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Factors which interfere in the results of the Fixed-Time Artificial Insemination in beef cattle.

Fatores que interferem nos resultados da Inseminação Artificial em Tempo Fixo em bovinos de corte.

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### Abstract

The objective of this study was to evaluate the effect of the animal category, the cyclicity, the CIDR reuse, the body condition score (BCS), the inseminator and the bull on the fertility rates of bovine submitted to a FTAI protocol. It was observed that pluriparous demonstrated a higher fertility. The group With Corpus Luteum obtained a higher conception rate. The CIDR of  $4^{th}$  use reduced (P < 0.05) the chance of conception. The chances of conception in cows of the Low BCS group were lower (P < 0.05), than the cows with intermediate and high BCS. The inseminator and the bull exerted influence on the fertility. Therefore, all factors are important in FTAI programs.

**Keywords:** Bos taurus indicus. Synchronization protocol. Conception rate.

#### Resumo

Objetivou-se avaliar o efeito da categoria animal, da ciclicidade, da reutilização do CIDR, do escore de condição corporal (ECC), inseminador e touro sobre os índices de fertilidade submetidas à IATF. De 8.496 fêmeas submetidas a um protocolo de IATF, observou-se que pluríparas demonstraram uma fertilidade superior. O grupo C/CL obteve taxa de concepção superior. O CIDR de  $4^{\circ}$  utilização reduziu (P < 0,05) a chance de concepção. As chances de concepção de vacas do grupo Baixo ECC foi menor (P < 0,05), que as vacas com ECC intermediário e alto. O inseminador e o touro exerceram influência na fertilidade. Assim, todos fatores devem ser levados em consideração nos programas de IATF.

Palavras-chave: Bos taurus indicus. Protocolo de sincronização. Taxa de concepção.

#### Introduction

In modern production systems, the insertion of reproductive biotechnology becomes essential to increase the production rates in the rural farms. Among these biotechnologies, is highlighted the fixed-time artificial insemination (FTAI). This technique corresponds to a hormonal protocol, based on progesterone (P4), estrogen, equine chorionic gonadotropin (eCG) and prostaglandin F2 alpha (PGF2 $\alpha$ ), designed to control follicular and luteal function, promoting follicular wave synchronization and ovulation induction, with subsequent artificial insemination in a predetermined period, without the need for estrus detection (VASCONCELOS et al., 2011).

The technological improvement of FTAI has provided advances in reproductive management, making the technique increasingly used in commercial farms in Brazil. According to ASBIA (2019), the inseminated Brazilian herd increased from 5.8% in 2002 to almost 16% of the herd in 2019, based on data on the amount of semen sold and the total number of cows suitable for reproduction.

Despite the evolution achieved with the employment of FTAI, the conception rates are stationed at 50%. It should be noted that these limited fertility rates can be justified by the fact that a high number of factors interfere in the results of this biotechnology (RODRIGUES et al., 2018).

Following this line of research, Sá Filho et al. (2009) carried out work in several farms in Brazil, determining the influence of the breed, the body condition score (BCS), the animal category, the bull and the artificial insemination technician on the results of FTAI programs, establishing that these factors may be responsible for the reproductive performance of animals submitted to this biotechnology and highlighted the bull and artificial insemination (AI) technician as critical variables that affect the reproductive rates in protocols for FTAI. Therefore, it is necessary to determine the effect of these factors on the results of FTAI programs, so that improvements and adjustments are implemented to this reproductive biotechnology (VIANA et al., 2015).

Therefore, the objective of this study was to evaluate the effect of the animal category, the cyclicity, the CIDR reuse, the BCS, the inseminator and the bull on the fertility rates of bovine females submitted to a FTAI protocol.

#### Material and methods

The study was carried out by means of the data analysis of a Technical Assistance in Bovine Reproduction company, located in the municipality of Montes Claros (16° 41' S and 43° 50' W), in the north of Minas Gerais, during the period of breeding season, from October 2017 to March 2018.

