

Reproductive performance of prepubertal and pubertal heifers submitted to timed artificial insemination protocols

Abstract

This study investigated the effect of using estradiol cypionate (ECP) and equine chorionic gonadotropin (eCG) as part of a hormonal protocol for timed artificial insemination (TAI) on the pregnancy rate (PR) in prepubertal and pubertal Nelore heifers pretreated with intravaginal progesterone (P4).

Methods: Two groups of animals were assessed: prepubertal heifers (no corpus luteum (CL) present and follicles <8mm diameter; n=130) and pubertal heifers (CL present; n=94). The beef heifers weighed between 297–320kg and body condition score was 2.3–3.5. On day 0 (d0), all heifers received an intravaginal device containing 0.558g of P4, and 1.0 mg of estradiol benzoate (EB). On d8, the P4 was removed and 0.075mg PGF2α 300IU of eCG, and 2 mg of ECP were administered. TAI was performed on day 10. Animals that return to show estrous signals post-TAI were re-inseminated (AI) 12h after estrus observation. Animals that returned to estrus after conventional AI were kept with bulls for 45days and after 30days, pregnancy diagnosis was performed.

Results: The PR in prepubertal and pubertal heifers were respectively for TAI 29.2% and 37.2% ($p>0.05$); for conventional AI, 13.0% and 33.9% ($P<0.05$); and for bulls' breeding, 36.2% and 35.9%, respectively. ($P>0.05$). At the end of the breeding season, the PR in prepubertal and pubertal heifers was 60.7% and 73.4% ($P=0.062$).

Conclusion: Use of the hormonal protocol was efficacious for both groups of animals due to the acceleration of puberty and this increased the PR at the beginning of the breeding season.

Keywords: *bos indicus*, nelore, timed-artificial insemination, breeding season

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Abbreviations: TAI, timed artificial insemination; PR, pregnancy rate; EB, estradiol benzoate; P4, progesterone; ECG, equine chorionic gonadotrophin; PGF2α, prostaglandin F2 alpha; CL, corpus luteum; CEAU, commission of ethics for animal use; PD, pregnancy diagnosis; BS, breeding season; AI, artificial insemination; US, ultrasonography

Introduction

High numbers of prepubertal heifers at the beginning of the breeding season (BS) reduces reproductive efficiency in beef cattle.¹ In Brazil, grazing *Bos indicus* heifers reach puberty between 22 and 36months, and the age at first calving is between 44 and 48months.² Seasonality, poor pasture management, and poor nutrition during the heifer's growth phase are some of the variables that lead to the delayed onset of puberty in *Bos indicus* cattle.³ Studies have been conducted to induce puberty in heifers, using either nutritional management strategies,⁴ or intravaginal progesterone (P4) devices.⁵ Sales et al.⁶ induced prepubertal *Bos indicus* heifers by administering P4 with estradiol cypionate (ECP) after 10days. Silveira et al.⁵ used prepubertal and pubertal zebu heifers for timed artificial insemination (TAI). They found a higher pregnancy rate (PR) in the groups that had received P4 with estradiol benzoate (EB) and ECP compared to prepubertal heifers only exposed to bulls. The exposure of prepubertal heifers to P4 for 8days induced and synchronized estrus. Similar effects were observed by Wheaton et al.⁷ and Demeterco et al.⁸ Treatment with a P4 intravaginal device induced prepubertal heifers to cycle and also

increased the uterine diameter⁶ even during anestrous.⁹ The addition of EB at the time of P4 removal potentiates this action.³

Peres et al.¹⁰ investigated the use of P4 intravaginal devices adding eCG at the time of P4 removal in Nelore heifers. SáFilho et al.³ used 400IU of eCG at the time of Norgestomet removal, achieving a PR of 50%. In another study, the same researchers achieved a 48.2% PR, using ECP to induce ovulation after previous treatment with Norgestomet, and concluded that ECP can be successfully employed.

Rodrigues et al.¹¹ used eCG and ECP after intravaginal P4 in prepubertal Nelore heifers. They observed an increase in the rate of estrus detection and ovulation, thus improving the PR earlier in the BS. Our group hypothesized that the administration of eCG and ECP to prepubertal and pubertal *Bos indicus* heifers, after P4 treatment and prostaglandin F2alpha (PGF2α) administration could lead to improvements in the PR of TAI.

