Sjournals
Scientific Journals
Scientific Journal of Biological Sciences (2015) 4(1) 10-17
ISSN 2322-1968
doi: 10.14196/sjbs.v4i1.1786

Contents lists available at Sjournals
Scientific Journal of
Biological Sciences
Journal homepage: www.Sjournals.com

Original article

The effect of recombinant bovine somatotropin on the pregnancy rate of resynchronized nellore cows submitted to fixed-time insemination

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ARTICLE INFO

Article history,
Received 06 December 2014
Accepted 21 January 2015
Available online 29 January 2015

Keywords,
Cattle
Growth hormone
Reproduction
Zebu

ABSTRACT

The objective of the study was to evaluate the effects of recombinant bovine somatotropin (rbST) during resynchronization on the pregnancy rates of Nellore cows submitted to fixed-time artificial insemination. A group comprised of 100 multiparae Nellore cows was submitted to a resynchronizing protocol: on day 0 the subjects received the Bovine Intravaginal Device with 1,0g of progesterone (P4), associated with intramuscular administration of 2,0mg of estradiol benzoate (IM); on day 8, the Bovine Intravaginal Device (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 subjects were then randomized into Control Group (n=50) and Treated Group (n=50), of which the latter received 500mg of rbST on the eighth day of the protocol. Sixty days after the

fixed-time artificial insemination, pregnancy diagnoses were conducted via rectal palpation. Blood samples were taken to measure plasma concentrations of glucose, triglycerides and cholesterol. Pregnancy rates were statistically evaluated through Generalized Linear Models Theory, and their significance was tested through Analysis of Deviance. No difference was found between groups, pregnancy rates were 40% and 48% for control and treated groups, respectively. Cholesterol plasma concentrations were also not affected by the treatment with rbST. There were significant increases in serum glucose and triglycerides between groups. The administration of rbST to multiparae Nellore cows, in a single dose, did not statistically interfere in the pregnancy rates. The resynchronization of estrus and re-insemination had a positive effect in cumulative pregnancy rates.

1. Introduction

The current economic situation demands maximum efficiency from livestock producers all over the world, in order to ensure economic return. Directly dependent on nutritional, health, genetic factors, as well as appropriate management, reproduction is identified as one of the most important factors associated with the profitability of cattle production (alexio et al., 2005). In this scenario, research has been conducted in order to find increasingly effective alternatives to increase pregnancy rates.

According to rosas et al. (2011), beneficial effects on reproductive performance have been observed with the use of recombinant bovine somatotropin (rbst) in a protocol for fixed-time artificial insemination (ftai). Aboin et al. (2013) and cerón e aguilar (2013) mention three mechanisms of action of bovine somatotropin (bst), as follows: direct action in the target tissue, independent of the growth factor type, similar to type-1 insulin (igf-1); indirect action, stimulating liver production of igf-1, which will act in target tissues via endocrine action; and also in an indirect manner, as bst stimulates local synthesis of igf-1 exerting localized biological action in the tissue.

Rbst enhances pituitary secretion of bst, as well as igf-1 synthesis by the liver, triggering 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 (starbuck et al., 2006); increase the population of small antral follicles; stimulate maturation of oocytes and increase fertilization rates (rosas et al., 2011); reduce serum concentrations of estradiol, around the 17th day of the estrous cycle in cattle; attenuate the production of prostaglandin f2a (pgf2a); increase embryonic survival and development; aid maternal recognition, thus increasing pregnancy rates (cerón and aguilar, 2013).

According to cerón and aguilar (2013) and rossetti et al. (2011), there are two physiological periods in which bst and igf-1 exert their effects, and that correspond to the stages of pregnancy when embryonic loss is greater. The first stage is related to the fertilization and embryo development during the first seven days, and the second is the period of maternal recognition of pregnancy (16 to 19 days after insemination).

Regarding the metabolism of carbohydrates, bst acts as a homeorhetic controller, delivering more glucose into the bloodstream. This is possible because somatotropin reduces the sensitivity of insulin receptors in peripheral tissues, thus resulting in a decrease in the uptake and oxidation of glucose by liver cells, muscle and adipose tissue, and consequently, glucose becomes available. This reduction in hepatic insulin response allows the liver to promote higher rates of gluconeogenesis (prado et al., 2003).