Information was used from 8.494 female bovines of different categories, breeds and average BCS of  $2.9 \pm 0.42$ , on a scale of 0 to 5, visually evaluated at the beginning of the protocol for FTAI (WRIGHT et al., 1986). All animals were kept on pasture with mineral supplementation and water *ad libitum*. Before starting the experiment, the females were submitted to a reproductive evaluation using transrectal ultrasonography with a 5Mhz linear transducer (DP10 Mindray, Shenzhen, China). The pattern of cyclicity was determined by classification into two groups according to the presence (W/CL) or absence of corpus luteum (Wo/CL). Only data from animals with no record of reproductive abnormalities were used for analysis.

The cows were submitted to the following synchronization protocol of estrus and ovulation: on a random day of the estrous cycle, called day zero (D0), the application of 2 mg of Estradiol Benzoate IM (Gonadiol®, Zoetis, São Paulo, Brazil) was performed and the intravaginal implant of

progesterone (CIDR®, Zoetis, São Paulo, Brazil) from first to fourth use was inserted; on day seven (D7), 12.5 mg of dinoprost trometamine IM (Lutalyse®, Zoetis, São Paulo, Brazil) was administered; on day nine (D9) the CIDR were removed and 0.6 mg of estradiol cypionate IM (ECP®, Zoetis, São Paulo, Brazil) and 300 IU of Equine Chorionic Gonadotrophin (Novormon®, Zoetis, São Paulo, Brazil) were administered and, finally, on day eleven (D11), artificial insemination was performed by 16 different AI technicians. For this purpose, commercial cryopreserved semen from 54 Nellore bulls was used. At the time of insemination, the semen was thawed at a temperature of 37°C for 30 seconds. The conception rate was diagnosed at 40 days after artificial insemination by transrectal ultrasonography with a 5 Mhz linear transducer (DP10 Mindray, Shenzhen, China). Being considered a positive gestation diagnosis, animals with the presence of a visible embryo with confirmed viability (heartbeat).

The data were divided into groups according to the animal category, the cyclicity, the reuse of the CIDR, the body condition score, the breed, the bull and the inseminator.

Regarding the animal category the data were divided into heifer, primiparous, pluriparous and single cow; being the term heifer designated for bovine females that never calved; primiparous cows for those that calved only once; pluriparous cows for those that calved more than once and single cows for those that were not pregnant and/or lactating and have calved more than once. The cyclicity was determined by the condition of the ovary being classified as With Corpus Luteum (W/CL), the animals with presence of corpus luteum (CL) and Without Corpus Luteum (Wo/CL), those with absence of CL, observed through transrectal ultrasonography, with a linear transducer of 5 Mhz (dp10 Mindray, Shenzhen, China) in the D0 of the FTAI protocol. Regarding the reuse of the CIDR, data were allocated according to the number of times the device was used, being distributed in four groups with first use (CIDR 1), second use (CIDR 2), third use (CIDR 3) and fourth use (CIDR 4) implants. The body condition score was classified from 1 to 5, with intervals of 0.25, being categorized as low (BCS: <2.5), intermediate (BCS: 2.5-3.0) and high (BCS: >3.0). Regarding the breed the animals were segmented in Nellore, Mixed-breed and Guzerá. Semen from 54 different bulls were used and 16 trained technicians performed the insemination procedure.

The animals were grouped according to the animal category, the cyclicity, the reuse of the CIDR, the body condition score, the breed, the bull and the inseminator, to verify the effect of these characteristics of interest on the rate of conception, and the binary logistic regression analysis was used. After this analysis the univariate chi-square test was performed to determine the effect of each of the variables under studied on the conception rate, for both analyses the software Statistical Package for Social Science (SPSS, version 19) with P<0.05 was used.

# Results and discussion

The fertility rates according to category, cyclicity, use of CIDR and the BCS are in Table 1. When analyzing the data obtained, it is observed that the fertility rates were impacted by the BCS, by the presence of CL and according to the reuse of the CIDR.

