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Comparison between recombinant bovine somatotropin and equine chorionic gonadotropin in timed artificial insemination protocols in *Bos indicus* cows under low body condition score

[*Comparação entre a somatotrofina recombinante bovina e a gonadotrofina coriônica equina em protocolos de inseminação artificial em tempo fixo em vacas* Bos indicus *com baixo escore corporal*]

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ABSTRACT

This study aimed to evaluate the efficiency of recombinant bovine somatotropin (rbST) on pregnancy rate (PR), dominant follicle diameter (DFD) pre-ovulatory follicle diameter (DFPO), and follicular growth rate (FGR) in *Bos indicus* cows with low body condition score (BCS; mean = 2.3) subjected to fixed-time artificial insemination (FTAI) protocols. The females were divided into four groups: rbST group day zero (GbSTd0; n=31) received a progesterone (P4) intravaginal device + estradiol benzoate + rbST on d0 of the protocol; on d8, P4 was removed and PGF2α + estradiol cypionate were administered; on d10, FTAI was performed; GbSTd8; n=31) received the same treatment as GbSTd0, except rbST administration on d8; the control group (GC; n=42) received the same treatment as GbSTd0 but without rbST; equine chorionic gonadotropin group (eCGG; n=46) received the same treatment as GC + eCG on d8. Ovarian follicles were evaluated by ultrasonography (US) on d0, d8, and d10 of the protocols. PRs (at d35) and FGR between d8 and d10 of the protocol were calculated. PRs resulted 16.1%, 19.4%, 30.9%, and 43.4% in GbSTd0, GbSTd8, GC, and GeCG, respectively. eCGG PR differed significantly (P<0.05) from rbST groups but not to the GC; no difference was observed between rbST groups and GC. eCGG resulted in larger DFPO (11.2±1.5 vs. 9.6±0.5 in GbSTd0; 9.9±0.8 in GbSTd8; 10.1±1.3 in GC; P=0.0001) and greater FGR (2.20±1.03 vs. 0.93±0.50 in GbSTd0; 0.94±0.52 in GbSTd8; 1.17±0.64 in GC) (P<0.0001). It was concluded that rbST groups resulted in lower PR, FGR, and DFPO compared to eCGG in cows with reduced BCS; therefore, eCG is recommended for use in cows with low BCS in FTAI programs.

Keywords: recombinant bovine somatotropin, equine chorionic gonadotropin, *Bos indicus*, follicular dynamics, FTAI

RESUMO

O estudo objetivou verificar a eficiência da somatotrofina recombinante bovina (rbST) sobre a taxa de prenhez (TP), o diâmetro do folículo dominante (DFD) e do pré-ovulatório (DFPO) e a taxa de crescimento folicular (TCF) de vacas Bos indicus *com baixo escore corporal (ECC médio=2,3) submetidas a protocolos de inseminação artificial em tempo fixo (IATF). As fêmeas foram distribuídas em quatro grupos: grupo bST dia zero (GbSTd0; n=31) recebeu, no d0 do protocolo, um dispositivo intravaginal com progesterona (P4) + benzoato de estradiol + rbST; no d8, houve a remoção da P4 e foi aplicado PGF2*α *+ cipionato de estradiol; no d10, executou-se a IATF; GbSTd8 (n=31) recebeu idêntico*

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tratamento ao GbSTd0, exceto a aplicação da rbST no d8; grupo controle (GC; n=42) recebeu o idêntico tratamento ao GbSTd0, exceto a não aplicação da rbST; grupo gonadotrofina coriônica equina (GeCG;n=46) recebeu idêntico tratamento ao GC + eCG no d8. Os folículos ovarianos foram avaliados por exames de ultrassonografia (US) em d0, d8 e d10 dos protocolos. Foram calculadas as TPs (no d35) e a TCF entre d8 e d10 do protocolo. As TPs resultaram respectivamente em 16,1%; 19,4%; 30,9% e 43,4% nos GbSTd0, GbSTd8, GC e GeCG. As TPs do GeCG mostraram diferença (P<0,05) em relação aos grupos da rbST, mas não ao GC; não houve diferença entre os grupos rbST e o GC. A eCG resultou em maior DFPO (11,2±1,5 vs. 9,6±0,5 do GbSTd0; 9,9±0,8 do GbSTd8 e 10,1±1,3 do GC; P=0,0001) e maior TCF (2,20±1,03 vs. 0,93±0,50 do GbSTd0; 0,94±0,52 do GbSTd8, 1,17±0,64 do GC) (P<0,0001). Concluiu-se que os grupos rbST resultaram em menor TP, TCF e DFPO que a eCG, em vacas com reduzido escore ECC; recomenda-se o emprego da eCG em vacas com baixo ECC em programas de IATF.

