of conception and pregnancy. In conclusion, crossbred animals have higher conception and pregnancy rates at the end of the breeding season. Besides, we found that Nellore females had a lower conception rate, with the use of MGA® Premix.

**Keywords:** Cattle; Conception; Crossbred; Nellore; Exogenous progesterone.

**Resumo**
Este estudo teve por objetivo avaliar a influência da suplementação de acetato de medroxiprogesterona (MGA) entre o 13º e 20º dia após a inseminação artificial em tempo fixo (IATF), sobre as taxas de concepção e gestação de vacas da raça Nelore e cruzadas (Nelore x Angus), pluríparas e lactantes. Para o estudo foram selecionadas 349 vacas Nelore e 270 vacas cruzadas, distribuídas em: 1) G1 (n=176): vacas Nelore submetidas a protocolo de IATF, sem suplementação de MGA; 2) G2 (n=173): vacas Nelore submetidas a protocolo de IATF, com suplementação por 7 dias de MGA® (0,28g/UA/dia) a partir do 13º pós IATF; 3) G3 (n=119): vacas cruzadas: protocolo de IATF, sem suplementação de MGA; 4) G4 (n=151): vacas cruzadas: protocolo de IATF suplementadas por 7 dias de MGA (0,28g/UA/dia), do 13º até 20º dia pós IATF. Para a análise estatística foi utilizado os programas MIXED e GLIMMIX do SAS. G3 apresentaram maiores taxas de concepção (TC=85,7%) e gestação (TG=95,8%) ao final da estação de monta (P<0,05), sem interferência da suplementação com MGA (P>0,05). G1 apresentaram menor taxa de concepção (TC=76,7%) (P<0,05), com o uso de MGA® (TC-MGA=59,5%), mas taxas similares de gestação (TG=90,3%; TG-MGA=92,5%) ao G2, ao final da estação de monta (P>0,05). G2 e G4 suplementadas com MGA apresentaram maior taxa de concepção e gestação. Conclui-se que animais mestiços apresentam maiores taxas de concepção e gestação ao final da estação de monta. Além disso, verificamos que fêmeas Nelore apresentaram menor taxa de concepção, com o uso de MGA® Premix.

**Palavras-chave:** Bovinos; Concepção; Cruzadas; Nelore; Progesterona exógena.

**Resumen**
Este estudio tuvo como objetivo evaluar la influencia de la suplementación con acetato de medroxiprogesterona (MGA) entre el día 13 y 20 después de la Inseminación Artificial de Tiempo Fijo (FTAI), sobre las tasas de concepción y gestación de vacas Nellore y mestizas (Nellore x Angus), pluríparas y lactante. Para el estudio se seleccionaron 349 vacas Nellore y
270 vacas mestizas, distribuidas en 1) grupo G1 (n = 176): vacas Nellore sometidas al protocolo FTAI, sin suplementación con MGA; 2) grupo G2 (n = 173): vacas Nellore sometidas al protocolo FTAI, con suplementación por 7 días de MGA® (0.28g / AU / día) a partir del 13 post-FTAI; 3) grupo G3 (n = 119): vacas cruzadas: protocolo FTAI, sin suplementación con MGA; 4) grupo G4 (n = 151): vacas cruzadas: protocolo FTAI seguido de suplementación con MGA durante 7 días (0,28 g / AU / día), desde el día 13 al 20 después del FTAI. Para el análisis estadístico se utilizaron los procedimientos MIXED y GLIMMIX de SAS. G3 tuvo mayores tasas de concepción (CR = 85,7%) y preñez (PR = 95,8%) al final de la temporada de reproducción (P <0,05), sin la interferencia de la suplementación con MGA (P > 0,05). Las mujeres G1 tuvieron una tasa de concepción más baja (TC = 76,7%) (P <0,05), con el uso de MGA® (CR-MGA = 59,5%), pero tasas similares de embarazo (PR = 90,3%; PR-MGA = 92,5 %) al G2, al final de la temporada de cría (P > 0,05). Cuando se compararon G2 y G4, encontramos que las hembras cruzadas suplementadas tenían tasas más altas de concepción y embarazo. En conclusión, los animales cruzados tienen mayores tasas de concepción y preñez al final de la temporada de reproducción. Además, encontramos que las hembras Nellore tenían una tasa de concepción más baja, con el uso de MGA® Premix.