In logistic regression analysis there was no significant difference between the primiparous, pluriparous, single and heifer categories in relation to the conception rate (P>0.05). However, in the chi-square test, distinctions between primiparous and pluriparous categories were observed.

Similar results were demonstrated by Sá Filho et al. (2009), who developed a work evaluating the effect of the animal category on fertility rates in female Nellore bovines and obtained significant

differences for conception rates with primiparous females presenting rates of 45.2% (3924/8677) and pluriparous females showed values of 51.8% (24,245/46,767).

In studies similar to this research, Grilo et al. (2015), Sá Filho et al. (2009) and Batista et al. (2012) also found that Nellore primiparae had a lower reproductive performance at FTAI than that shown by other categories. These authors associated the low performance of the primiparae compared to other categories to low BCS, being justified by the association of body growth factors, first lactation and stress at first delivery, reinforcing the need for a strategic supplementation for heifers, with the objective that they have a better BCS when primiparae and can reduce the interference of these factors.

Table 1 - Conception rate of Nellore cows submitted to FTAI protocol according to the animal category, the cyclicity, the CIDR reuse and the body condition score.

Parameters	Percentage (%)	Odds ratio	Confidence Interval	P-value
Animal Category				
Heifer	55.44 (392/707)			
Primiparous	51.23 (458/894)	ns	ns	0.09
Pluriparous Single	57.59 (3462/6011) 55.00 (330/600)			
Corpus luteum				
Without corpus luteum	55.3 (392/707) <sup>b</sup>	1.17	1.068-1.285	0.001
With corpus luteum <i>CIDR</i>	59.14 (1586/2682) <sup>a</sup>			
CIDR 1 <sup>(1)</sup>	57.9 (2043/3524) <sup>a</sup>			
CIDR 2 <sup>(2)</sup>	55.7 (1379/2473) <sup>ab</sup>			
CIDR 3 <sup>(3)</sup> CIDR 4 <sup>(4)</sup>	55.5 (9011/1623) <sup>ab</sup> 54.34 (473/874) <sup>b</sup>	0.807	0.693-0.940	0.006
Body condition score				
Low BCS <sup>(5)</sup>	52.9 (1563/2952) <sup>b</sup>			
Intermediate BCS <sup>(6)</sup> High BCS <sup>(7)</sup>	58.5 (2914/4981) <sup>a</sup> 57.2 (321/561) <sup>a</sup>	1.227	1.117-1.348	0.001

Values followed by distinct letters "a, b and c" for the same variable differ from one another P<0.05, through the chi-square test. (1) CIDR 1: first use implants. (2) CIDR 2: second use implants. (3) CIDR 3: third use implants. (4) CIDR 4: fourth use implants. (5) Low BCS= animals with body condition score  $\leq$ 2.5. (6) Intermediate BCS= animals with body condition score between 2.5 and 3.0. (7) High BCS= animals with body condition score >3. ns= non-significant.

With regard to cyclicity, the W/CL group outlined a significantly higher conception rate than that presented by the Wo/CL group (Table 1). It was also observed that the presence of corpus luteum increased the chances of conception by 1.17 times (P <0.05), compared to cows that did not have corpus luteum at the beginning of the FTAI protocol. Similar results were found by Batista et al.

(2017) suggesting that the determination of the functional activity of the ovary may be important before starting an estrus synchronization protocol.

Previous gynecological screening based on the evaluation of cyclicity may contribute to the increase in conception rates by adjusting the best FTAI protocol according to ovarian function (GRILLO et al., 2015). With hormonal manipulation it is possible to exclude the influence of CL, by the application of PGF2 alpha at a more appropriate moment in the protocol, as was done in this study, which allowed a prolongation of the proestrus and a more appropriate endocrine environment in cyclic females, thus determining a greater functionality of the dominant follicle and favoring a new ovulation (RODRIGUES et al., 2018).