Palavras-chave: somatotrofina recombinante bovina, gonadotrofina coriônica equina, Bos indicus, *dinâmica folicular, IATF*

INTRODUCTION

Advances in ovarian physiology and follicular dynamics in cattle have led to a significant increase in hormonal protocols for estrus and ovulation synchronization, resulting in an increase in artificial insemination in cattle (Sirois and Fortune, 1988).

Progesterone (P4) combined with estradiol (E2) suppresses follicular growth (FG), leading to the emergence of a new follicular wave between 3 and 4 d after administration (Tribulo *et al*., 1995; Caccia and Bó, 1998) and, together with luteolytic agents (PGF2α) applied in P4 withdrawal, provides the synchronization of estrus and ovulation (Bó *et al*., 2002; Cavalieri *et al*., 2002).

Equine chorionic gonadotropin (eCG) is also used to maximize results in fixed-time artificial insemination (FTAI) programs, especially when applied to *Bos indicus* cows (Baruselli *et al*., 2012; Bó *et al*., 2016). Because of its similar activity to luteinizing hormone (LH) and follicle-stimulating hormone (FSH), eCG can optimize ovarian FG, resulting in a larger corpus luteum (CL) diameter, as well as higher P4 concentrations, leading to higher reproductive efficiency (Lonergan *et al*., 2013; Bó *et al*., 2016). When applied at the time of removal of the P4 implant, eCG increases ovulation rates (ORs), follicular growth rates (FGRs), and pregnancy rate (PR), mainly in cows with low body condition score (BCS) and/or postpartum anestrus (Sales *et al*., 2011; Pessoa *et al*., 2016).

In turn, positive effects have been verified when recombinant bovine somatotropin (rbST) is associated with bovine reproduction (Lucy *et al*., 1994; Kirby *et al*., 1997b; Kaminski *et al*., 2019). The rbST has been associated with elevated serum levels of insulin growth factor 1 (IGF1) (Bartolome *et al*., 2002; Baruselli *et al*., 2019; Gong *et al*., 1997). Indirectly, rbST binds to hepatic growth hormone receptors (GHRs), stimulating the synthesis and serum release of IGF1 (Gong *et al*., 1997; Etherton and Bauman, 1998; Baruselli *et al*., 2019) and promotes FG together with pituitary gonadotropins (Moreira *et al*., 2000; Gong, 2002; Fortune *et al*., 2004). Through IGF1, rbST acts on the cells of the granulosa and theca interna of the follicle, stimulating cell proliferation, increasing E2 production and, consequently, promoting better positive feedback on the release of gonadotropin releasing hormone (GnRH). It leads to more efficient recruitment of the preovulatory LH peak and better ovulation synchrony (Gong *et al*., 1997; Lonergan *et al*., 2013; Kaminski *et al*., 2019). Furthermore, the rbST was positively correlated with an increased number of recruited follicles, early emergence of the second follicular wave (Lucy *et al*., 1994; Kirby *et al*., 1997a), higher FGR, and higher oocyte growth and maturation. The rbST has also been correlated with higher PR, better embryonic development, better CL function, and maternal recognition of pregnancy (Figueiredo *et al*., 1997; Hernández-Cerón and Gutierrez-Aguilar, 2013). Kaminski *et al*. (2019) correlated the use of rbST administered on d0 of the protocol to the highest FGR, preovulatory follicle diameter (POF), and OR.

In another study Burton *et al.* (1994) reported that the increase in IGF1 in response to rbST manifests only in animals with good BCS. Therefore, the response to rbST is directly influenced by positive and/or negative energy balance (PEB/NEB) (Lucy *et al.*, 2001, 2013; Armstrong *et al.*, 2002). Furthermore, the plasma concentrations of IGF1 differ between bovine genetic groups (Simpson *et al.*, 1994; Alvarez *et al.*, 2000). Studies have shown that although *Bos taurus* females have larger POF and CL structures, *Bos indicus* females have higher concentrations of E2 and IGF1 hormones (Bastos *et al.*, 2010; Sartori *et al.*, 2016). In *Bos taurus*, the use of rbST has been correlated with improved embryonic development through the actions of IGF1 (Hasler *et al.*, 2003; Martins *et al.*, 2012). However, the beneficial effects of rbST in *Bos indicus* animals have not yet been verified, suggesting that there are differences in the response to this hormone (Martins *et al.*, 2012). Thus, there are physiological differences between *Bos taurus* and *Bos indicus* that must be considered to establish times and doses that may result in better reproductive efficiency.