In cows, especially the fetus and the placenta depend on the metabolic environment (mainly glucose). Glucose appears to be a major metabolite which promotes early embryonic development (green et al., 2009).

Since glucose is used as the primary energy metabolite, and as a substrate for the synthesis of milk constituents, the energy needed for other peripheral body tissues is derived from the products of lipolysis. The effects on lipid metabolism vary according to the energetic balance of the animal, wherein the exogenous

somatotropin promotes the immediate use of the energy reserves of the body (lipolysis), as well as a reduction in the formation of new reserves (lipogenesis) (gülay and hatipoglu, 2005; etherton and bauman, 1998).

Studies with rbst in beef cows are limited. The results observed in such studies are dissimilar due to the use of synchronization protocols, which differ in regard to the day of rbst application (cerón and aguilar, 2013; rossetti et al., 2011; alexio et al., 2005).

In view of the mechanisms that rbst can influence in the reproductive physiology of cows, and the need for further studies with nellore cattle, the present study aimed to evaluate the effect of rbst during resynchronization on the pregnancy rates of nellore cows, submitted to fixed-time insemination.

2. Material e methods

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

The study was conducted between the months of April and June 2012, at a property named Maratá, in the municipality of Pio XII, state of Maranhão. The farm 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, and mean body condition index of 3,0 were selected (Houghton et al., 1990). They were kept under extensive grazing conditions with Brachiaria Brizantha, water and mineral salt at will. A group of 274 Nellore cows underwent a protocol for estrus synchronization, and then the FTAI. After pregnancy diagnosis by ultrasonogram (30 days after FTAI), 100 cows, which were not pregnant, were selected and underwent a protocol of resynchronization and re-insemination.

This group of 100 cows was submitted to a protocol of estrus resynchronization and randomized into control group (n = 50) and Treatment Group (n = 50) with administration of 500mg of rbST (Boostin®, Intervet - Schering Plough Animal Health) on the eighth day of the resynchronization protocol.

The estrus resynchronization protocol was the same as used in the synchronization (Figure 1).

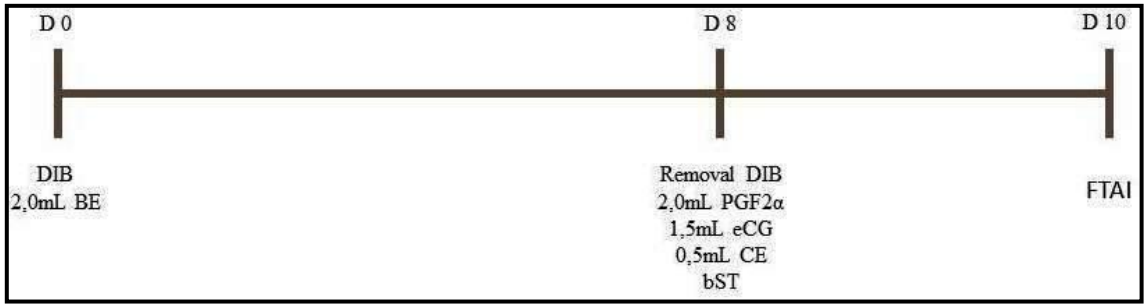

Fig. 1. Schematic representation of estrus synchronization and resynchronization protocols and ovulation induction.

The implantation of the bovine intravaginal device with 1,0g P4 (DIB®, Schering-Plough Coopers), associated with the administration of 2,0mg of intramuscular (IM) estradiol benzoate (Gonadiol®, Coopers Schering-Plough), was conducted on day 0.

On day 8, the DIB® was removed, 1,0mg of estradiol cypionate de (ECP®, Pfizer), 0,15mg prostaglandin F2α (PGF2α- Veteglan® Luteolic, Hertape Calier), as well as 300UI de equine chorionic gonadotropin (Folligon®, Schering-Plough) were administered, all IM.

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

On day number 8 and 10 of the resynchronization protocol, blood samples were collected, as well as on the day of pregnancy diagnosis. The blood was collected from the coccygeal vessels, into 10mL Vacutainer® tubes, without anticoagulant, and stored at a temperature between 5 and 8 ºC 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 -18 ºC for later analyses.