The present study aimed to evaluate the efficacy of eCG and ECP to improve reproductive performance in prepubertal and pubertal Nelore heifers pretreated with intravaginal P4.

Material and Methods

This study was based on the principles of ethics in research involving animals, applicable in the country having consonance with the Commission of Ethics for Animal Use (CEAU). In this study, 224 (prepubertal and pubertal Nelore) heifers for commercial replacement

were used. The animals were on average 25 months old (range, 24–27 months) and weighed an average of 312 kg (range, 297–320 kg) with a body condition score (BCS) of 3.0 (2.25–3.5; 1=thin to 5=obese). At the start of the study, 42.0% of the heifers were pubertal (presence of corpus luteum [CL] or follicles >8 mm diameter) and 58.0% were prepubertal (absence of CL or follicles >8 mm diameter). The animals grazed on *Brachiaria brizantha* and mineral salt and water were available ad libitum.

The experimental protocol for all animals is presented in Figure 1, and was as follows: at day 0 (d0), a single-use intravaginal P4 device (0.558 g P4, Cronipres, Biogenes Bago, Curitiba-Brazil) was inserted as well as EB (1.0 mg, IM; Cronibest, Biogenes Bago); on d8, P4 removal+PGF2 α (Croniben, 0.075 mg, IM, Biogenes Bago)+eCG (300 IU, IM; Folligon, MSD Animal Health, São Paulo)+ECP (Estradiol cypionate, 2 mg, IM; E.C.P., Zoetis, São Paulo,

Brazil) were administered. On d10, TAI was performed using frozen semen. Ovarian ultrasonography (US) to evaluate CLs or follicles was performed on d45. Animals that were repeat breeders after TAI were re-inseminated using conventional AI (AI 10–12 hours after estrus observation) and pregnancy diagnosis (PD) was performed 35 days later. Animals that came into estrus after AI were kept with bulls for 45 days. After 30 days with the bulls, ultrasound (Tringa model, Probe 5.0; Pyomedical Co., The Netherlands) was performed for PD.

Statistical analysis

To verify the difference between prepubertal and pubertal heifers the data corresponding to TAI, conventional artificial insemination (AI), and natural breeding were analyzed using ANOVA and the Fisher's tests; (P value lower than < 0.05 was considered statistically significant). The statistical analysis was performed by means of GraphPad Prism Software, version 5.0



Figure 1 Hormonal protocol scheme used for the animals conducted in Nellore.

US, ultrasound; P4 (Cronipresmonodosis, 0.558 g progesterone, Biogenes Bago, Curitiba-Brazil); EB (Cronibest, 1.0 mg, IM, Biogenes Bago); BCS (1–5); PGF2 α (Croniben, 0.075 mg, IM, Biogenes Bago); ECP (Estradiol cypionate, 2 mg, IM, Zoetis, São Paulo, Brazil); eCG (Folligon, 300 IU, IM, MSD Animal Health, São Paulo); d10=TAI; d45=Pregnancy Diagnosis (PD); from d46 to d76 detection of estrus+conventional AI in repeat breeding animals; ED=Estrus detection; d107 to d152 heifers were submitted to natural service with bulls; PD occurred at d182.

Results and Discussion

The results are shown in Table 1. The PR percentages are based on the TAI, conventional AI, and natural breeding results. The protocols used for TAI in *Bos indicus* heifers using a long-acting P4 intravaginal device followed by the administration of eCG or ECP after device removal have been previously reported by SáFilho, et al.³ Claro Junior et al.,¹ Rodrigues et al.,¹¹ and Silveira et al.⁵ These studies highlighted the importance of hormonal TAI protocols for this category of animal, with an emphasis on animal replacement. Our research group hypothesized that using ECP and eCG in heifers previously “sensitized” by exogenous P4 and PGF2 α could have a more positive effect on the PR than other protocols.⁵