In addition, the rbST was originally developed with the aim of increasing milk production in dairy cows (*Bos taurus*), in which metabolism is faster than in beef cows (Sangsritavong *et al.*, 2002; Shingu *et al.*, 2002). It has been suggested that, in crossbred *Bos taurus Bos indicus* cows, doses lower than 500 mg would be sufficient to promote a satisfactory response to the hormone (Fontes *et al.*, 1997; Phipps *et al.*, 1997). However, doses for application in beef cattle to improve reproductive performance have not yet been established, emphasizing the reduction of reproductive efficiency were observed when excessive doses (650 mg) were utilized (Oosthuizen *et al.*, 2018). Thus, this study hypothesizes that the use of 250 mg of rbST in cows with reduced BCS for FTAI results in better reproductive efficiency than eCG.

The present study aimed to compare the effects of 250 mg of rbST in different moments of the FTAI protocols *versus* 300 UI eCG in multiparous *Bos indicus* cows with reduced BCS on PR, largest follicle diameter, and FGR.

MATERIAL AND METHODS

The project was approved by the ethical committee of Pontifícia Universidade Católica do Paraná (approval number 01608). The study was carried out in a commercial beef cattle farm under a humid subtropical climate, at coordinates (25° 02’ 3″ S, 52° 40’ 49″W), with an average temperature of 18.3°C and an annual rainfall of 1,752mm. A total of 150 multiparous *Bos indicus* (Nelore) cows with mean age of 4.5±0,8 years, mean of 45±3 d postpartum (DPP), and BCS (1.0 = very thin, 2.0 = thin, 3.0 = ideal, 4.0 = fat, and 5.0 = obese (Lowman *et al.*, 1976). Animals between 1.5 and 2.5 were maintained ad libitum on a pasture predominantly containing Cynodon dactylon and Brachiaria decumbens, water, and mineral salt. The BCS of the animals was measured at the beginning of the study (d0). The health status of the farms was routinely monitored with updated vaccination protocols to ensure that it was free of brucellosis and bovine tuberculosis.

Only sanitarily healthy cows capable of reproduction with a BCS between 1.5 and 2.5 (scale from 1 to 5, (Lowman *et al.*, 1976)) were included.

On d0 of protocol, cows with BCS higher than those presented above, as well as females with reproductive problems, such as metritis, endometritis, delayed uterine involution, ovarian cysts, and atrophic ovaries, among others, were excluded. Cows with follicles ≤ 4.0 mm and without CL were considered anestrus (Ferreira, 2010) and were not included in the experiment. Throughout the synchronization protocol, cows that had follicles between 5.0 and 6.0mm on d0, but that did not show larger follicles on d8, were also excluded from the experiment.

The animals were randomly distributed into the following groups: rbST group on d0 (bSTGd0, n = 31), rbST group on d8 (bSTGd8, n = 31), control group (CG, n = 42), and eCG group (eCGG, n = 46). bSTGd0 received an intravaginal device with P4 (1.0g, Cronipres® monodose, Biogenesis Bagó, Curitiba, Brazil) + EB (2.0mg, im, Cronibest®, Biogenesis Bagó, Curitiba, Brazil) + rbST (250mg, sc, Lactotropin InjectableTM, ElancoTM, Georgia, USA) on protocol d0. On d8, the device was removed and D-cloprostenol (150mg, im, Croniben®, Biogenesis Bagó, Curitiba, Brazil) and EC (0.5mg, im, Croni-CIP®, Biogenesis Bagó, Curitiba, Brazil) were applied. On d10, TAI was performed. The bSTGd8 received the same protocol, except for rbST, which was administered on d8. The CG received the same treatment as that of the bSTGd0, except that rbST was not applied. The eCGG received the same treatment as that of the CG, with the addition of 300 IU eCG (im, Ecegon®, Biogenesis Bagó, Curitiba, Brazil) on d8 (Figure 1).

Commercially available frozen semen was used for insemination. Pregnancy diagnosis was performed on d45 via transrectal ultrasonography (SonosCape® A6v, L531v 3.5 to 7.5 MHz rectilinear transducer, China).