Serum glucose, cholesterol and triglyceride concentrations were obtained by using commercial colorimetric reagent kits (Labtest®), as per manufacturer's instructions, using Bioplus 2000.

The Generalized Linear Models theory was used for the statistical analysis of the pregnancy rates (McCullagh and Nelder, 1989), and the significance of the effect of rbST was tested through Deviance test ProcLogistic (SAS, 2001).

For plasma glucose, cholesterol and triglycerides, the data structure is comprised of repeated measures over time, for each cow, within the groups. The data is analyzed by applying the repeated measurements technique using SAS Proc Mixed (Littell et al., 1998).

3. Results e discussion

Of a total of 274 cows submitted to the estrus synchronization protocol and timed insemination, 58,7% became pregnant (161). Such percentage is within normal rates observed in other studies (Silva et al., 2007; Penteado et al., 2005). To increase the final or cumulative pregnancy rate, there synchronization of estrus is an important tool, which provides a second chance for pregnancy on the same breeding season.

The additional costs of resynchronization are offset by the increase in pregnancy rate. Under this light, the user of FTAI is essential for achieving success, as it enables the rationalization of labor and reduces losses, resulting from failure indetecting estrus (Giraldo, 2008; Freitas et al., 2007).

rbST was used to achieve better results in the resynchronization process, as rbST stimulates increased concentrations of GH and IGF-1, which exert positive effects on fertility (Rossetti et al., 2011), promoting the differentiation of CL and embryonic development (Santos et al. 2004). Additionally, there is evidence that bST plays a role in the specific time of maternal recognition of the embryo (Badinga et al., 2002), and is also associated with faster fetus growth. There is a positive correlation between the size of the fetus and the amount of interferon-τ secreted, which is a potent inhibitor of PGF2α synthesis (Rossetti et al., 2011).

Physiologically, bST reduces the sensitivity of insulin receptors in peripheral tissues, which results in a decrease in the uptake and oxidation of glucose by liver cells, muscle and adipose tissue. This results in greater availability of glucose in the bloodstream (Gulay et al, 2004; Etherton and Bauman, 1998). Glucose is considered to be a key source of energy for the production of adenosine triphosphate (ATP), through oxidative phosphorylation in mitochondria, which the embryo uses for the formation of new cells, thus being considered a major factor driving both fetal and placental development (Green et al., 2009).

In fact, in the present study, rbST significantly influenced serum concentrations of glucose (P<0,05) (Figure 2).

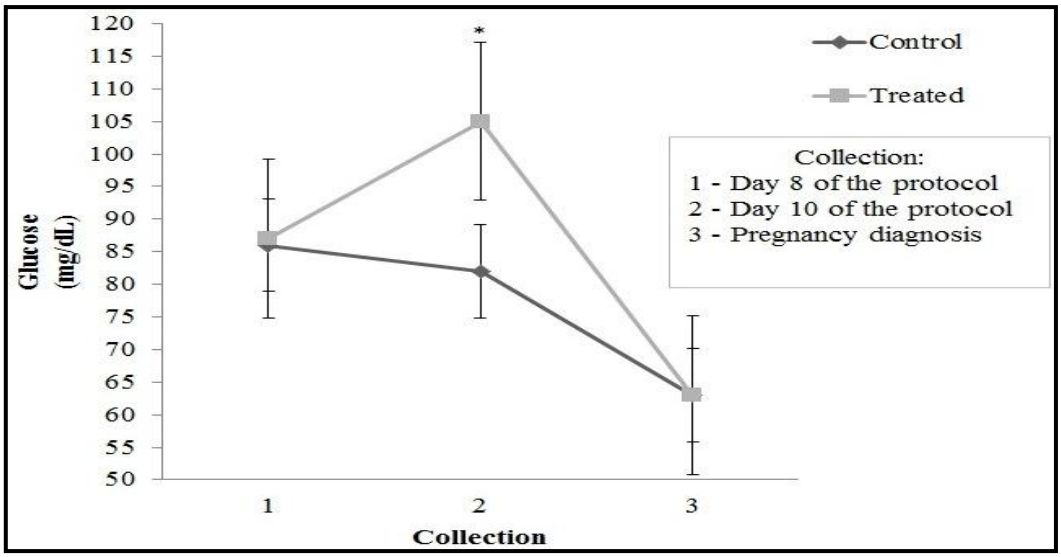

Fig. 2. Serum glucose concentrations in Nellore cows, on different days of the resynchronization protocol, with and without rbST (P <0.05); (*) Indicates the difference between treatments.

