Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2022v43n6p2643

Reproductive performance of fixed-time artificial insemination in heifers supplemented with a homeopathic complex

Desempenho reprodutivo à inseminação artificial em tempo fixo em novilhas suplementadas com complexo homeopático

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Highlights

The homeopathic product increased the diameter of the dominant follicle on D8.
The homeopathic product increased the diameter of the preovulatory follicle on D10.
Heifers treated with homeopathy had a larger area and CL vascularization score.
The homeopathic product did not influence the antral follicle count in animals.

Abstract

For this study, ½ blood Nelore × Aberdeen Angus heifers (n = 40; 14 months) were divided into two groups: control (n=20; 310 kg) and Pró-Cio (n=20; 304 kg). For Pró-Cio group, 20g of homeopathic product (Pró-cio®, Real H, Campo Grande, Brazil) was supplied together with corn eighteen days prior (D-18) to the beginning of the fixed-time artificial insemination protocol (FTAI). On D0, all females received an intravaginal P4 device and 2 mg BE. On D5, the AFC was assessed by ultrasound and blood was collected for AMH dosage. On D8, P4 device was removed; 300 IU eCG, 0.530 mg of cloprostenol sodium and 0.5 mg of EC were administered; an estrus-identifying adhesive was fixed at the tail insertion and the diameter of the dominant follicle (DF) was measured. On D10, all heifers were inseminated, estrus manifestation was assessed and the preovulatory follicle (POF) was measured. On D20, the CL area was measured and blood flow was assessed using Doppler ultrasound. Data were analyzed by Student, Mann-Whitney or Friedman test. The rates of heat expression, ovulation, and blood flow score with Doppler were analyzed using Fisher's exact test and pregnancy rate by binary logistic regression model (P ≤ 0.05). There was no difference in AFC or AMH

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Received: Oct. 14, 2022 - Approved: Dec. 13, 2022

dosage (P > 0.05). Pró-Cio group had greater diameters of the DF (9.94 ± 0.42 mm) and POF (11.61 ± 0.56 mm) than the controls (DF: 7.72 ± 0.34 mm and POF: 9.91 ± 0.37 mm), as well a larger CL area (3.26 ± 0.26 *versus* 2.35 ± 0.16 cm^2) and a higher mean CL vascularization score (3.06 *versus* 2.26; P < 0.05). There was no significant difference (P > 0.05) between estrus manifestation or conception rate for control and Pró-Cio groups. Heifers supplemented with the homeopathic product showed larger follicular diameters at the end of the FTAI protocol and CL with better blood flow scores compared to the control group.
Key words: Homeopathy. Dominant follicle. Insemination. Corpus luteum. Cattle.

Resumo

Para esse estudo, novilhas ½ sangue Nelore x Aberdeen Angus (n = 40; 14 meses) foram divididas em dois grupos: Controle (n = 20; 310 kg) e Pró-cio (n = 20; 304 kg). Para o grupo Pró-Cio, 20 g/animal de produto homeopático (Pró-cio®, Real H, Campo Grande, Brazil) foi fornecido juntamente com o milho dezoito dias antes (D-18) de inicar o protocolo de inseminaçãoa artificial em tempo-fixo (IATF). No D0, todas as fêmeas receberam um dispositivo intravaginal de P4 e 2 mg BE. No D5, avaliou-se a contagem de folículos antrais (CFA) por ultrassonografia e realizou-se coleta de sangue para dosagem de AMH. No D8, procedeu-se a retirada do dispositivo de P4; aplicação de 300 UI de eCG, 0,530 mg de cloprostenol sódico e 0,5 mg de CE; um adesivo identificador de estro foi fixado na inserção da cauda e foi feita a mensuração do diâmetro do folículo dominante (FD). No D10, todas as novilhas foram inseminadas, foi realizada a avaliação da manifestação de estro e mensuração do folículo pré-ovulatório (FPO). No D20, realizou-se mensuração da área e avaliação do fluxo sanguíneo do CL por ultrassonografia Doppler. Os dados foram analisados pelos testes de Student, Mann-Whitney ou Friedman. As taxas de expressão de estro, ovulação e escore de fluxo sanguíneo com Doppler foram analisadas usando o teste exato de Fisher e taxa de prenhez pelo modelo de regressão logística binária (p ≤ 0,05). Não houve diferença na CFA nem na dosagem de AMH (p > 0,05). O grupo PróCio apresentou maior diâmetro do FD (9,94 ± 0,42 mm) e FPO (11,61 ± 0,56 mm) em relação ao controle (FD: 7,72 ± 0,34 mm e FPO: 9,91 ± 0,37 mm), além de maior área do CL (3,26 ± 0,26 *versus* 2,35 ± 0,16 cm^2; p < 0,05) e maior escore de vascularização médio do CL (3,06 *versus* 2,26; p < 0,05). Não houve diferença (p > 0,05) na taxa de manifestação de estro e nem na taxa de concepção entre os grupos Controle e Pró-Cio, respectivamente. Novilhas suplementadas com o produto homeopático apresentaram maiores diâmetros foliculares ao final do protocolo de IATF e CL com melhores escores de fluxo sanguíneo em relação ao grupo controle.
Palavras-chave: Homeopatia. Folículo dominante. Inseminação. Corpo lúteo. Bovinos.