The ovaries were evaluated using transrectal ultrasonography on d0, d8, and d10 of the protocols. The diameter (mm) of the dominant follicle (DF) and POF were measured, considering a larger diameter plus smaller diameter divided by 2 (Figueiredo *et al*., 1997). The FGR was calculated using the difference in the diameters of DF on d8 and d10 of the protocols (size of POF at d10 less size of FD at d8; mm; (Viana *et al*., 2000; Coutinho *et al*., 2007). Follicles were growing when they showed an increase greater than 5% between d8 and d10.

Statistical analyses were performed using the IBM SPSS Statistics software, version 24 (IBM Corp., Armonk, NY, USA). Levene's test was used to assess the homogeneity of variance, a fundamental premise for the subsequent use of ANOVA, followed by the Tukey or Bonferroni test according to homoscedasticity. Analysis of variance included the group factor at d0, d8, and d10 of the protocol to compare the means of DF, POF, and FGR. The PR and percentage of animals with FG between the groups were compared using the Chi-square test with correction for unweighted samples, aiming at a more reliable result between the groups with different numbers of animals. Correlations among the variables were verified using Pearson's test. For all statistical analyses, significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents data related to PR for each group.

Table 1 shows the results of PR of different groups. The PR was better ($P < 0.05$) in eCGG than in both the groups treated with rbST and is similar to CG. Reports by Cutaia *et al*., (2003) confirmed the beneficial effects of eCG even in animals with reduced BCS, such as those employed in the present study.

Table 1. Pregnancy rate (PR) in the recombinant bovine somatotropin (rbST) groups administered on d0 (bSTGd0), d8 (bSTGd8), eCG group (eCGG), and control group (CG) in *Bos indicus* cows

Pregnancy rate on d45 n (%)			
bSTGd0	bSTGd8	eCGG	CG
5/31 (16.1)^{b}	6/31 (19.4)^{b}	20/46 (43.4)^{a}	13/42 (30.9)^{ab}

Different letters indicate significance at $P < 0.05$

Pearson's correlation test showed that BCS and DPP were positively correlated with PR in all groups with the correlations between BCS and PR ($P < 0.0001$) and DPP and PR ($P = 0.0002$) being highly significant. The impact of BCS on reproductive performance was demonstrated by Gottschall *et al*. (2011), who obtained PR values of 55, 47, and 31 using animals under BCS > 2.5, equal to 2.5, and < 2.5, respectively, corroborating the differences between the PR observed in the present study.

Table 2 shows data from the animals in the protocols that showed growth of DF between d8 and d10.

Table 2 shows the percentage of animals in each group showing FG between d8 and d10. The eCGG treatment resulted in greater follicular growth in comparison to the other treatments, whereas the bSTGd0 treatment had the lowest growth. At the beginning of the study, follicles with dimensions of < 10 mm were observed in all groups and were even smaller than those reported by Ferreira (2000). The cows used in the present study were in the puerperium phase (45 d) under reduced BCS due to NEB resulting from the lack of good pastures at parturition time. The cows were also considered nursing. In addition, there is a reduced supply of forage, adversely influencing postpartum ovarian activity, resulting in reduced FG in these animals (Table 2; Ferreira *et al.*, 2000; Vanholder *et al.*, 2006). Animals under NEB have a lower frequency of LH pulses, lower FGR, and lower DF diameter, in addition to reduced serum concentrations of IGF1, glucose, and insulin (Bastos *et al.*, 2004). Cows with low BCS in the postpartum period require 60–120 d for the first ovulation (Stagg *et al.*, 1998). The prolongation of the postpartum period (influenced by nutrition and low BCS) is also due to the presence of the suckling calf, which interferes with the re-establishment of LH storage, generating low PR and anestrus (Williams, 1990). Additionally, a reduced circulating LH concentration up to d45 postpartum may be caused by a deficiency in the number of GnRH receptors in the anterior pituitary (Nett *et al.*, 1988).

