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ANIMAL REPRODUCTION

# One or two cyclicity inductions on TAI pregnancy rate and pregnancy loss in precocious Nellore heifers

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**ABSTRACT**: The objective was to evaluate the pregnancy rate and pregnancy loss of precocious Nellore heifers submitted to one or two previous cyclicity inductions to TAI (timed artificial insemination). A total of 572 Nellore nulliparous aged 12 to 14 months-old were used, divided into two treatments: on D-50, T1- one cyclicity induction (n = 313): 1 ml of 0.9% NaCl and T2- two cyclicity induction (n = 259): 1 ml of injectable progesterone. From that date, the two groups received the same management as follows: On D-26, oral supply of 2.28 g/head/day (MGA® PREMIX) for twelve days and on D-12, IM application 0.6 mg of estradiol cypionate (E.C.P.®) in all. Subsequently carried out the TAI protocol and pregnancy diagnosis was performed on D41 and pregnancy confirmed on D101. The uterus and ovary were evaluated in D-50, D-26, and D0. There was no difference in pregnancy rate and pregnancy loss ($P > 0.05$), besides in T2, better grades of uterine and ovarian scores were observed between the beginning of cyclicity induction and D0 ($P < 0.05$). The two-induction group had better uterus and ovary scores, which did not interfere with pregnancy and pregnancy loss rates.
**Key words**: inductions, progesterone, pregnancy loss, precocious.

## Efeito de uma ou duas indução de ciclicidade na taxa de prenhez à IATF e na perda gestacional de novilhas nelore precoces

**RESUMO**: O objetivo foi avaliar a taxa de prenhez e perda de prenhez de novilhas Nelore precoces submetidas a uma ou duas induções prévias à IATF (inseminação artificial em tempo fixo). Foram utilizadas 572 nulíparas nelore com idade entre 12 e 14 meses de idade, divididas em dois tratamentos: no D-50, T1- uma indução (n = 313): 1 ml de NaCl 0,9% e T2- duas induções (n = 259): 1 ml de progesterona injetável. A partir dessa data, os dois grupos receberam o mesmo manejo: no D-26, administração oral de 2,28 g/cabeça/dia (MGA® PREMIX) por doze dias e no D-12, aplicação IM de 0,6 mg de cipionato de estradiol (E.C.P.®) em todos. Posteriormente foi realizado o protocolo de IATF e o diagnóstico de gestação foi realizado no D41 e a gestação confirmada no D101. O útero e o ovário foram avaliados em D-50, D-26 e D0. Não houve diferença na taxa de prenhez e perda gestacional ($P > 0,05$), apesar de observadas melhores notas dos escores uterino e ovariano entre o início da indução e o D0 no tratamento T2 ($P < 0,05$). O grupo das duas induções teve melhor desenvolvimento de útero e ovário, não interferindo na taxa de prenhez e perda gestacional.
**Palavras-chave**: induções, progesterona, perda gestacional, precoces.

## INTRODUCTION

The Brazilian commercial cattle herd is approximately 215 million head, considered the largest in the world (IBGE, 2019). As a result, reproductive biotechnologies have emerged in this sector to increase reproductive efficiency, among which artificial insemination (AI) and timed artificial insemination (TAI) are the most widely used (FERRAZ et al., 2008). The main advantage of TAI is that it synchronizes estrus, eliminating the problems of conventional insemination such as failures to observe estrus. This makes it possible to inseminate a greater number of animals and reduces postpartum anestrus (NOGUEIRA et al., 2019).

In tropical countries, biotechniques are used during a restricted period of the year called the breeding season, a period when there is a greater supply of food and consequently, greater cyclicity, but for heifers this time becomes restricted, requiring these animals to attain puberty earlier in order to have good conception results. This was confirmed

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by FUNSTON et al. (2012) who observed a higher pregnancy rate in heifers with a corpus luteum at the beginning of the breeding season, (i.e., females at puberty).

In order to reach puberty earlier, hormonal protocols have been developed for heifers, resulting in an increase in the herd's reproductive rates. The most commonly used hormones are progesterone and estrogen, which are responsible for inducing ovulation and altering the hypothalamic-pituitary axis (SANTOS et al., 2018). The pregnancy rate of cyclicity induced animals compared to non-induced animals has shown better results according to ARAUJO et al. (2019) who demonstrated ovulation rates of 82.22% in prepubertal heifers and studies by This is explained by RIBEIRO et al. (2020) who, when carrying out studies with heifers in two consecutive reproductive seasons, observed pregnancy rates for induced females of 53.2 and 54.2% and for non-induced females of 45 and 46%. Noting that progesterone has a positive effect and according to MAULEON (1974) progesterone administered over a long period have the power to induce cycle synchrony with up to 98% success. This trait is predicted by several breeding programs that select animals based on weaning weight, age at puberty, age at first conception and age at first calving (OLIVEIRA et al., 2007; FERRAZ & ELER, 2007).