Introduction

Homeopathy has increased in multiple areas of human and veterinary medicine, mainly in Internal Medicine. Homeopathy has been used for decades as an alternative therapy in veterinary medicine mainly in Europe, as demonstrated by Williamson et al. (1991). Hahn (2013) reported the results of clinical treatments based on homeopathy for reproductive disorders in men and women. More recently, homeopathic products have been used in livestock, but scientific investigations about homeopathy are quite scarce.

The use of homeopathy is based on natural compounds, which stimulate the immune system and have a low cost. Studies have already tested the effectiveness of homeopathy for the prophylaxis of endometritis in cattle and the use of homeopathic products for the treatment of endometritis in cows during the postpartum period and obtained positive results (Arlt et al., 2009). In anestrus cows, homeopathy reduced the interval from treatment to onset of estrus, the service per conception and increased the overall conception rate and serum estradiol concentration compared to the control group (Rajkumar et al., 2006). For herd treatment, the herd is understood as a single organism, where each group has unique characteristics such as race, temperament, and geographical distribution (Souza, 2002).

In beef and dairy cattle fixed-time artificial insemination (FTAI) programs represent one of the main reproductive techniques (Baruselli et al., 2022). This practice aims to synchronize estrus and ovulation in a large number of animals, favoring the optimization of reproductive management and increasing conception rates and reproductive efficiency of the herd (Meneghetti et al., 2009; Wiltbank et al., 2015). Although satisfactory results are already a reality in most FTAI programs, in many situations the reproductive performance is still limited, which points to the influence of factors other as nutritional management, semen, inseminator, sanitary aspects, well-being, and others (Baruselli et al., 2017; Marques et al., 2018).

Considering that reproductive performance is linked to multifactorial issues and that many may result from subclinical disorders, homeopathy can contribute to improvements in the rates of reproductive programs (Rajkumar et al., 2006). This is due to the fact that homeopathy is a science that works on healing, prevention and health promotion, which can contribute not only to better organic homeostasis but also to reducing the stress resulting from disease and guarantee animal welfare in production systems (Souza, 2002).

Another aspect highly related to the efficiency of reproductive programs in cattle is the antral follicle count (AFC). The AFC is also an important indicator because it is related to fertility and has been the focus of several studies (Burns et al., 2005; Ireland et al., 2011; Morotti et al., 2018). AFC is a highly variable characteristic among animals, but it is highly repeatable in the same individual (Ireland et al., 2007), which allows the classification of females into low, medium, or high AFC groups (Morotti et al., 2022, 2018; Silva-Santos et al, 2014). In addition, AFC is strongly correlated with anti-mullerian hormone (AMH) concentrations in both *Bos indicus* and *Bos taurus* (Guerreiro et al., 2014), and can be a reliable method for predicting AFC (Ireland et al., 2011; Rico et al., 2011). To the best of our knowledge, to date, there are no studies that have evaluated the relationship between homeopathic treatment and AFC or serum AMH concentration.

The objective of the present study was to evaluate the effects of supplementation with homeopathic products on the reproductive performance of heifers submitted to FTAI, under the hypothesis that supplementation with the homeopathic product, Pró-Cio, may contribute to follicular development and increase the pregnancy by artificial insemination (P/AI).

Material and Methods

Local

The experiment was conducted in Parana State, Brazil (latitude 23º °22′ 24″ S and longitude 50º °50′ 29″ W at an altitude of 605m). The present study was conducted according to the standards of the Ethics Committee for Animal Experimentation which is based on the Federal Law 11.794 of October 8, 2008, and approved under number 027.2020.

