Effect of recombinant bovine somatotropin on pregnancy rates of Nellore cows resynchronized with the use of new DIB® and third use, and inseminated at fixed-time

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ABSTRACT

The objective of the experiment was to evaluate the effects of recombinant bovine somatotropin (rbST), and the reuse of the progesterone (P4) releasing devices in resynchronization, on the pregnancy rates of Nellore cows submitted to fixed-time artificial insemination. A group of 123 multiparae Nellore cows, was submitted to a resynchronization protocol: on day 0 a Bovine Intravaginal Device (DIB®) with 1,0g of P4 was implanted, associated with intramuscular administration of 2,0mg of estradiol benzoate (IM); on day 8 DIB was removed; and 1,0mg of estradiol cypionate, 0,15mg of prostaglandin F₂α and 300 UI of equine chorionic gonadotropin were administered; on day 10, fixed-time artificial insemination was conducted (FTAI). The cows were randomized into G1 (n=12) – without rbST / with used Bovine Intravaginal Device, G2 (n=50) – without rbST / with new DIB, G3 (n=11) – with rbST / with used DIB and G4 (n=50) – with rbST / with new DIB. rbST was introduced on the eighth day of the protocol. Sixty days after TAI, pregnancy diagnoses were conducted, via rectal palpation. Blood samples were taken on day 0, 8 and 10 of the protocol, in order to assess P4 plasma concentrations. Pregnancy rates were statistically evaluated through Generalized Linear Models Theory and their significance was tested with Analysis of Deviance. Pregnancy rates were 58%, 40%, 81% and 48% for G1, G2, G3 and G4, respectively, with significant statistical difference for G3. Plasma concentrations of P4 were not statistically different among groups, or collections. In view of the results obtained, we concluded that the administration of rbST in association with P4 DIB, used for the third time, improves pregnancy rates. Estrus resynchronization and re-insemination positively impacted pregnancy rates.

Keywords: Cattle; growth hormone; progesterone; reproduction; zebu.
INTRODUCTION

The growing demand for food in the world requires efficiency in beef cattle production. The profitability of the production system is closely related to the reproduction efficiency of the herd. The goal is to get a calf per cow per year [11]. However, that the best definition would be to have one calf per cow, with a twelve-month interval between births.

Beneficial effects in the performance are achieved through the use of recombinant bovine somatotropin (rbST) in fixed-time artificial insemination (FTAI) protocol [27].

Among the functions studied by several authors [6; 28; 31; 4] the action of rbST in the FTAI protocol is probably the one which best relates to the improvement of the pregnancy rates. As rbST enhances pituitary secretion of bST, as well as IGF-1 synthesis by the liver, it triggers a cascade of events that may directly or indirectly: accelerate corpus luteum (CL) growth and the secretion of progesterone (P4), during the luteal phase of the estrous cycle [31]; increase the population of small antral follicles; stimulate maturation of oocytes, and increase fertilization rates [27]; reduce serum concentrations of estradiol, around the 17th day of the estrous cycle in cattle; attenuate the production of prostaglandin F2α (PGF2α); increase embryonic survival and development; and aid maternal recognition, thus increasing pregnancy rates [6].

Studies with rbST in beef cows are limited. The results observed in these studies are dissimilar due to the use of synchronization protocols, which differ in regard to the day of rbST administration [6; 28; 1].

In cattle breeding programs, intravaginal P4-releasing devices are now widely known and used. They represent 50% or more of the costs of a synchronization program for FTAI [12]. A strategy to reduce the costs of these programs is the reuse of such devices.

In beginning, the devices were used only once and discarded. However, the reuse of these devices has demonstrated satisfactory efficiency without compromising pregnancy rates [9].

According to Pereira et al. (2011) 1g P4 devices (DIB®, Sincrogest®, Primer®, Cronipres®) began to be used up to three times with cattle, while 1.9g devices (CIDR®) are already being used four times in some properties. Dias et al. (2009) observed higher conception rates as the number of device uses increased.

P4 serum concentrations are almost undetectable until about 30 days postpartum. However, they demonstrate the activity of the corpus luteum when the concentration is higher than 1ng/ml. P4 serum profiles have been suggested to be a reliable method to objectively assess ovarian activity [16].

Some studies show that high concentrations of P4 can influence the pulsatility of luteinizing hormone (LH), which is responsible for the growth, maturation and ovulation of the dominant follicle (DF) [5].