Therefore, by associating selection for sexual precocity with hormonal induction, hypotheses have arisen about the number of inductions to be carried out on heifers, knowing that the results of one induction are already satisfactory for this category, now two may or may not improve ovulation rates, pregnancy, gestational losses and decrease the age of cyclicity of *Bos indicus*, which begins at around 22 to 36 months (MALHADO et al., 2013).

Progesterone vaginal devices, injectable P4 or MGA (Melengestrol Acetate - an orally-delivered synthetic progestational steroid) are options as inducers, as they provide a supply of progesterone, improving uterine function and ovulation (BERTAN et al., 2005; SÁ FILHO et al., 2007; SILVA JUNIOR et al., 2014) and the induction supply of this compound has proved beneficial in improving the pregnancy rate according to studies carried out by NOGUEIRA et al. (2021), who observed a higher pregnancy rate at end of breeding season in the group treated with MGA associated with estradiol cypionate compared to the control group, 74.4% and 56.6%, respectively.

In view of the above, this study evaluated the effect of one or two cyclicity inductions on the pregnancy rate and pregnancy loss and the effect on the development of the reproductive tract in precocious Nelore heifers. Therefore, that heifers receiving two hormonal inductions will exhibit a higher pregnancy rate and lower pregnancy loss compared to those receiving a single cyclicity induction. Additionally, expect that two inductions will result in enhanced reproductive tract development, as reflected by improved uterine and ovarian scores.

## MATERIALS AND METHODS

The study was conducted in Mato Grosso do Sul, Brazil (20°24'02.0"S 1935 56°18'11.2"W). A total of 572 nulliparous Nellore heifers with weight = 238.02 ± 20.58 and BCS = 3.01 ± 0.13, from 12 to 14 months of age, kept in 3 management lots, were evaluated. All animal procedures were approved by the Animal Care and Use Committee of the EMBRAPA (Protocol 02-2015), Brazil.

The pastures were *Urochloa brizantha* cv. Marandu, using the rotational grazing method, water *ad libitum* and protein-energy supplementation (PROBEEF URÉIA ADT), plus a mixture with ground whole corn, and the indicated consumption 3 g/kg of LW (0.3%), always supplied at the same time.

*Reproductive management*

The experiment started from November 2020 to January 2021, 50 days before the beginning of the TAI protocol and ended 90 days after insemination (D-50 to D101). There were 4 lots, with the two treatments being carried out on all the lots and the heifers were randomly assigned to the treatment: T1) Control group (n= 313) one cyclicity induction: IM injection of saline solution (Saline; 0.9% NaCl; 1 ml/matrix); T2) Group two cyclicity induction (1 dose of injectable progesterone; n = 259): IM injection of 1 ml (150 mg) of long-acting progesterone (Sincrogest®, Ouro Fino). Also in this management, the vaccination against the reproductive and respiratory vaccine (CattleMaster® GOLD FP 5/L5; Zoetis) was applied to both treatments. On D-26, heifers (T1 and T2) was submitted to ciclicity induction with MGA® PREMIX (Zoetis) - were supplied in the trough, along with the mineral supplement, 2.28 g/head/day of melengestrol acetate (MGA® PREMIX) for twelve consecutive days, and in the following day, the trough was emptied (D-13) and then 0.6 mg of estradiol cypionate (ECP®; Zoetis) was applied for synchronized ovulation induction in the group, aiming increase presence of corpus luteum on D0 of the protocol.

On day 0 (D0) an intravaginal progesterone device (CIDR®) was inserted, then 1.0 mg of estradiol benzoate (Gonadiol®, Zoetis, Campinas, Brazil) was

administered intramuscularly (IM) and application of the 2nd dose of reproductive and respiratory vaccine (CattleMaster® GOLD FP 5/L5; Zoetis). On day nine (D9) the progesterone implant was removed and administered by IM 12,5 mg of PGF2α (Lutalyse®), 0,6 mg of estradiol cypionate (ECP®), 200 IU of eCG (Novormon®) and use of the marker stick to paint the sacrum-caudal region according to NOGUEIRA et al. (2019). All hormones were from Zoetis (Campinas, Brazil). On day eleven (D11) the TAIs were performed with 2 Nellore bulls' semen, using the targeted mating of the genetic improvement program.

On day forty-one (D41), the pregnancy diagnosis was performed and the pregnancy confirmed on day one hundred one (D101), with the aid of an ultrasound device (Mindray DP 2200 VET with a 7.5 MHz transducer, Shenzhen, China).