Animal selection, nutritional management, and experimental design

This study involved 40 bovine females (½ Nellore x ½ Aberdeen Angus). They were aged between 13 and 15 months and had an average weight of 300 kg and body condition scores (BCS) between 3.5 and 4.0 (on a scale of 1 to 5) (Houghton et al., 1990). All heifers were pubertal (based on uterine diameter and presence of CL) based on gynecological examination with transrectal ultrasound. AFC was determined (follicles ≥ 3 mm in diameter) based on ultrasound examination.

The animals were grouped 18 days before the beginning of the ovulation synchronization protocol. The heifers were homogeneously distributed according to weight, BCS, and AFC into two groups: control group (untreated animals) and treatment group (animals that received the treatment with the homeopathic product Pró-cio®, Real H, Campo Grande, Brazil). For both groups, 500 g of ground corn per animal was offered daily in a linear trough area of approximately 30 cm per animal (to avoid competition and restriction of consumption by some animals). For the treated group, 20 g of Pró-Cio®/animal/day was added to the ground corn from Day -18 to Day 20.

The heifers were kept in paddocks side by side; the paddocks had similar dry matter and quality of pasture. The heifers also had *ad libitum* access to water and mineral supply, which was already established by the farm. After the treatment period (D-18 to D20), all the heifers were relocated to a large group during the breeding season of the farm.

Ovulation synchronization protocol, estrus detection, and artificial insemination

After an 18-day adaptation period, all the females received an intravaginal progesterone (P4) device (Fertilcare 600®, Vallée, Montes Claros, Brazil) and 2 mg of intramuscular (IM) estradiol benzoate (EB; Fertilcare Benzoato Sincronização®, Vallée). Eight days later (D8), the P4 device was removed, and 300 IU of equine chorionic gonadotropin hormone (eCG; Folligon®, MSD Animal Health, São Paulo, Brazil), 530 µg of sodium cloprostenol (Ciosin®, MSD Animal Health), and 0.5 mg of estradiol cypionate (EC; Fertilcare Ovulation®, Vallée) were administered intramuscularly (Figure 1).

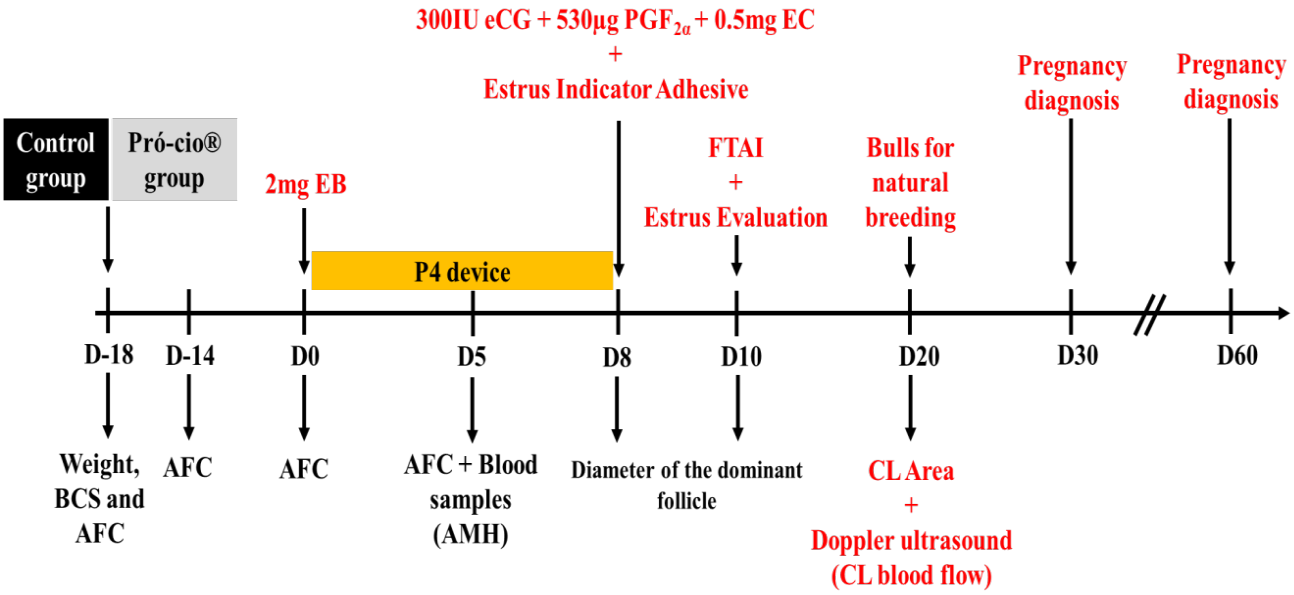

Figure 1. Experimental design and chronology of events.
BCS - body condition scores; AFC - antral follicle count; AMH - Anti-Mullerian hormone; EB - estradiol benzoate; P4 - progesterone; eCG - equine chorionic gonadotropin; $PGF_{2\alpha}$ - prostaglandin/sodium cloprostenol; EC - estradiol cypionate; FTAI - fixed-time artificial insemination; CL - corpus luteum.