Table 2. Diameters of the largest follicle (⦸>F), dominant follicle (⦸DF) and preovulatory follicle diameter (⦸POF), follicular growth rate (FGR), and pregnancy rate (PR) of *Bos indicus* cows subjected to different protocols showing follicular growth (FG) between d8 and d10

Groups	FG between d8 to d10 n (%)	⦸>F d0 (mm) (x ± s)	⦸DF d8 (mm) (x ± s)	⦸POF d10 (mm) (x ± s)	FGR d8 to 10 (mm) (x ± s)	⦸POF pregnant cows (mm) (x ± s)	⦸POF Non-pregnant cows (mm) (x ± s)	PR n (%)
bSTGd0	12/31 (38.7)^{c}	9.6±1.1	8.6±0.5	9.6±0.5^{b}	0.93±0.50^{b}	9.9±0.7^{b}	9.4±0.3	5/31 (16.1)^{b}
bSTGd8	18/31 (58.0)^{bc}	9.5±0.9	8.9±0.8	9.9±0.8^{b}	0.94±0.52^{b}	10.6±0.2^{b}	9.6±0.7	6/31 (19.4)^{b}
eCGG	42/46 (91.3)^{a}	9.6±0.9	9.0±1.1	11.2±1.5^{a}	2.20±1.03^{a}	12.1±0.9^{a}	10.3±1.5	20/46 (43.4)^{a}
CG	29/42 (69.0)^{b}	9.4±1.7	8.9±1.4	10.1±1.3^{b}	1.17±0.64^{b}	11.4±0.7^{ab}	9.3±0.9	13/42 (30.9)^{ab}
P value	< 0.05	0.9712	0.8507	0.0001	< 0.0001	0.0002	0.0616	< 0.05

Different letters in the same column represent a significant difference ($P < 0.05$). d = day.

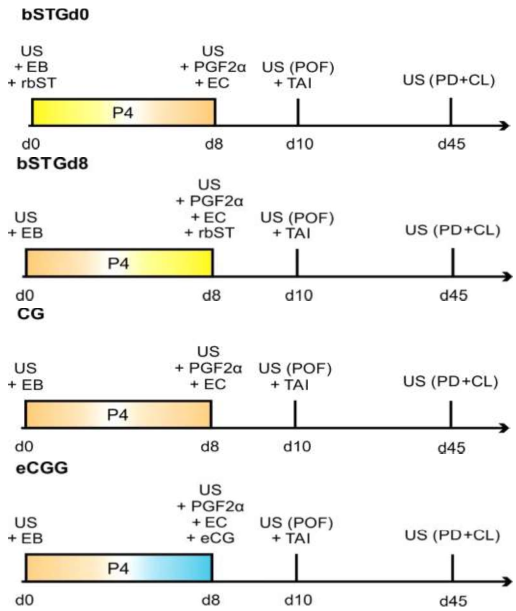
On the other hand, cows with follicles ≥10mm at the beginning of a hormonal FTAI protocol showed improved response to hormone therapy when they were under adequate BCS (Martinez *et al.*, 1999; Amaral *et al.*, 2008). Animals with good BCS have LH stores reestablished between 15 and 30 d after parturition (Yavas and Walton, 2000), with the first ovulation usually occurring between 27 and 37 d after parturition (Stagg *et al.*, 1998). Higher PR was verified in cows under high BCS at d56 postpartum compared to those under low BCS (McDougall and Compton, 2005). Silveira *et al.* (2010) used eCG in cows at d35 and d40, and later (from d45 postpartum) obtained a PR of 37.9 and 51.7%, respectively. In the present study, eCGG partially confirmed the findings of these authors, with a PR of 43.4%. The lowest FG responsiveness rates were observed in both rbST groups compared to eCG (Table 2). Could the release and metabolic rates of rbST acting on follicles be slower than the effects of eCG? Could eCG be a more specific gonadotropin that acts more directly on the hypothalamic-gonadal axis than rbST? Could a likely cause be the composition of eCG (FSH and LH)? It is believed that these questions can be answered through further studies.

The differences in diameter of the DF on d8 were not verified among all groups (Table 2).

However, a significant difference ($P < 0.05$) was observed in the diameter of the POF in the eCGG (11.2 mm) compared to those of other groups, which also showed a higher FGR between d8 and d10 (2.20 mm). The use of eCG at the removal of the P4 implant (d8) led to an increase in OR and PR, mainly in cows with low BCS and/or postpartum anestrus (Sales *et al*., 2011; Pessoa *et al*., 2016), corroborating the best eCG performance (Table 2). It is possible that OR and PR are related to the larger diameter of the POF and FGR verified in the eCGG, with more efficient hormonal responsiveness and release of the preovulatory peak of LH. Larger follicles have a higher intrafollicular concentration of E2, leading to a higher release of GnRH and LH, favoring ovulation synchrony (Atkins *et al*., 2010; Ferreira, 2010). In addition, the larger diameter of the POF is positively correlated with PR by increasing the number of LH receptors and responsiveness to this hormone (Dias *et al*., 2009), leading to an increase in follicular diameter and OR (Simões *et al*., 2012).