According to Perez et al. (2009), the availability of exogenous P4 close to ovulation reduces the growth rate of the DF. Elevated P4 plasma concentrations (between 5 and 8ng/mL) result in low LH pulsatility, which is in line with other studies, that found that high serum concentrations of P4 decrease the ovulatory capacity of the DF, thus justifying the reuse of intravaginal devices.

Considering the mechanisms by which rbST can influence the reproductive physiology of cows, the efficiency of P4 reused devices, and several protocols proposed, with different results, the objective of the present experiment was to evaluate the effect of rbST and of reused P4 devices, in resynchronization, on the pregnancy rates of fixed-time inseminated Nellore cows.

MATERIAL AND METHODS

This study was authorized by the Ethics Committee on Animal Use of Universidade Federal do Tocantins, authorization procedure number 23101.003935/2012-57.

The experiment was conducted between the months of April and June 2012, at Maratá farm, in the municipality of Pio XII, state of Maranhão, Brazil. The property is located at 3°52'30" latitude S and 45°07'30" longitude W, with an annual average temperature of 27°C.

 Clinically healthy multiparae females, suitable for reproduction, more than 90 days postpartum, aged 42-48 months, with mean body condition index of 3.0, in a scale of 1 to 5, were selected [15]. The animals were kept under extensive grazing conditions with Brachiaria Brizantha, water and mineral salt at will.

A group of 337 Nellore cows underwent a protocol for estrus synchronization, and then FTAI. After pregnancy diagnosis by ultrasonogram (30 days after FTAI), 123 cows, which were not pregnant, were selected and underwent a protocol of resynchronization and re-inseminatin.

This group of 123 cows was subjected to a protocol of estrus resynchronization and randomized into four groups: G1 (n = 12) - without rbST and reused DIB, G2 (n = 50) - without rbST and new DIB, G3 (n = 11) - with rbST and reused DIB, and G4 (n = 50) - with new DIB and rbST.

On day 8 of the estrus resynchronization protocol, 500 mg of rbST (Boostin®; Intervet - Schering Plough Animal Health) was administered. The estrus synchronization protocol was the same used in the resynchronization (Figure 1).
Figure 1 – Schematic representation of estrus synchronization and resynchronization protocols and ovulation induction.

The bovine intravaginal device impregnated with 1.0g P4 (DIB®, Schering-Plough Cooper) was implanted on day 0. Implantation was associated with the administration of 2.0mg of estradiol benzoate (Gonadiol®, Coopers Schering-Plough) intramuscularly (IM).

On day 8, the DIB® was removed, and 1.0mg of estradiol cypionate (ECP®, Pfizer), 0.15mg of prostaglandin F$_2$α (Veteglan® luteolic, Hertape Calier) and 300 IU of equine chorionic gonadotropin (Folligon®, Schering Plough), were administered all IM.

On day 10, all the TAIs were performed by the same technician. The semen used had been obtained from an artificial insemination center, from seventeen breeding Nellore. Rectal palpation was used for pregnancy diagnosis, 60 days after FTAI.

To determine P4 serum concentrations, blood samples were taken from 10 animals in each group, on day zero, eight and ten of the resynchronization protocol. The blood was collected from the coccygeal vessels, into 10mL Vacutainer® tubes, without anticoagulant, and stored at a temperature between 5 and 8ºC, until they were centrifuged at 1422 x g for 20 minutes, in order to completely separate the serum, which was then stored in microtubes (Eppendorf tubes) and kept frozen at -18ºC for later analyses.

Serum P4 concentrations were obtained by using commercial kits (EIA Cayman Chemical Company, Ann Arbor, MI; EIA kits No. 0447860), as per manufacturer’s instructions. The progesterone assay had a sensitivity of 6.5pg/ml, with a coefficient of variation of 4.4% intra-assay and 2.7% inter-assay.

Generalized Linear Models theory was used for the statistical analysis of pregnancy rates [18], and the significance of the effect of bSTr was tested through Deviance test Proc Logistic [29].

For P4 concentrations, the data structure is comprised of repeated measures over time, for each animal, within the groups. The data is analyzed by applying the repeated measurements technique using SAS Proc Mixed [17].

RESULTS AND DISCUSSION

Of a total of 337 cows submitted to the estrus synchronization protocol and fixed-time artificial insemination, 59.05 % (199) became pregnant. This percentage is within normal rates found in other studies [30; 23]. Just over 40% were not pregnant. In order to increase the rate of late or cumulative pregnancy, resynchronization of estrus was performed, thus providing these animals with a second chance of pregnancy within a breeding season.