At the time of removal of the P4 device, an estrus-identifying adhesive (Fasco Ap®, Alta Genetics, Uberaba, Brazil) was also attached to the sacrococcygeal region. On the tenth day after the beginning of the protocol (D10), the manifestation of estrus was evaluated, and all the heifers were inseminated with conventional semen from a single Brangus bull, as shown in Figure 1. The manifestation of estrus was evaluated by a scale adapted from Nogueira et al. (2019). Briefly, females as high estrus (the adhesive was completely scraped), low estrus (partially scraped) and no estrus (the adhesive was not scraped). Ten days after the treatment (D20), the heifers were exposed to a bull until completing 60 days of the breeding season.

Ultrasound evaluations

Using transrectal ultrasonography (6 MHz linear transducer; A5V, Sonoscape, Shenzhen, China), the ovarian structures, as well as AFC, were evaluated on D-18, D0, and D5 (follicles ≥ 3 mm in diameter). On D8 and D10, the diameter of the dominant follicle was measured.

The area of the corpus luteum (CL) was measured on D20 (10 days after artificial insemination) using B-mode ultrasonography (6 MHz linear transducer; S2V, Sonoscape, Shenzhen, China). During ultrasound scanning, the largest diameter from central area of the CL was identified and measured using the ellipse function of the ultrasound itself. Blood flow was also evaluated using the

Doppler mode (S2V, Sonoscape, Shenzhen, China). The proportion of luteal blood perfusion was determined using a scale of five vascularization scores (0-4) (Pugliesi et al., 2017). Briefly, a score of 0 to 1 (poor or no flow) represents less than 10% of the CL area, a score of 2 represents flow in up to 25% of the CL area, a score of 3 represents flow in 35% or more, but less than 45% of the CL area, and a score of 4 represents flow in more than 50% of the CL area. Scores 3 and 4 were analyzed together as being high flow. The diagnosis of gestation by ultrasonography in mode B was performed 30 days after FTAI, which was confirmed by the identification of the gestational vesicle and viability of the concept (with heartbeat).

Serum collection and anti-Müllerian hormone dosage

On D5 of the synchronization protocol, blood samples were collected using the coccygeal vein puncture method and stored in a thermal container to preserve their properties. The samples were centrifuged at 3000 rpm for 10 min to allow plasma separation. AMH dosing was performed using the AMH kit for cattle (Elisa AL-114, Ansh Labs, Webster, TX, USA), and the concentration was expressed as pg/mL.

Data analysis

Quantitative data were previously analyzed for normal distribution, using the Anderson-Darling test, and homogeneity of variances, using the Levene test. For parametric data, comparisons of the control and treated groups were performed using Student's t-test. The nonparametric data were compared using the Mann-Whitney test (independent variable or unpaired) or Friedman's test (dependent variable). The proportions of cows that expressed estrus, as well as the different blood flow scores of the CL, were analyzed using Fisher's exact test. The pregnancy rate was analyzed using a binary logistic regression model, including treatment as the main effect and the diameter of the dominant follicle, the expression of heat, and the size of the corpus luteum as covariates. The quantitative data are presented as the mean and standard error of the mean (M ± SE), and the qualitative data are presented as percentages. All statistical analyses were performed using the Minitab® 18.1.1 statistical program, and $P \leq 0.05$ denoted statistical significance.

Results and Discussion

Heifers treated with the homeopathic product showed a larger (P = 0.0001) diameter of the dominant follicle on D8 of the FTAI protocol than the heifers of the control group (9.94 ± 0.42 vs. 7.72 ± 0.34 mm). Likewise, the group that received homeopathic treatment had a larger diameter of the dominant follicle on D10 (11.61 ± 0.56 vs. 9.91 ± 0.37 mm; P = 0.016), as shown in Table 1.

The number of antral follicles on D5 did not differ (P = 0.74) across the groups studied (Control Group: 29.1 ± 3.0 vs. Pró-Cio Group 31.4 ± 3.3 follicles); the expression of estrus and ovulation rates were similar (P = 0.54) in the groups (Table 1).