In the other groups, as the POF decreased, the ovulation capacity was reduced, resulting in reduced PR (Table 2). Follicles with smaller diameters at the time of FTAI are immature and less fertile, resulting in a lower PR (Atkins *et al*., 2010; Perry *et al*., 2007). According to Schams and Berisha (2002), bovine follicles with a diameter of 5–7, 8–10, 10–12, 13–14, and > 14 mm have < 0.5, 0.5–5, 10–12, 5–20, 20–180, and > 180ng/mL of intrafollicular E2, respectively. Follicles measuring 8–10 mm acquire the ability to respond to the preovulatory peak of LH with ovulation (Driancourt, 2001), although follicles with smaller diameters can ovulate and form CL with lower weight and dimensions, resulting in lesser production of P4 and ability to maintain pregnancy (Driancourt, 2001; Siqueira *et al*., 2009).

In contrast, in the rbST groups, smaller POF diameters were observed, especially in bSTGd0, but without any difference. The respective FGR and PR were lower ($P < 0.05$) than those of the eCGG (Table 2). It was expected that the rbST administered on d0 or d8 would lead to the maximization of FGR, PR, and mean diameters of DF and POF owing to the stimulation of intrafollicular IGF1 and E2 production (Jones and Clemmons, 1995; Acosta *et al*., 2017), which was not verified in this study.



US: transrectal ultrasonography; P4: intravaginal device with controlled-release progesterone (1.0 g of P4, Cronipres® monodose, Biogenesis Bagó, Curitiba, Brazil); EB: estradiol benzoate (2.0 mg IM, Cronibest®, Biogenesis Bagó, Curitiba, Brazil); rbST: recombinant bovine somatotropin (250mg, Lactotropin Injectable™, Elanco™, Georgia, USA); PGF2: D-cloprostenol (150mg im, Croniben®, Biogenesis Bagó, Curitiba, Brazil); EC: estradiol cypionate (0.5 mg im, Croni-CIP®, Biogenesis Bagó, Curitiba, Brazil); eCG: equine chorionic gonadotropin (300 IU im, Ecegon®, Biogenesis Bagó, Curitiba, Brazil); POF: preovulatory follicle; FTAI: fixed-time artificial insemination; PD: pregnancy diagnosis; CL: corpus luteum.

Figure. 1. Diagrams showing the hormonal protocols administered in multiparous *Bos taurus indicus*, beef cows for fixed-time artificial insemination, under ovarian monitoring using ultrasonography. (a) Recombinant bovine somatotropin group on d0 (bSTGd0). (b) Recombinant bovine somatotropin group on d8 (bSTGd8). (c) Control group. (d) Equine chorionic gonadotropin group on d8 (eCG).

The effect of rbST on the reproductive system occurs indirectly, mainly through the somatotropic axis (Etherton and Bauman, 1998). The rbST binds to hepatic GHRs (Butler *et al*., 2003) and stimulates the production and serum release of IGF1 (Etherton and Bauman, 1998). IGF1, together with gonadotropins, promotes FG (Fortune *et al*., 2004) by increasing E2 production and acting on the granulosa and theca interna layers of the follicle, leading to more efficient LH recruitment and better ovulation synchrony (Lonergan *et al*., 2013; Acosta *et al*., 2017). However, for this to occur, the somatotropic axis needs to be coupled (Fig. 2); that is, the response mechanism to synthetic GH depends on the availability and sensitization of GHRs (Hauser *et al*., 1990), which is directly influenced by the balance of positive or negative energy (Lucy *et al*., 2001, 2013; Lucy, 2011); In addition, IGF1 acts as an endocrine regulator of GH through negative feedback. That is, the higher the concentration of IGF1, the lower the production and release of GH by the adenohypophysis, and the opposite is also true (Le Roith *et al*., 2001; Etherton, 2004).