Another effect of the administration of rbST is an increase in triglycerides concentrations, due to the reduction in lipogenesis, inhibiting the formation of new reserves in the animal, which is in positive energy balance, thus meeting the energy needs during the reproductive state (Etherton and Bauman, 1998; Renaville et al., 2002). In the present work, administration of rbST yielded an increase in triglycerides serum concentrations (P<0,05) (Figure 3).

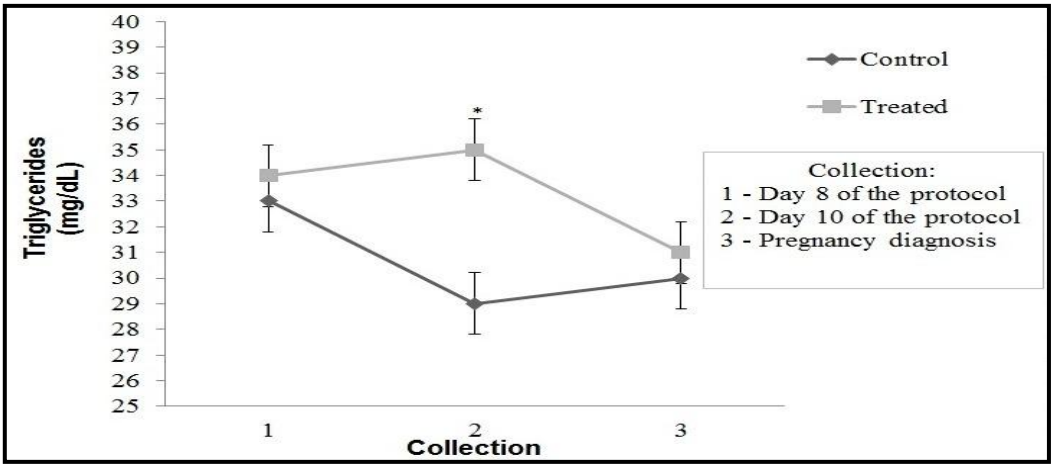

Fig. 3. Serum triglyceride concentrations in Nellore cows, on different days of the resynchronization protocol, with and without rbST (P <0.05); (*) Indicates the difference between treatments.

The administration of rbST did not promote an increase in serum cholesterol concentrations (Figure 4). However, the animals had serum concentrations above the reference values, for such animals under normal conditions (80-180 mg/dL) (Reece, 2006). Such concentrations are justified by the postpartum phase the animals were going through. This shows a direct relationship between serum concentration of cholesterol and the number of days postpartum, i.e., as the days pass, there is a decrease in body condition, and an elevation in the concentration of circulating cholesterol (Souza, 2005; Ruas et al., 2000); also, due to the fact that the animals are not in the luteal phase of the estrous cycle, there is no lipoprotein uptake or use for the synthesis of ovarian P4 (Borges et al., 2001).