The  $4^{th}$  use CIDR provided a lower fertility index than the first use CIDR, while the  $2^{nd}$  and  $3^{rd}$  use CIDR were indifferent to the first use CIDR as outlined in Table 1 and Figure 1. It was observed that the  $4^{th}$  use CIDR reduces by 0.807 times (P <0.05) the chance of conception when compared to the  $1^{st}$  use CIDR.

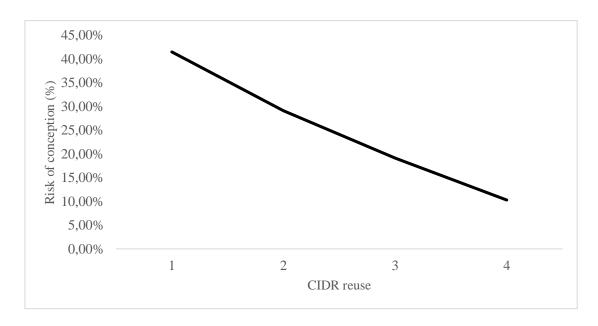


Figure 1 - Risk of conception of Nellore females in a FTAI protocol according to the CIDR use. CIDR 1: first use implants, CIDR 2: second use implants, CIDR 3: third use implants and CIDR 4: fourth use implants.

The CIDR reuse is a practice that reduces the cost of the FTAI protocol, and this technique has been tested by several research groups. In the result of the study by Carvalho et al. (2019) there was no significant difference between the groups that reused CIDR up to 4 times, on the fertility in an FTAI program in bovine females. However, similarly to the present study Gottschall et al. (2012) employed synchronization protocols in beef cows and found that the CIDR should be reused only twice, without generating negative impacts on the conception rates. However, it is important to note that in both studies the number of animals evaluated was limited, compared to this study.

The conception rate was affected by the body condition score. Cows in the Low BCS group had a lower conception rate (P < 0.05) than those presented by animals in the intermediate and high ECC group as outlined in Table 1 and Figure 2. The chances of conception of the cows in the low

BCS group are 1.227 times lower (P < 0.05) than those cows in the intermediate BCS group. Corroborating with several works demonstrating that cows with BCS >2.5 have a higher fertility rate ( $S\acute{A}$  FILHO et al., 2009; GOTTSCHALL et al., 2012; TORRES et al., 2015; FERREIRA et al., 2013; PEREIRA et al., 2018).

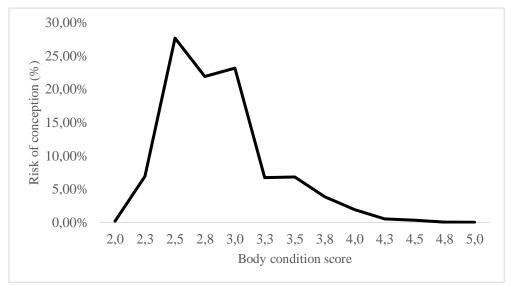


Figure 2 - Risk of conception after FTAI in Nellore females according to the body condition score.

The BCS is responsible for variations in reproductive performance of cows submitted to the FTAI protocol, directly affecting conception rates. This result may be associated with negative energetic balance, which influences the estrous cycle and the response to the protocol (SÁ FILHO et al., 2009; D'OCCHIO et al., 2018). Ferreira et al. (2013) observed that cows with BCS below 2.5 have low conception rates. Therefore, the visual evaluation of the BCS should be used as a tool to define supplementation strategies associated with hormonal manipulations, with the formation of lots in accordance with the BCS, in addition to adjustments in the BCS of the animals to be inserted in the synchronization program and consequently the promotion of improvements in the reproductive rates of the herd.

In figure 2 it can be observed that animals with BCS above 3.5 demonstrated minimal risk of conception. In this context, Gottschall et al. (2005) in their studies also observed that very high BCS can compromise the reproductive efficiency of the herd, leading cows to present difficulties in calving, increase in the neonatal losses, weight reduction at weaning and increased nutritional costs.