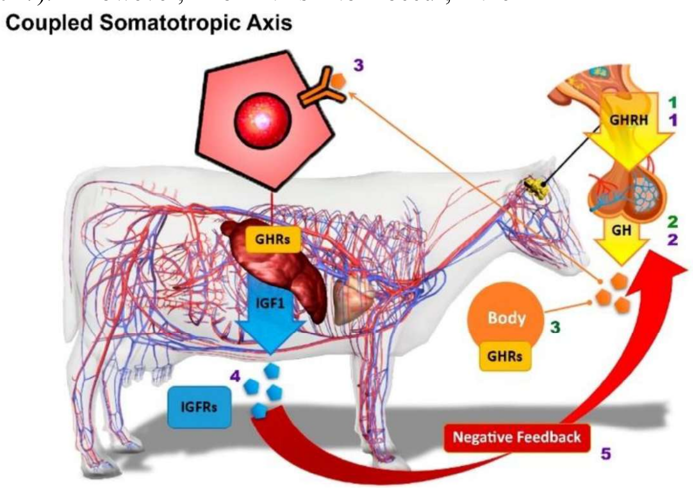
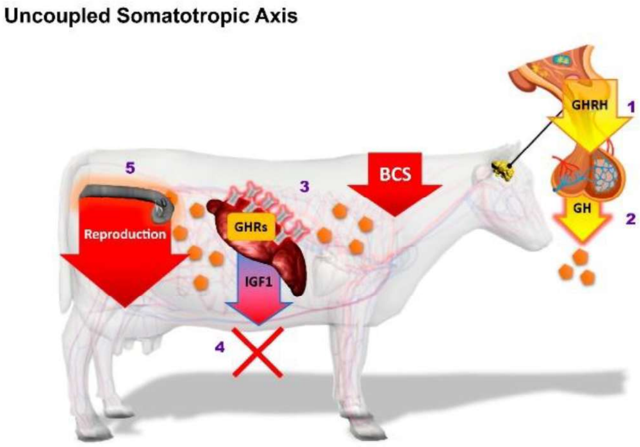


Figure 2. Schematic diagram of the coupled somatotropic axis and its chain of direct and indirect physiological mechanisms. GHRH: growth hormone releasing hormone; GH: growth hormone; body: animal organism as a whole; GHRs: growth hormone receptors; IGF1: insulin-like growth factor type 1; IGFRs: insulin-like growth factor receptor type 1; negative feedback: negative feedback mechanism caused by IGF1; green numbers: sequence of mechanisms via direct action of GH; purple numbers: sequence of mechanisms via the indirect action of GH. Source: Gassenferth, 2019; Modified 3D figure from Bovine Anatomy Software (Biosphera.org).

It is likely that almost all animals had an altered GH:IGF1 ratio due to low BCS (2.3 ± 0.2) and NEB (Armstrong *et al*., 2002; Lucy *et al*., 2013). Consequently, it is possible that the somatotropic axis was “uncoupled” (Fig. 3), causing a decrease in the synthesis of IGF1, with no negative feedback on GH occurring, and thereby, increasing serum levels of GH (Fenwick *et al*., 2008; Lucy, 2011). This mechanism is commonly observed in animals during the postpartum period (especially in dairy), resulting in detrimental reproductive functions (Lucy, 2011). Bossis *et al*. (1999) demonstrated this mechanism by nutritionally inducing anestrus in beef heifers, where GH levels increased during two consecutive reproductive cycles before females entered the anestrus phase. The same authors demonstrated that the levels of IGF1 were reduced, with non-responsive hepatic GHRs to GH due to malnutrition.

It is possible that an even higher quantity of GH was induced by rbST treatment in both rbST-treated groups, which could have led to insufficient IGF1 production, resulting in unsatisfactory reproductive performance. Reduced IGF1 levels combined with elevated GH levels are directly associated with impaired reproductive function (Lucy, 2011). This statement is consistent with the low reproductive efficiency observed in the rbST groups, especially in bSTGd0 (Table 2).



IGF1: insulin-like growth factor type 1; reproduction: negative influence on reproductive factors; purple numbers: sequence of mechanisms. Source: Gassenferth, 2019; Modified 3D figure from Bovine Anatomy Software (Biosphera.org).

Figure 3. Schematic diagram of the uncoupled somatotropic axis and its chain of mechanisms. GHRH: growth hormone releasing hormone; GH: growth hormone; BCS: body condition score; GHRs: growth hormone receptors;

Another aspect to be considered is that cows treated with rbST probably had different degrees of lipolysis, even more compromising NEB and low BCS, affecting the non-responsiveness or non-recruitment of the LH preovulatory peak. Muller *et al*. (1999) and Butler *et al*. (2003) reported that high GH levels stimulate lipolysis to reduce reproductive function. Elevated GH synthesis may be associated with the blockade of hypothalamic GnRH synthesis (Chagas *et al*., 2007), similar to the occurrence of reduced levels of IGF1 and insulin leading to insufficient E2 production by the POF and resulting in a lack of preovulatory LH and ovulation (Beam and Butler, 1999). Low concentrations of IGF1, combined with high levels of GH, result in ovulation failure (Bossis *et al*., 1999; Ferreira, 2010). This supports the fact that cows in such groups have smaller follicles in the ovaries, low FGR, and reduced PR. In all groups, positive correlations ($P < 0.05$) were observed between PR and BCS, POF diameter and BCS, and POF diameter and PR. Data related to cows presenting follicular atresia between d8 and d10 are shown in Table 3.