Palabras clave: Vacas; Concepción; Cruzadas; Nellore; Progesterona exógena.

1. Introdução

According to official information released by the Brazilian Association of Artificial Insemination (Asbia, 2019), only 16% of beef cows are subjected to artificial insemination (AI). Thus, in view of the low adoption of AI, it is imperative to look for strategies to improve reproductive performance and guarantee the economic return and genetic improvement of the Brazilian herd (Torres-Junior, 2009).

In this context, the Brazilian herd is mostly composed of Zebu breeds, with emphasis on the Nellore breed (Bos taurus indicus), which presents greater rusticity, adaptation to heat, and greater resistance to parasites (Rodrigues, et al., 2017). Also, it is a breed adapted to the extensive production system (Baracellos, et al., 2011). However, it has lower sexual precocity than Taurus animals, for example, the Aberdeen Angus breed (Reggiori, et al., 2016). Thus, to improve the productive and reproductive efficiency of herds, breeding biotechniques are used, such as the Fixed-Time Artificial Insemination (FTAI), with the aim of crossing zebu breeds and taurine breeds, for better adaptability to the Brazilian climate (Sousa, et al., 2017). Besides, the advantages of crossbred animals are represented by better weight gain, early age
at first birth, a shorter interval between deliveries, and, mainly, a higher pregnancy rate (Artmann, et al., 2014).

Regarding the hormonal protocols used in FTAI programs, exogenous progesterone after AI has a fundamental role in improving the conception rate (Yamamoto, et al., 2019). Studies show that cows have low progesterone (P4) concentration in the luteal phase after AI and, consequently, embryos with developmental delay (Mann & Lamming, 1999). In this sense, during early pregnancy, embryos must inhibit the luteolytic mechanism to maintain the progesterone secretion necessary for continuous development. Thus, embryos must secrete a protein called interferon tau (IFN-t), which acts locally in the endometrium, inhibiting prostaglandin secretion (PFG2a), inactivating the development of oxytocin receptors in the luminal epithelium (Robinson, et al., 1999; Bazer, et al., 2008), and by inducing an inhibitory prostaglandin synthesis (Thatcher, et al., 1995).

The correct understanding of embryonic development and IFN-t production is of paramount importance in determining strategies to reduce high early embryonic mortality. In addition, progesterone plays an important role in stimulating the production of a variety of endometrial secretions necessary for successful embryo development. Many studies show lower concentrations of progesterone in cows with embryonic mortality (Henricks, et al., 1971; Nyman, et al., 2018). Studies in sheep have shown that low concentrations of progesterone can lead to poor embryonic development (Nephew, et al., 1991) and, in cows subjected to progesterone supplementation, have shown better embryonic development (Garrett, et al., 1988). Thus, studies show that the use of a vaginal implant impregnated with progesterone can be used to improve the fertility of dairy cows after AI. In beef cows, on the other hand, information about the effects of post-AI treatment with a P4 device on fertility is limited.

With this, we report the use of melengestrol acetate (6α-methyl-6-dehydro-16-methylene-17-acetoxyprogesterone: MGA), a synthetic progestational steroid for oral administration, developed from the addition of a methyl radical to the medroxyprogesterone (MAP), with a considerable increase in its pharmacological activity (MAP: 968 mg/day vs. MGA: 0.5 mg/day), to improve the implantation and conception rates of cows (Wood-Follis, et al., 2004). Although several previous studies have demonstrated the effectiveness of MGA for synchronizing the bovine estrous cycle, few studies developed to date have evaluated the effect of this substance as an adjuvant to luteal function when administered to beef cattle submitted to FTAI.
In this context, this work aimed to evaluate the reproductive efficiency of Nellore cows (Bos taurus indicus) and crossbred cows (Nellore - Bos taurus indicus X Angus - Bos taurus taurus) submitted to a fixed-time artificial insemination protocol, supplemented or not with MGA® post artificial insemination.