There was no interference of the breed of animals on the conception rate (P >0.05). Whereas the results found by Sá Filho et al. (2009), in a research evaluating FTAI protocols, observed that fertility differed between *Bos taurus taurus*, *Bos taurus indicus* and *Bos taurus x Bos taurus indicus* crossbred cows, where *Bos taurus taurus* cows had a higher conception rate than the others. There are particularities within the *Bos taurus indicus* group that must be considered for the choice of the best protocol, and therefore the achievement of better results (SÁ FILHO et al., 2010). However, in the present work there was no effect of the breed on the conception of bovine females, and it can be justified by the discrepancy in the number of animals per group where there was a great predominance of Nellore cows 96.4% over the crossbred 3.4% (*Bos taurus taurus x Bos taurus indicus*) and Guzerá cows 0.2%, besides the presence only of zebu and mixed-breed animals in the evaluation.

Regarding the inseminator, 16 AI technicians were evaluated, and influence on fertility rates (P < 0.05) was observed, with a 1.02-fold increase in the conception rate among the 16 AI technicians. Corroborating the study by Sá Filho et al. (2009), who evaluated data from more than 64 thousand animals observed that the AI technician can critically influence the conception rate. Soethe et al. (2014) also highlighted the importance of using a skilled workforce to achieve a higher conception rate. The authors associated low performance with experience time, in a way that with the increase of the experience time, the skill of the technician and the results of FTAI would also improve.

Regarding the bull, frozen semen from 54 different Nellore sires was used, where there was interference on the conception rate (P<0.05), with an increase of 1.00 times in the chances of conception among the bulls. These findings corroborate those reported by Sá Filho et al. (2009), who showed that fertility rates were consistently affected by the bull. The work attests that semen quality is directly related to satisfactory results.

Fertilization is a complex process that involves several reactions and factors, and the spermatic parameters must be within those considered ideal by the Brazilian College of Animal Reproduction (CBRA) (HENRY et al., 2013) for the semen to be commercialized. It is of great importance that the semen used in FTAI has a good viability, to resist long periods in the female genital tract, in addition, spermatozoa require a period of capacitation, which occurs after ejaculation and before the acrosome reaction and fertilization of the egg, being this stage of great importance so that the spermatozoa can successfully ascend in the reproductive tract to the site of fertilization (LARSSON et al., 2000; JANUSKAUSKAS et al., 2001; BRAUNDMEIER et al., 2001). However, Oliveira et al. (2014), state that the evaluation of sperm parameters of morphology and progressive sperm motility, as well as some complementary tests, are insufficient to prognosticate the fecundating potential of bovine semen batches.

However, following the whole methodology for performing the andrological examination there may be individual variations among the bulls, which may impact the results of the FTAI programs. Although techniques and equipment are increasingly advanced, sperm characteristics may be altered during the cryopreservation process, and there may be variations between lots. Moreover, during the evaluation, the semen is analyzed based on the average, taking into account that the ejaculate is a homogeneous sample, but it is already known that the ejaculate has heterogeneous subpopulations, both in morphology and kinetics, and may be related to the change in individual performance of the bull, affecting the final results, and the application of additional techniques for semen evaluation helps to improve quality control (FLOREZ-RODRIGUEZ et al., 2014).

## Conclusion

Primiparous cows have a lower reproductive performance than the other categories, being necessary a strategic supplementation still when heifers, so that they can provide adequate reproductive performance after the first delivery. Regarding the cyclicity, cows that have the presence of the corpus luteum at the beginning of the FTAI protocol have a greater chance of conception. The CIDR must be used for a maximum of three times during the execution of the synchronization technique. Cows with low BCS have lower conception rates than cows with intermediate and high BCS, and strategies should be established to improve the BCS of breeding animals. The AI technician and the bull influenced the results of the FTAI, and experienced technicians and bulls with high viability semen should be used during the execution of the technique.

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