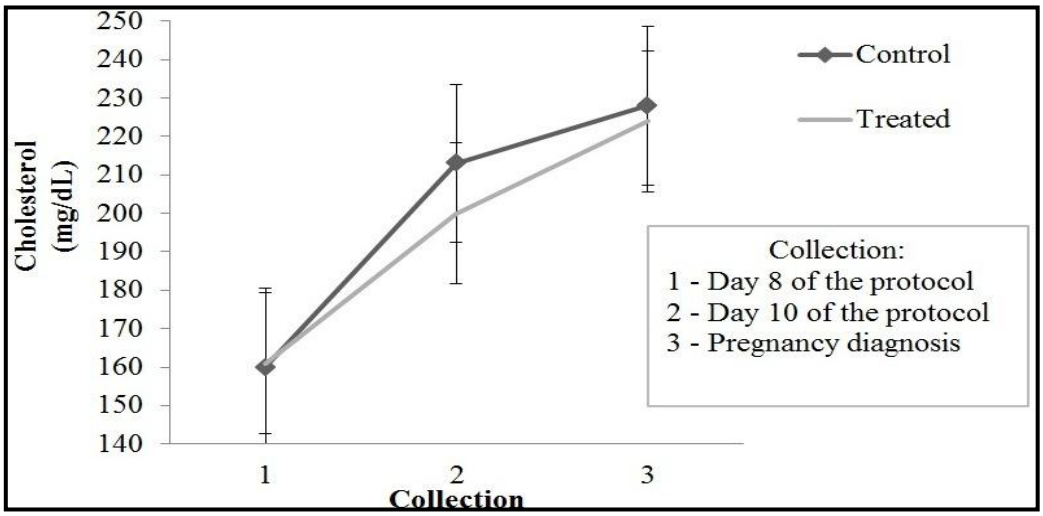

Fig. 4. Serum cholesterol concentrations in Nellore cows, on different days of the resynchronization protocol, with and without rbST (P <0.05).

Although rbST treatment had a positive effect on serum glucose and triglyceride levels, this hormone administered on day 8 of the resynchronization protocol did not produce any significant increase (P = 0.41) in

pregnancy rates, which were 40% and 48%, for control and treated groups, respectively. Such results are corroborated by Rossetti et al. (2011), who administered rbST to Nellore cows, seven days after FTAI; and Santos et al. (2004), who worked with Holstein cows, with rbST treatment 14 days before FTAI.

However, the absence of a significant effect in this study may have been due to the day of rbST administration. Further research will be necessary in order to establish the dosage and the best time for administration of this hormone.

Although rbST did not have a positive effect increasing pregnancy rates in resynchronization, it was possible to obtain, after synchronization and resynchronization, an accumulated pregnancy rate of 74.8% (n = 205), within a period of 45 days, without the need for estrus observation, thus providing two opportunities for the animals to be inseminated, within the same breeding season, which resulted in a gain for the farmer (Figure 5).

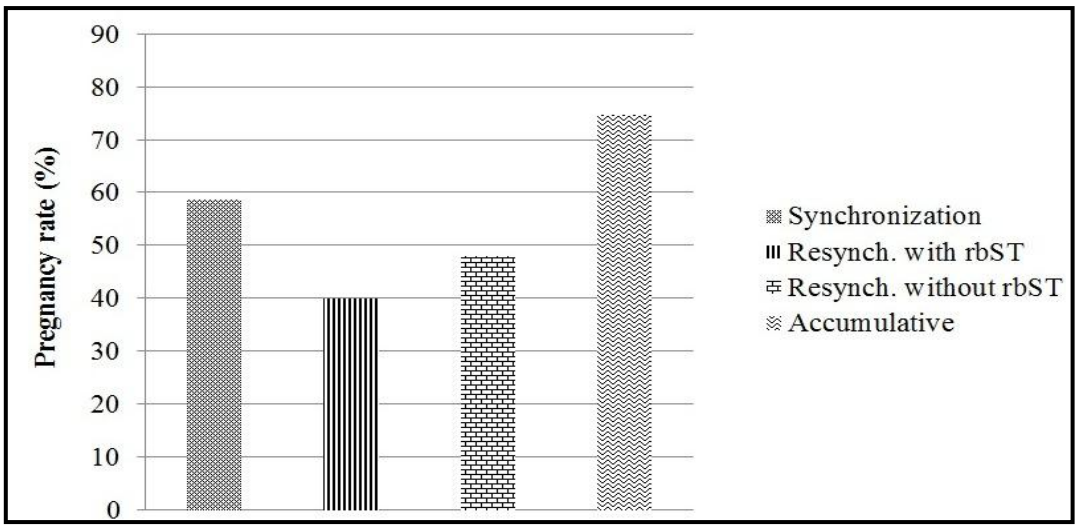

Fig. 5. Pregnancy rates in Nellore cows subjected to synchronization, resynchronization, with or without rbST, and accumulated pregnancy rate.

Meneghetti et al. (2012), Nascimento (2009), Giraldo (2008) and Freitas et al. (2007) also obtained similar results to those found in the present study.

4. Conclusion

The administration of rbST in multiparae Nellore cows, in a single dose, on day 8 of the resynchronization protocol increased the concentrations of glucose and triglycerides, and had no effect on cholesterol concentrations and pregnancy rates.

Resynchronization of oestrus and re-insemination had a positive effect, increasing cumulative pregnancy rates.

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