The protocol used in this study was administered to 130 prepubertal and 94 pubertal heifers (Table 1). The TAI protocol was able to induce puberty in the heifers, resulting in a PR of 29.2% in prepubertal and 37.2% in pubertal heifers. Conventional AI was performed (45 days after the BS commenced), and the prepubertal and pubertal PR reached 38.5% and 58.5%, respectively ($P < 0.05$) which can be attributed to the protocol. These results demonstrate the importance of using a protocol to achieve such PR percentages in prepubertal heifers, because it is unlikely that these results could be achieved in the BS without the protocol, as previously reported.⁵

The final PRs when TAI, AI and the bull were used in prepubertal and pubertal heifers were 60.7% and 73.4% respectively, which only approached a significant difference ($P > 0.06$). This results indicates

that exposure to the hormonal protocol for TAI, is primarily beneficial for prepubertal animals. It is plausible that ECP and eCG were more effective in prepubertal than in pubertal heifers, balancing the ovarian response at the beginning of the BS.¹¹ According to Rodrigues et al.,¹¹ isolated treatments with eCG and/or ECP after 12 days of previous exposure to intravaginal P4 in prepubertal heifers resulted in a higher induction of ovulation when compared to the control group, and the isolated use of ECP performed moderately and did not differ from other treatments.

At the end of the BS, TAI, conventional AI, and natural breeding resulted in a combined PR of 66.0%, and there was no significant difference in the PRs among pubertal and prepubertal heifers. The results of this study are an important finding and could be applied to commercial farms. A study by Claro Junior et al.¹ in beef cattle in Brazil achieved a PR of 83.7%. The lower PR results observed in our study may have been due to the shorter time that the intravaginal P4 device remained in the heifers in our study compared to that in the study by Claro Junior et al.¹ (8 days versus 12 days). Other possibilities include factors associated with differences in nutrition, BCS, age, and environment conditions. Silveira et al.⁵ administered ECP after P4 removal (d8) in prepubertal and pubertal heifers and achieved PRs of 37.0% and 49.0%, respectively. This result is higher than the results of the present study (Table 1). We hypothesized that these differences could be attributed to the breed, because Silveira et al.⁵ worked with crossbred (*Bos indicus* x *Bos taurus*) heifers. This hypothesis is supported by Rodrigues et al.¹¹ who reported that

heifers that were heavier at the beginning of the protocol, showed the highest percentages of estrous detection and the greatest number of ovulations. Studies by Carvalho et al.¹² demonstrated that crossbred heifers had a larger dominant follicle (DF) on d8, a larger ovulatory follicle, and a higher DF growth rate compared with pure *Bos indicus* or *Bos taurus* heifers. Rodrigues et al.¹¹ administered P4

for 12 days and ECP and eCG after P4 removal, and reported better reproductive performance in *Bos indicus* heifers than the other groups that received only eCG or no treatment (control group). SáFilho et al.³ reported that eCG is an important tool to increase follicular growth and ovulation, and the size and function of the subsequent CLs, thus improving the PR.

Table 1 Pregnancy rate (PR) after application of the hormonal protocol using estradiol cypionate and equine chorionic gonadotropin in Nellore heifers pretreated with an intra vaginal device P4 (n=224)

| Heifers | Number of Animals (%) | PR TAI (%) | PR AI (%) | PR TAI+AI (%) | PR Bulls (%) | PR TAI +AI + Bull (%) |
|-------------|-----------------------|---------------------|---------------------|---------------------|---------------------|-----------------------|
| Prepubertal | 130/224 | 38/130 | 12/92 | 50/130 | 29/80 | 79/130 |
| | (58.0) | (29.2) ^a | (13.0) ^a | (38.5) ^a | (36.2) ^a | (60.7) ^a |
| Pubertal | 94/224 | 35/94 | 20/59 | 55/94 | 14/39 | 69/94 |
| | (42.0) | (37.2) ^a | (33.9) ^b | (58.5) ^b | (35.9) ^a | (73.4) ^a |

PR, pregnancy rate; TAI, timed artificial insemination

Different letters in the same column are statistically significant (P<0.05)

Conclusion

In conclusion, the application of a hormonal protocol such as that utilized in this study is beneficial to heifers because it can hasten the onset of puberty in prepubertal animals and subsequently the PR in pubertal heifers at the beginning of the BS was maximized.

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None.

Conflict of interest

The authors declare they have no conflicts of interest regarding the work presented in this study.

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