Table 1
Diameter of the dominant follicle, estrus manifestation, luteal body area and pregnancy rate in heifers treated or not with Pró-Cio®

Variables	Control (M ± SE)	Pró-Cio® (M ± SE)	P-value
Number of females (n)	20	20	-
Antral follicle count on D5 (n)	29.1 ± 3.0	31.4 ± 3.3	0.745
Diameter of dominant follicle on D8 (mm)	7.72 ± 0.34	9.94 ± 0.42	0.0001
Diameter of dominant follicle on D10/FTAI (mm)	9.91 ± 0.37	11.61 ± 0.56	0.016
Estrus manifestation			
No estrus (%)	20 (4)	15 (3)	0.66
Low estrus (%)	30 (6)	30 (6)	0.91
High estrus (%)	50 (10)	55 (11)	0.75
Ovulation rate (%)	95% (19/20)	90% (18/20)	0.54
Corpus luteum area 10 days after FTAI (cm^2)	2.35 ± 0.16	3.26 ± 0.26	0.005
Pregnancy rate (FTAI)	55% (11/20)	70% (14/20)	0.20
Pregnancy rate (FTAI + Bull)	65% (13/20)	85% (17/20)	0.084

M = mean
SE = standard error of the mean.

The area of the CL, evaluated 10 days after the FTAI (D20), proved to be larger (P = 0.005) for heifers in the group treated with Pró-Cio (3.26 ± 0.26 cm^2) than in the controls (2.35 ± 0.16 cm^2). However, the pregnancy rate did not differ between the control and treated groups (P = 0.2) (Table 1).

Doppler ultrasonography on D20 demonstrated that the animals in the Pró-cio group had a higher (P = 0.01) proportion of CLs with blood flow scores of 3 and 4 (83.3%; 15/18) than those in the control group (38.9%; 7/18) (Table 2).

Table 2
Corpus luteum blood flow score measured by Doppler ultrasound in heifers treated or not with Pró-Cio®

Blood flow score with Doppler US	Control (N = 18)	Pró-Cio® (N = 18)	P-value
Score 1	11.1% (2)	0	0.486
Score 2	50% (9)	16.7% (3)	0.075
Score 3	38.9% (7)	61.1% (11)	0.318
Score 4	0	22.2% (4)	0.104

The AFC remained constant in all the animals on all the days ultrasound evaluations were performed (Table 3). The dosage values of the anti-Mullerian hormone (AMH) are shown in Table 4, and they were not different (P ≥ 0.05) in the heifers treated and untreated with homeopathic products on any of the days of blood sample collection (D0 and D5).

Table 3
Antral follicle count in heifers treated or not with Pró-Cio®

Days of FTAI protocol	Control (M ± SE)	Pró-cio® (M ± SE)	P-value
Number of females	20	20	-
Day -18 (pre-synchronization)	25.1 ± 2.9	24.0 ± 2.6	0.93
Day 0 (FTAI)	28.3 ± 3.1	31.5 ± 2.5	0.38
P-value	0.38	0.02	
Day -14 (pre-synchronization)	24.1 ± 3.0	30.3 ± 2.9	0.07
Day 5 (FTAI)	29.1 ± 3.0	31.4 ± 3.3	0.74
P-value	0.26	0.63	

M = mean
SE = standard error of the mean.

Table 4
Plasma concentration of Anti-Mullerian hormone (AMH) in heifers treated or not with Pró-Cio® during the fixed time artificial insemination protocol (FTAI)

Days of FTAI protocol	Control (M ± SE)	Pró-cio® (M ± SE)	P-value
Number of females	10	10	-
Day 0	1148.39 ± 248.67	1301.72 ± 214.61	0.64
Day 5	1322.03 ± 241.15	1274.44 ± 210.67	0.88
P-value	0.15	0.46	

M = mean
SE = standard error of the mean.

The results of the present study indicate a more suitable development of the dominant follicle on D8, as well as a greater diameter of the preovulatory follicle on D10. These data are promising because a larger diameter of the dominant follicle is associated with greater pregnancy rates in FTAI (Morotti et al., 2018; Pfeifer et al., 2015; Sá Filho et al., 2010). Follicular growth has been reported to be positively associated with plasma estrogen concentrations (Staigmiller et al., 1982). Studies indicate that a follicle that reaches a larger diameter after the divergence phase has a greater ovulatory potential, which results in a larger CL and greater rates of progesterone (Gimenes et al., 2008; Pfeifer et al., 2009; Sá Filho et al., 2015).