*Weight, BCS, and uterus and ovary* a*ssessments*

Weight and BCS were analyzed on the D-50, D-26, D0 and D41 according to MACHADO et al. (2008), on a scale of 1 to 5 with intervals of 0.25 points. The reproductive tract score evaluation was performed on days D-50, D-26, and D0, using transrectal ultrasonography.

The methodology for reproductive tract score was adapted for Nellore heifers according to GUTIERREZ et al. (2014), with a classification of 1 to 3 for the uterus and ovary on D0, with 1 being an immature uterus and without the presence of a dominant follicle in ovary and 3 with the presence of a corpus luteum. In the experiment, this method was the scale of 1-3, uterus: 1- flaccid, 2- tonic and 3- swollen; and ovary: 1- smooth appearance, 2- presence of dominant follicle (> 7 millimeters) and 3- presence of corpus luteum. The heifer was considered pubertal with the presence of a corpus luteum without interfering with uterine tone.

*Statistical analysis*

All evaluations were performed by a single evaluator. The SAS (SAS Inst. Inc., Cary, NC, USA; version 9.0) was used and the data was analyzed using the SAS GLIMMIX procedure. The statistical model had the treatment as a fixed effect and the animal as a random effect (treatment×lot). Initial BCS, initial live weight, sire and inseminator data were included as covariates, but were removed from the model when P was > 0.10 for each specific covariate. Means were separated using the PDIFF function and all 22 results were reported as LSMEANS followed by EPM. Also, a logistic regression between live weight and pregnancy rate was performed, using the SAS LOGISTIC procedure, with the function link = logit. Significance was defined when $P \leq 0.05$. In figure 1, the main activities of the experiment are presented, from the beginning of the cyclicity induction of the heifers until D0. In figure 1, the main activities of the experiment are presented, from the beginning of the cyclicity induction of the heifers until D0.

## RESULTS AND DISCUSSION

The pregnancy rate of heifers that underwent two cyclicity inductions was 38.61%, and heifers with only one cyclicity induction was 45.05%, with no significant difference (P = 0.13) on table 1. Thus, the use of hormonal protocols with TAI, aiming at pregnancy after one or two cyclicity inductions, is considered a good alternative to prolong the reproductive life of the bovine female and, as a consequence, increase the production of calves, reduce intervals between generations and provide greater profitability for the farmer (SÁ FILHO et al., 2010).

Induction using P4 acts by reducing the concentration of hypothalamic E2 receptors, thus decreasing the negative feedback on GnRH, thereby leading to an increase in LH secretion (ANDERSON et al., 1991). Showing the effect of cyclicity induction observed in the experimental groups. SARTORI et al. (2021), submitted precocious bovine females to two cyclicity inductions before artificial insemination, and obtained positive results, with final pregnancy rates close to 50%. Different from the above that there was no difference between the control group and the group of two cyclicity inductions.

The pregnancy loss of heifers that underwent two cyclicity inductions was 8%, and heifers with only one induction was 10%, there was no significant difference between treatments (P = 0.43). Results like those of SARTORI et al. (2021), who did not observe a difference in the rate of pregnancy loss, concluding that the protocols were able to induce cyclicity in females and remain cyclical, showing a higher concentration of progesterone and presence of corpus luteum corresponding to the number of cyclicity inductions.

Regarding the evaluation of the uterus and ovary score, the group with two cyclicity inductions obtained an improvement from 1.56 to 1.95 (P = 0.02) and 1.61 to 2.35 (P = 0.06) from the beginning of trial to D0, respectively. This rapid development of the reproductive system after the induction of puberty is related to the stimulus exerted by the increase in estradiol and progesterone concentrations during the post-pubertal period (DESJARDINS & HAFS, 1969; HONARAMOOZ et al., 1999).