2. Material and Methods

All procedures performed during the study were approved by the Ethics Committee (Universidade Paranaense, UNIPAR (CEPEEA Protocol: 35552/2018).

The experimental stages were conducted in a single private property located in the municipality of Iguatemi, Mato Grosso do Sul (Latitude 23° 40' 49" S and Longitude 54° 33' 40" W). For this purpose, 619 multiparous and lactating bovine females (average weight of 450 Kg; ECC = 3.5) were used, according to (Ayres, 2009), with an average of 61.4 ± 1.3 days postpartum, being 349 Nellore cows (Bos taurus indicus) and 270 crossbred cows, half Nellore blood and half Angus blood (Bos taurus indicus X Bos taurus taurus).

The animals were handled in an extensive breeding system, in paddocks with brachiaria grass mg5 and mg4, and a central food court, consisting of a drinking fountain with chlorinated water and troughs for mineral supplementation (Fosbovi® 20).

In a period of 20 days, before the beginning of the FTAI protocol, the product Megalac E® (Elanco Brasil, São Paulo, SP) was supplied, together with mineral salt for the animals, at a concentration of 100 g per cow/day.

The cows were randomly assigned to the following treatments:

Group 1 (n = 176): Nellore cows submitted to FTAI protocol, without supplementation with MGA® Premix.

Group 2 (n = 173): Nellore cows submitted to FTAI protocol, with MGA Premix® supplementation (2.28 g/AU/day) for 7 days from the 13th post-FTAI.

Group 3 (n = 119): group composed of crossbred cows (Nellore x Angus) submitted to a conventional FTAI protocol, without progestogen supplementation.

Group 4 (n = 151): crossbred cows (Nellore x Angus) submitted to FTAI protocol, followed by supplementation of MGA® Premix (2.28 g/AU/day) for 7 days from the 13th.

To control the intake of MGA® Premix, the animals were accommodated in individual troughs (average of 1 m / animal unit) and monitored visually during delivery, to assess the intake of the right amount of product. For better palatability, the product was mixed with mineral salt.
All animals underwent the same progesterone-based FTAI protocol on day 0 (D0), with the insertion of an intravaginal implant with progesterone (Fertilcare® Implante 1200 PT®, 1.2 g of P4; MSD Saúde Animal) and administration of 2 mg of Estradiol Benzoate (Benzoato Sincronização®, MSD Saúde Animal, IM). On day 9 (D9), the P4 implant was removed and 4 mg of estradiol cypionate (E.C.P.® Zoetis Saúde Animal; IM), 300 IU eCG (Folligon®, MSD Saúde Animal) and 0.5 mg of prostaglandin PGF2α (Ciosin® MSD Saúde Animal). On day 11 (D11) artificial insemination was performed.

The pregnancy diagnosis was made with the aid of transrectal ultrasonography on day 30 (D30). Those animals with a negative diagnosis at 30 days after AI were again submitted to the FTAI protocol, described above, and a new pregnancy diagnosis was made 30 days after the second AI (D60).

**Figure 1** - Design of the experimental protocol used in Nellore and crossbred cows, supplemented with MGA® Premix.

The results were analyzed by the computer program Statistical Analysis System (SAS Institute Inc., 2001), and the normality of the residues was verified by the Shapiro-Wilk Test (Proc Univariate) and the homogeneity of the variances compared by the Chi-Square Test. The data were submitted to analysis of variance (Proc GLM), considering as main effects the effect of MGA supplementation, in addition to the possible interactions with the ECC and genotype of females synchronized to FTAI.
3. Results and Discussion

It was found that the animals in the Nellore MGA Premix® group had a lower conception rate when compared to the Nellore group (P < 0.05). However, when comparing pregnancy rates between these two groups, no statistical differences were observed (P > 0.05). In the groups of crossbred females, there were no differences, either between conception rates or between pregnancy rates when using MGA Premix® (P > 0.05; Table 1).

When comparing the Nellore and crossbred animals, we found that the Nellore females had a lower conception rate compared to the other groups (P < 0.05), and the crossbred animals, both without and submitted to the administration of MGA Premix®, showed higher rates of pregnancy (P < 0.05; Table 1).