The additional resynchronization costs are offset by the increase in the pregnancy rate [13]. The use of FTAI is essential for achieving success, as it enables the rationalization of labor and reduces losses, due to the failure in estrus detection [13; 12].

rbST was used with the aim of achieving better results in resynchronization, as it stimulates increased concentrations of GH and IGF-1, which exert positive effects on fertility [28].

And, seeking to increasingly lower the cost per pregnancy, the reuse of P4 devices was adopted, as they may correspond to more than half of the total cost per pregnancy [12; 9].

Pregnancy rates after resynchronization of estrus, using new DIB®, and in third use, with or without rbST treatment, were satisfactory (TABLE 1). This shows that the protocols adopted were effective for the resynchronization of ovulation, resulting in an average conception rate of 56%. Such rate is within the percentage normally found in FTAI studies, for beef cows with reused devices [24; 14; 25].
Table 1 – Pregnancy rates of Nellore cows submitted to estrus resynchronization protocol, with new and reused P4 devices, with or without rbST administration.

| Experimental Groups | Pregnancy Rates (%) |
|---------------------|---------------------|
| G1 – without rbST and with used DIB | 58% (7/12)\* |
| G2 – without rbST and with new DIB | 40% (20/50)\* |
| G3 – with rbST and with used DIB | 81% (9/11)\* |
| G4 – with rbST and with new DIB | 48% (24/50)\* |

Averages followed by different lowercase letters in the column differ, as per Chi-square test (P<0.05).

The reuse of DIB® in the present study did not affect pregnancy rates, similarly to what was observed by Peixoto & Ulian Jr. (2007) and Chesta et al. (2005). This leads to the conclusion that there is sufficient residual P4 in these third use devices for synchronization of estrus and ovulation in the cows treated, as found by Pereira et al. (2011), Meneghetti et al. (2009) and Freitas et al. (2007). This shows that the reuse of other types of P4 devices does not compromise the reproductive efficiency of this category of animals.

Pregnancy rates in the groups treated with third use devices (G1 and G3) exceeded the others, especially G3, in which the result may be associated with the administration of rbST.

According to Rossetti et al. (2011), this hormone stimulates the concentrations of GH and IGF-1, which, in turn, accelerate embryonic development, on the eighth day after fertilization, through proliferation and cell differentiation [2]. They also modulate its metabolism to increase glucose transport [31], thus resulting in increased secretion of interferon-τ, at the time of maternal recognition of pregnancy [3]. Badinga et al. (2002) also found a potential of bST for inhibiting the synthesis of prostanoids, which may also be associated with improved embryo survival.

Plasma concentrations of P4 did not differ between groups or collection days (P<0.05) (Figure 2). Such P4 concentrations at the time of FTAI were lower than those reported by Perez et al. (2009), who concluded that concentrations of P4 between 5 and 8ng/ml at the time of ovulation, impairs LH pulsatility, and therefore, the diameter of the dominant follicle (DF) [8]. The positive correlations between the diameter of the ovulatory follicle and pregnancy rates are well-known, both being influenced by P4 concentrations [5].

Figure 2 – Serum progesterone concentration in Nellore cows submitted to estrus resynchronization, collected in different days of the protocol. G1 without rbST and used DIB; G2 without rbST and new DIB; G3 with rbST used DIB; and G4 with rbST and new DIB (P <0.05).

After synchronization and resynchronization of estrus, a cumulative pregnancy rate of 76.8% (n = 259) was obtained, over a period of 45 days, without the need for observation of estrus, thus representing a gain for the producer (Figure 3).
Figure 3 – Pregnancy rates in Nellore cows subjected to synchronization, resynchronization, and accumulated pregnancy rate.

Nowadays, obtaining acceptable conception rates after using estrus and ovulation synchronization protocols for FTAI has become a reality, however, not all synchronized cows become pregnant. That is the justification for the practice of providing a second chance to those cows, which did not conceive after FTAI, in the same breeding season. This may result in a service rate of 100% [20].

Meneghetti et al. (2012), Nascimento (2009), Silva et al. (2007) and Penteado et al. (2005) also found results, which were similar to those presented here in.

CONCLUSION

The administration of rbST in multiparae Nellore cows, in a single dose, on day 8 of the resynchronization protocol, in association with P4 devices, reused for the third time, improved pregnancy rates.

Resynchronization of estrus and re-insemination exerted positive effects in the increase of cumulative pregnancy rates.

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