Table 3. Diameters of the largest follicle (⦰>F), dominant follicle (⦰DF) and preovulatory follicle (⦰POF) and follicular atresia rate (FAR) in *Bos indicus* cows subjected to different protocols and presenting follicular atresia between d8 and d10

Group	Cows with follicular atresia between d8 and d10 n (%)	⦰> F d0 (mm) (x ± s)	⦰> DF d8 (mm) (x ± s)	⦰POF d10 (mm) (x ± s)	FAR between d8 and d10 (mm) (x ± s)
bSTGd0	19/31 (61.3)[a]	9.1 ± 0.8	8.2 ± 0.4[b]	7.0 ± 0.6[b]	−1.16 ± 0.50
bSTGd8	13/31 (42.0)[ab]	9.0 ± 1.3	8.8 ± 0.7[ab]	7.9 ± 0.9[a]	−0.87 ± 0.76
eCGG	4/46 (8.7)[c]	8.7 ± 0.7	9.3 ± 0.5[a]	8.8 ± 0.7[a]	−0.51 ± 0.46
CG	13/42 (30.9)[b]	9.2 ± 1.6	8.8 ± 1.1[ab]	8.1 ± 1.0[a]	−0.63 ± 0.56
P value	< 0.05	0.9189	0.0203	0.0007	0.0592

Different letters in the same column represent a significant difference at the level of $P < 0.05$.

The animals in the rbST groups showed higher follicular atresia rates (FARs) between d8 and d10 of the protocol compared to eCGG (Table 3; $P < 0.05$). These data suggest that cows with low BCS treated with rbST (mainly on d0) had an increase in serum GH levels. This may have increased the GH:IGF1 ratio and blocked the release of GnRH (Chagas *et al*., 2007) resulting in insufficient production of E2 by the POF, failure of the preovulatory LH peak, and ovulation (Beam and Butler, 1999). This was evident when observing the dimensions of the POF diameter, showing the lowest value in the bSTGd0 between the groups ($P < 0.05$; Table 3).

The responsiveness of the DF to the ovulation inducer depends on the dimensions of DF, which explains the significant variation in results of PR between protocols (Gimenes *et al*., 2008). Considering that most animals of the bSTGd0 and bSTGd8 groups presented follicles in atresia and dimensions of the POF, it was concluded that the animals in these groups had reduced POF to exert an adequate response to the hormonal stimulus. A considerable percentage of animals in these groups showed follicular atresia (61.3 and 42.0% for bSTGd0 and bSTGd8, respectively), resulting in reduced PR (Table 1). Carvalho *et al*. (2008), working with *Bos indicus* cows, verified a high percentage of non-ovulation because the POFs were still immature at the end of the protocol. In addition, the large quantity of small POF led to a considerable percentage of ovulation failures and delays, as well as a lower steroidogenic capacity.

Studies on the use of rbST in beef cattle for FTAI are scarce. Martins *et al*. (2012) found beneficial effects on the reproductive performance of *Bos taurus* (Brangus and Holstein Black and White) cows but not of *Bos indicus* (Nellore) cows. Thus, it is possible to deduce that the physiological differences between *Bos taurus* and *Bos indicus* animals must be considered. Few studies have established ideal dosages, moments (timing), and mode of administration regarding the use of rbST in favor of its efficiency in FTAI protocols.

The effects of rbST can be influenced by several variables related to NEB, BCS, and genetic aspects, among others, where significant correlations between PR and BCS, POF diameter, and PR ($P < 0.05$) have been found (Etherton and Bauman, 1998; Lucy, 2011). However, further studies are required in this area. Our research group will continue to conduct rbST research on FTAI protocols.

CONCLUSION

In conclusion, rbST used in FTAI protocols did not increase the PR, FGR, or POF diameter compared to the protocol that used eCG in *Bos indicus* beef cows under low BCS. Therefore, the use of eCG in beef cows with reduced BCS in FTAI programs is recommended.

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