Rajkumar et al. (2006) treated cows in anestrus with a homeopathic complex based on *Calcarea phosphorica 30c, Aletris farinosa 30c, Pulsatilla 30c, Aurum muriaticum natronatum 30c, Sepia 30c*, and *Phosphorus 30c.* The treatment proved to be effective in inducing estrus in the treated cows, and it increased their estrogenic concentrations (20.88 ± 5.60 to 27.80 ± 7.28 pg / mL). The authors of this study attributed the gradual increase in estrogen to the effect of the *Pulsatilla* and *Aletris farinosa* compounds present in the homeopathic complex, which is known to increase estrogenic activity in women (Boericke, 2001).

In our study, using healthy animals, we observed better follicular development, larger CL area, and a greater proportion of CL with high blood flow (scores of 3 and 4). The development of a functional CL capable of maintaining pregnancy depends on the granulosa cells, the amount of LH receptors in the teak and granulosa cells, and the capacity of granulosa cells for synthesizing progesterone after luteinization (Mcnatty et al., 1979). The structure and function of the CL are known to be associated with the characteristics of the dominant follicle (Pfeifer et al., 2009). On the other hand, smaller dominant follicles have fewer cells in the granulosa before ovulation, which results in fewer larger luteal cells in the CLs during their formation (Perry et al., 2007). This may explain the larger diameter of the preovulatory follicle found in cows treated with the homeopathic product, with larger diameters and higher vascularization scores for their CLs.

The larger diameter of the dominant follicle positively influences pregnancy rates; there is a greater ovulatory potential for cows with larger follicles and, consequently, larger CLs (Meneghetti et al., 2009). In the present study, although there were no differences in the ovulatory rates of heifers in the control group and the group that received homeopathy ($P \leq 0.05$), the diameter of the dominant follicle was greater in the treated group.

The heifers did not show differences in the number of follicles on the days of counting; it was similar across the individuals, which reiterates the findings of several studies on AFC in bovine females (Burns et al., 2005; Ireland et al., 2007; Morotti et al., 2018). Cardoso et al. (2018) suggested that there is a high correlation between the population of antral follicles and the measurement of AMH; therefore, the quantification of AMH in plasma can be used to predict AFC. Just as AFC did not differ in the present study, AMH dosages were also similar for the heifers that received the homeopathic treatment and those that did not.

Although this subject needs to be further studied with a greater number of animals, possibly the combination of pharmacological treatments of estrus synchronization and homeopathy can be effective at improving pregnancy rates in IATF, considering the results for the diameter of the dominant and pre-ovulatory follicle and the area and vascularization of CL, which are factors intricately linked to fertility.

Conclusion

Under the conditions of the present study, heifers supplemented with homeopathic product (Pró-Cio®) had dominant follicles with larger diameters on D8 of the protocol, as well as preovulatory follicles with larger diameters on the day of the FTAI. In addition, the heifers that received the homeopathic treatment had a larger corpus luteum diameter and a higher CL vascularization score. This study enables the use of another tool that can contribute to improvements in the reproductive performance of herds, contributing to make livestock more productive and efficient.

Acknowledgments

We thank the Santa Maria Farm for the collaboration in this work.

References

Arlt, S., Padberg, W., Drillich, M., & Heuwieser, W. (2009). Efficacy of homeopathic remedies as prophylaxis of bovine endometritis. *Journal of Dairy Science, 92*(10), 4945-4953. doi: 10.3168/jds.2009-2142

Baruselli, P. S., Santos, G. F. F. D., Crepaldi, G. A., Catussi, B. L. C., & Oliveira, A. C. D. S. (2022). IATF em números: evolução e projeção futura. *Revista Brasileira de Reprodução Animal, 46*(2), 76-83. doi: 10.21451/1809--3000.RBRA2022.007

Baruselli, P. S., Ferreira, R., Colli, M., Elliff, F., Sá Filho, M., Vieira, L., & Freitas, B. (2017). Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. *Animal Reproduction, 14*(3), 558-571. doi: 10.21451/1984-3143-AR999

Boericke, W. (2001). *Pocket manual of homeopathic materia medica* (9a ed.). Indian Books and Periodicals Publishers.