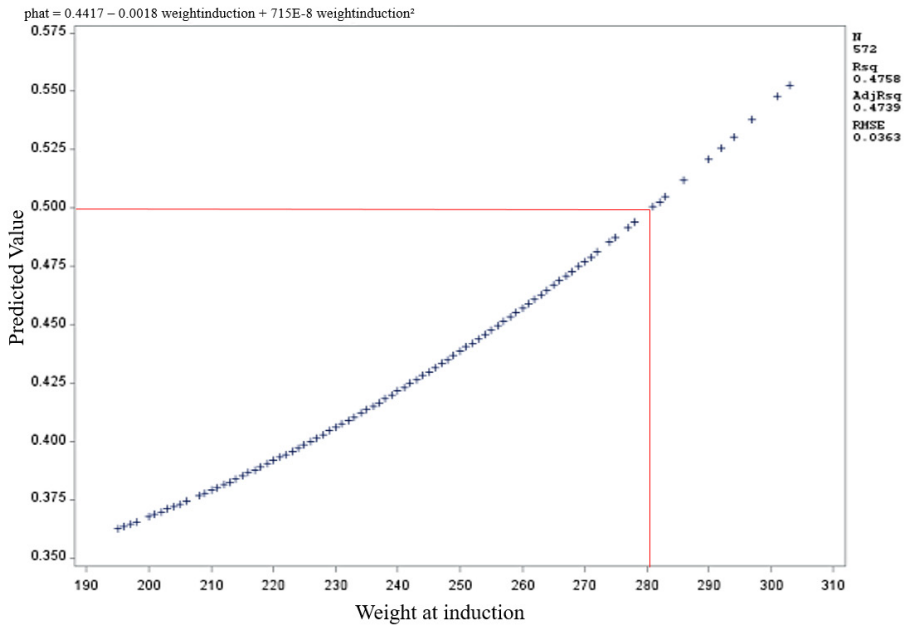


Figure 1 - Logistic regression analysis of initial body weight at the onset of breeding season and its influence on pregnancy probability 30 days Post-TAI in precocious Nellore heifers.

Studies evaluating endometrial thickness and uterine tone have observed greater development in pubescent heifers when compared to non-pubescent heifers (MONTEIRO et al., 2013). In this research, an increase in uterine scores was observed in animals from the group with two cyclicity induction, showing the effect of progesterone for cyclicity induction.

After puberty and endocrine and reproductive competence have been established, studies state that with a greater number of subsequent ovulations there is a progressive increase in reproductive performance, with higher pregnancy rates being observed (BYERLEY et al., 1987). Therefore, longer intervals (> 40 days) between cyclicity induction and the beginning of the breeding season are suggested (FREITAS, 2021). An interval of 50 days was used in the study.

In comparison with other studies, when using only a cyclicity induction, it is noted that the uterus and ovary score is an important tool, as it evaluates the maturity of the reproductive tract of the females and then, selecting those that are suitable for early conception.

FERNANDES et al. (2019) noted that the groups of induced heifers had a greater proportion of

Table 1 - Comparative effects of single vs. double cyclicity inductions on pregnancy outcomes, weight gain, and reproductive indicators (uterine and ovarian scores).

| | | ------Control (313)------ | --2 Inductions (259)-- | --------SEM-------- | ------P-value------ |
|---|---|---|---|---|---|
| Pregnancy (%) | | 45.05 | 38.61 | . | 0.13 |
| Pregnancy Loss (%) | | 10 | 8 | . | 0.43 |
| Weight gain | | 30.39 | 26.29 | 1.38 | 0.53 |
| Uterus Score (1-3) | Induction | 1.57 | 1.56 | 0.12 | 0.91 |
| | D0 | 1.78 | 1.95 | 0.13 | 0.02 |
| Ovary Score (1-3) | Induction | 1.56 | 1.61 | 0.09 | 0.13 |
| | D0 | 2.2 | 2.35 | 0.11 | 0.06 |

Pregnancy loss: from day 30 after TAI to 5 months gestation.
Weight Gain (first day of treatment - AI): weight gain from the first day of treatment to the day of artificial insemination.

corpus luteum and greater reproductive tract score. Knowing that adequate nutrition directly influences the reproductive function, since establishing a good body condition added to the positive energy balance of bovine females causes leptin levels to increase due to the deposition of subcutaneous fat, stimulating Kisspeptin, responsible for increasing the release of GnRH and consequently LH and FSH (MICHAEL et al., 2019).

Body weight is as decisive for a good result as the characteristics of the reproductive system, which can be understood in a research carried out by GREGIANINI et al. (2021), reporting that weight is a factor that confers sexual precocity and when added to age and good reproductive conditions, confers excellent gestational rates. However, according to SARTORI et al. (2021) the age and weight of the females on D0 did not interfere with the probability of pregnancy.

TORRES et al. (2015) stated that these indices can also be high when a body condition score greater than 3 is achieved, positively interfering in the TAI results.

A positive shift between body weight at the beginning of the breeding season and the probability of pregnancy can be verified, since in this property the heifers were selected with BCS close to 3.0 at the beginning of the breeding season, with a regression being traced, indicating that to obtain pregnancy rates of 50%, the heifers should have a body weight equal to or greater than 282 kg (Figure 1). ENDECOTT et al. (2013) stated that to have a better economic return from the activity, females must begin their reproductive activity with 50 to 57% of their adult weight. This study showed that the 50% probability of pregnancy is around 282 kg in cyclicity induction, corresponding to 62.6% of 450 kg, which is considered 1 AU.

## CONCLUSION

The use of two cyclicity induction previous to TAI (timed artificial insemination), improved the reproductive tract score, but did not influence the pregnancy rate and pregnancy loss of precocious Nellore heifers.

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## DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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