Table 1. Conception (%) and pregnancy rates (%) among the Nellore, Nellore MGA, Crossbred, and Crossbred MGA groups, obtained during the experimental period.

| Group            | Conception rate (%) | Pregnancy rate (%) |
|------------------|---------------------|--------------------|
| Nellore G1       | 76,7\(^a\) (134/176)| 90,3\(^a\) (159/176) |
| Nellore MGA G2   | 59,5\(^b\) (103/173)| 92,5\(^a\) (160/173) |
| Crossbred G3     | 85,7\(^c\) (102/119)| 95,8\(^b\) (114/119) |
| Crossbred MGA G4 | 88,7\(^c\) (134/151)| 98\(^b\) (148/151)  |

\(^{a,b}\) Values followed by different letters in the same column differ from each other (P < 0.05). Source: Authors.

Table 2. Conception (%) and pregnancy rate (%) of control animals (C) and animals treated (T) with MGA Premix®, regardless of genotype, obtained during the experimental period.

| Group     | Conception rate (%) | Pregnancy rate (%) |
|-----------|---------------------|--------------------|
| Control   | 80\(^a\) (236/295)  | 92,5\(^a\) (273/295) |
| Treated   | 73,1\(^b\) (237/324)| 95,1\(^a\) (308/324) |

\(^{a,b}\) Values followed by different letters in the same column differ from each other (P < 0.05). Source: Authors.
Several researchers evaluate the performance characteristics of purebred and crossbred animals, showing that, in general, crossbred animals have higher rates of body development and greater reproductive efficiency (Engle, et al., 2018). Therefore, understanding the differences between zebu and crossbred females is important for the establishment of correct procedures, regarding the management and evaluation of reproductive parameters, for later adoption of adequate reproductive biotechnologies (Pereira, et al., 2015).

Zebu animals have particular reproductive characteristics, which represent a lower performance in relation to Taurus animals (Sá Filho & Vasconcelos, 2011). Studies report that *Bos indicus* animals have smaller follicle size and corpus luteum, smaller amount, and diameter of follicles before follicular divergence, and smaller size of the dominant follicle, thus interfering in ovulatory capacity (Sartorelli, et al., 2005; Sartori, et al., 2001). Also, studies show that *Bos taurus* females have a higher serum progesterone concentration than Zebu females (*Bos indicus*) (Segerson, et al., 1994).

Thus, when using *Bos indicus x Bos taurus* crossbred cows, we observed higher conception and pregnancy rates compared to Nellore cows. This is probably due to the combined advantages of heterosis and adaptation to the tropical climate of crossbred animals, as reported by studies by (Engle, et al., 2018).

The protocols associating MGA and GnRH have been shown to be effective in inducing cyclicity in cows in the postpartum anestrus, with increased estrus expression, conception, and pregnancy rates (Patterson, et al., 2003; Mingoti, et al., 2018; Knickmeyer, et al., 2019). Thus, we found that the FTAI protocol used in the present study brought excellent conception and pregnancy rates since the average conception and pregnancy rates reported in the literature vary between 40 to 70% (Sales, et al., 2019; Dhami, et al., 2015).

According to the particularities between Nellore and crossbred cows, it is justified that the conception and pregnancy rates were higher in crossbred animals, due to the characteristics of the Zebu breeds, which despite being well adapted to the tropics, present late puberty (Nogueira, 2004), long intervals of postpartum anestrus (Abeygunawardena & Dematawewa, 2004) and a lower percentage of fertility Ferreira Junior (2018). Therefore, breeding systems between breeds are used in order to adapt the genotype of the animals more quickly, so that they have good productive and reproductive performance in the most diverse environments (Teixeira, et al., 2006), in addition to the heterosis obtained in crossings (Ferreira, et al., 2013).

Contrasting our results of conception and pregnancy rates in Nellore females, in the work carried out by Aono, et al., (2008) and Silva Junior, et al., (2014) it was found that the
supply of MGA® for seven days (14th to the 20th) after FTAI in Nellore cows provided a higher pregnancy rate.