Burns, D. S., Jimenez-Krassel, F., Ireland, J. L., Knight, P. G., & Ireland, J. J. (2005). Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. *Biology of Reproduction, 73*(1), 54-62. doi: 10.1095/biolreprod.104.036277

Cardoso, C. J. T., Oliveira, J. S., Jr., Kischel, H., Silva, W. A. L., Arruda, E. D. S., Souza-Cáceres, M., B., Oliveira, F. A. M., Nogueira, E., Nogueira, G. P., & Melo-Sterza, F. A. (2018). Anti-Müllerian hormone (AMH) as a predictor of antral follicle population in heifers. *Animal Reproduction, 15*(1), 12-16. doi: 10. 21451/1984-3143-2017-AR887

Gimenes, L. U., Sá Filho, M. F., Carvalho, N. A. T., Torres, J. R. S., Jr., Souza, A. H., Madureira, E. H., Trinca, L. A., Sartorelli, E. S., Barros, C. M., Carvalho, J. B. P., Mapletoft, R. J., &

Baruselli, P. S. (2008). Follicle deviation and ovulatory capacity in *Bos indicus* heifers. *Theriogenology, 69*(7), 852-858. doi: 10.1016/j.theriogenology.2008.01.001

Guerreiro, B. M., Batista, E. O., Vieira, L. M., Sá Filho, M. F., Rodrigues, C. A., Castro Netto, A., Silveira, C. R. A., Bayeuxe, B. M., Dias, E. A. R., Monteiro, F. M., Accorsi, M., Lopes, R. N. V. R., & Baruselli, P. S. (2014). Plasma anti-mullerian hormone: an endocrine marker for in vitro embryo production from *Bos taurus* and *Bos indicus* donors. *Domestic Animals Endocrinolgy, 49*(1), 96-104. doi: 10.1016/j. domaniend.2014.07.002

Hahn, R. G. (2013). Homeopathy: meta-analyses of pooled clinical data. *Forsch Komplementmed, 20*(5), 376-381. doi: 10.1159/000355916

Houghton, P. L., Lemenager, R. P., Moss, G. E., & Hendrix, K. S. (1990). Prediction of postpartum beef cow body composition using weight to height ratio and visual body condition score. *Journal of Animal Science, 68*(5), 1428-1437. doi: 10.2527/1990.6851428x

Ireland, J. J., Ward, F., Jimenez-Krassel, F., Ireland, J. L. H., Smith, G. W., Lonergan, P., & Evans, A. C. O. (2007). Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. *Human Reproduction, 22*(6), 1687-1695. doi: 10.1093/humrep/dem071

Ireland, J. J., Smith, G. W., Scheetz, D., Jimenez-Krassel, F., Folger, J. K., Ireland, J. L., Folger, J. K., Ireland, J. L. H., Mossa, F., Lonergan, P., & Evans, A. C. (2011). Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Mullerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. Reproduction, *Fertility and Development, 23*(1), 1-14. doi: 10.1071/RD10226

Marques, M. O., Morotti, F., Lorenzetti, E., Bizarro-Silva, C., & Seneda, M. M. (2018). Intensified use of TAI and sexed semen on commercial farms. *Animal Reproduction, 15*(3), 197-203. doi: 10.21451/1984-3143-AR2018-0070

Mcnatty, K. P., Smith, D. M., Makris, A., Osathanondh, R., & Ryan, K. J. (1979). The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *Journal of Clinical Endocrinology and Metabolism, 49*(6), 851-60. doi: 10.1210/jcem-49-6-851

Meneghetti, M., Sá, O. G., Fº., Peres, R. F. G., Lamb, G. C., & Vasconcelos, G. L. M. (2009). Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: basis for development of protocols. *Theriogenology, 72*(2), 179-189. doi: 10.1016/j.theriogenology.2009.02.010

Nogueira, E., Silva, M. R., Silva, J. C. B., Abreu, U. P. G., Anache, N. A., Silva, K. C., Cardoso, C. J. T., Sutovsky, P., & Rodrigues, W. B. (2019). Timed artificial insemination plus heat I: effect of estrus expression scores on pregnancy of cows subjected to progesterone-estradiol-based protocols. *Animal, 13*(10), 2305-2312. doi: 10.1017/S1751731119000442

Morotti, F., Moretti, R., Santos, G. M. G., Silva-Santos, K. C., Cerqueira, P. H. R., & Seneda, M. M. (2018). Ovarian follicular dynamics and conception rate in *Bos indicus* cows with different antral follicle counts subjected to timed artificial insemination. *Animal Reproduction Science, 188*(1), 170-177. doi: 10.1016/j.anireprosci.2017.12.001

