Reprouctive 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 Nellore 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. At the end of the breeding season, the PR in prepubertal and pubertal heifers was 66.7% 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, nellore, timed-artificial insemination, breeding season

Abbreviations: TAI, timed artificial insemination; PR, pregnancy rate; EB, estradiol benzoate; P4, progesterone; ECG, equine chorionic gonadotropin; 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. In Brazil, grazing Bos indicus heifers reach puberty between 22 and 36months, and the age at first calving is between 44 and 48months. 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. Studies have been conducted to induce puberty in heifers, using either nutritional management strategies, or intravaginal progesterone (P4) devices. Sales et al. induced prepubertal Bos indicus heifers by administering P4 with estradiol cypionate (ECP) after 10days. Silveira et al. 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. and Demeterco et al. Treatment with a P4 intravaginal device induced prepubertal heifers to cycle and also increased the uterine diameter even during anestru.

Peres et al. investigated the use of P4 intravaginal devices adding eCG at the time of P4 removal in Nellore heifers. SáFilho et al. 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. investigated the use of P4 intravaginal devices adding eCG at the time of P4 removal in Nellore 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 Nellore 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 Nellore) 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 >8mm diameter) and 58.0% were prepubertal (absence of CL or follicles >8mm 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 as follows: at day 0 (d0), a single-use intravaginal P4 device (0.558 g P4, Cronipres, Biogenesis Bagó, Curitiba-Brazil) was inserted as well as EB (1.0 mg, IM; Cronibest, Biogenesis Bagó); on d8, P4 removal+PGF2α (Croniben, 0.075 mg, IM, Biogenesis Bagó)+ECP (Estradiol cypionate, 2 mg, IM; Zoetis, Sao Paulo, Brazil) was 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; Pyemedical 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.
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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. 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. 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. 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)

<table>
<thead>
<tr>
<th>Heifers</th>
<th>Number of Animals (%)</th>
<th>PR TAI (%)</th>
<th>PR AI (%)</th>
<th>PR TAI+AI (%)</th>
<th>PR Bulls (%)</th>
<th>PR TAI +AI + Bull (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepubertal</td>
<td>130/224</td>
<td>38/130</td>
<td>12/92</td>
<td>50/130</td>
<td>29/80</td>
<td>79/130</td>
</tr>
<tr>
<td></td>
<td>(58.0)</td>
<td>(29.2)a</td>
<td>(13.0)a</td>
<td>(38.5)a</td>
<td>(36.2)a</td>
<td>(60.7)a</td>
</tr>
<tr>
<td>Pubertal</td>
<td>94/224</td>
<td>35/94</td>
<td>20/59</td>
<td>55/94</td>
<td>14/39</td>
<td>69/94</td>
</tr>
<tr>
<td></td>
<td>(42.0)</td>
<td>(37.2)a</td>
<td>(33.9)a</td>
<td>(58.5)a</td>
<td>(35.9)a</td>
<td>(73.4)a</td>
</tr>
</tbody>
</table>

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.

**Acknowledgements**

None.

**Conflict of interest**

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

**References**