Another study evaluated the influence of the administration of 100 mg of exogenous progesterone in the first four days of pregnancy on the development and growth of the bovine conceptus and concluded that treated animals had higher plasma concentrations of progesterone at the beginning of pregnancy and more proteins in the uterine fluid to 14 days of gestation, in addition to better embryonic development (Garrett, et al., 1988). These results found may be one of the reasons for the increase in pregnancy rates, with the use of MGA®, in crossbred cows.

Mann & Lamming (1999) reported that the performance of exogenous progesterone supplementation led to a decrease in the rates of embryonic loss, on average 5.0%, and in the protocols in which supplementation was performed before the sixth day of pregnancy, there was an increase in 10% in pregnancy rates. These authors also showed that in herds with a history of embryonic loss, supplementation with progesterone can provide an increase of up to 19% in pregnancy rates. However, in our study, we found that the use of MGA Premix® did not bring benefits to the conception rate at 30 days, but when submitting the animals to the new FTAI protocol, there was a 22% increase in pregnancy rates with exogenous supplementation of progesterone.

As in the present study, Rodrigues, et al., (2018) did not observe an improvement in pregnancy rates in lactating Nellore cows when using the same dosage of MGA/cow/day (2.28 g). However, when providing two days less than in the present study, from days 13 (D13) to 18 (D18) after FTAI, they observed that in the control group there was a pregnancy rate of 42.16% and in the progesterone group of 50%. When using exogenous progesterone from day 5 (D5) to day 10 (D10) after FTAI, they observed a decrease in conception rates when using 2.28 g of MGA/cow/day. This may be due to greater nutritional requirements in this species and, consequently, to the body condition score (BCS) in which the animals are found, which may lead to a low conception rate due to nutritional deficiencies.

Studies on the reproduction of cattle, using cows in the postpartum period, show that the body condition scoring (BCS) is an indicator of the energy condition and cyclicity in these animals (Sartori, et al., 2001). In the present study, we found that all animals maintained an adequate body condition scoring (BCS) (3.5) and did not present weight loss, similar between the four groups (P > 0.05), with an average ECC of 3.5 ± 0.1 and an average weight of 450 ± 1.2 kg. Thus, the total nutrient requirement is of great importance in cattle breeding, especially in the case of larger cows within a breed or between breeds or crossbreeds, since
they produce heavier calves at weaning, but have higher maintenance requirements and normally produce more milk, which increases their nutritional requirements (Ribeiro, et al., 2001).

According to the results obtained in this study, it is concluded that the main advantages of using MGA consist of its easy administration of the product, in which the animals do not need to be handled individually, the cost of the product relatively low when compared with other progestogens and have zero toxicity, in addition to presenting similar results in estrus response and synchronization, conception and pregnancy rates, regardless of the animals' cyclicity (Lamb, et al., 2010). However, there are disadvantages, since MGA intake is not always uniform and constant for all animals, especially when the product is used in grazing systems, without supplementation with concentrate (Martins, et al., 2001). In this work we observe the individual intake of MGA by each animal, being able to verify the amount ingested by all animals.

Treatment with a CIDR® from day 4 to day 19 (day 0 = day of AI) showed an increase in the conception rate of Japanese black cows with constant heat repetitions. However, treatment with a P4 device from days 5 to 14 and days 7 to 20 did not affect the conception rate in beef cows. In dairy cattle, the conception rate has been reported to decrease after post-AI treatment with a P4 device from day 4 to 9 and from day 2 to 9, suggesting that P4 supplementation in the initial period has a potential risk of deleterious effect on corpus luteum (CL) function (Yamamoto, et al., 2018). As in the present study, we used supplementation from the 13th to the 20th post-AI, that is, for a later period, we cannot confirm this hypothesis.

4. Conclusion

It can be concluded that crossbred animals have higher conception and pregnancy rates at the end of the breeding season. Besides, we found that Nellore females had a lower conception rate, with the use of MGA® Premix. When comparing the use of exogenous P4 supplementation, without interference from the studied genotype, we found that the MGA® Premix product did not improve the pregnancy rates of Nellore and crossbred females, that is, the product did not act as adjuvant to luteal function when it was supplied to beef cattle submitted to the FTAI.
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