Morotti, F., Miguez-Gonzalez, S., Cerezetti, M. B., & Seneda, M. M. (2022). Evaluation of three classification methods of antral follicle count and fertility to the timed artificial insemination in cattle. *Animal Reproduction, 19*(1), 1-12. doi: 10.1590/1984-3143-AR2021-0121

Perry, G. A., Smith, M. F., Roberts, A. J., Macneil, M. D., & Geary, T. W. (2007). Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *Journal of Animal Science, 85*(3), 684-689. doi: 10.2527/jas.2006-519

Pfeifer, L. F. M., Castro, N. A., Melo, V. T. O., Neves, P. M. A., Cestaro, J. P. A., & Schneider, A. (2015). Timed artificial insemination in blocks: a new alternative to improve fertility in lactating beef cows. *Animal Reproduction Science, 163*(1), 89-96. doi: 10.1016/j.anireprosci.2015.10.002.

Pfeifer, L. F. M., Mapletoft, R. J., Kastelic, J. P., Small, J. A., Adams, G. P., Dionello, N. J., & Singh, J. (2009). Effects of low versus physiologic plasma progesterone concentrations on ovarian follicular development and fertility in beef cattle. *Theriogenology, 72*(9), 1237-1250. doi: 10.1016/j.theriogenology.2009.07.019

Pugliesi, G., Rezende, R. G., Silva, J. C. B., Lopes, E., Nishimura, T. K., Baruselli, P. S., Madureira, E. H., & Binelli, M. (2017). Uso da ultrassonografia Doppler em programas de IATF e TETF em bovinos. *Revista Brasileira de Reprodução Animal, 41*(1), 140-150.

Rajkumar, R., Srivastava, S. K., Yadav, M. C., Varshney, V. P., Varshney, J. P., & Kumar, H. (2006). Effect of a homeopathic complex on oestrus induction and hormonal profile in anoestrus cows. *Homeopathy, 95*(3), 131-135. doi: 10.1016/j.homp.2006.03.002

Rico, C., Medigue, C., Fabre, S., Jarrier, P., Bontoux, M., Clement, F., & Monniaux, D. (2011). Regulation of antiMullerian hormone production in the cow: a multiscale study at endocrine, ovarian, follicular, and granulosa cell levels. *Biology of Reproduction, 84*(3), 560-571. doi: 10.1095/biolreprod.110.088187

Sá Filho, M. F., Nasser, L. F. T., Penteado, L., Prestes, R., Marques, M. O., Freitas, B. G., Monteiro, B. M., Ferreira, R. M., Gimenes, L. U., & Baruselli, P. S. (2015). Impact of progesterone and estradiol treatment before the onset of the breeding period on reproductive performance of Bos indicus beef heifers. *Animal Reproduction Science, 160*(1), 30-29. doi: 10.1016/j.anireprosci.2015.06.024

Sá Filho, M. F., Crespilho, A. M., Santos, J. E. P., Perry, G. A., & Baruselli, P. S. (2010). Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. *Animal Reproduction Science, 120*(1-4), 23-30. doi: 10.1016/j.anireprosci.2010.03.007

Silva-Santos, K. C., Santos, G. M., Koetz, C., Jr., Morotti, F., Siloto, L. S., Marcantonio, T. N., Urbano, M. R., Oliveira, R. L., Lima, D. C. M., & Seneda, M. M. (2014). Antral follicle populations and embryo production in vitro and in vivo of Bos indicus-taurus donors from weaning to yearling ages. *Reproduction in Domestic Animals, 49*(2), 228-232. doi: 10.1111/rda.12255

Souza, M. F. (2002). Homeopatia veterinária. *Anais da Conferência Virtual Global sobre Produção Orgânica de Bovinos de Corte,* Corumbá, MS, Brasil, 1.

Staigmiller, R. B., England, B. G., & Webb, R. (1982). Estrogen secretion and gonadotropin binding by individual follicles during estrous cycle. *Journal of Animal Science, 55*(6), 1473-1482. doi: 10.2527/jas1982.5561473x

Williamson, A. V., Mackie, W. L., & Craford, W. J. (1991). A study using sepia 200C given prophylatically postpartum to prevent anoestrus problems in the dairy cow. *Br Homeopathic Journal, 80*(3), 149-156. doi: 10.1016/S0007-0785(05)80226-1

Wiltbank, M. C., Baez, G. M., Cochrane, F., Barletta, R. V., Trayford, C. R., & Joseph, R. T. (2015). Effect of a second treatment with prostaglandin F2alpha during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. *Journal of Dairy Science, 98*(12), 8644-8654. doi: 10.3168/jds.2015-9353