

Effect of Recombinant Bovine Somatotropin on Plasma Concentrations of Insulin-like Growth Factor I, Insulin and Membrane Integrity of Bull Spermatozoa

MB Vieira, I Bianchi, EM Madeira, VFB Roll, CA Oliveira, P Viau, I Pivato, NC Severo, FAB Del Pino, A Schneider and MN Corrêa

Federal University of Pelotas, Pelotas, Brazil

Contents

This study aimed to evaluate the effect of the exogenous recombinant bovine somatotropin (rbST) on plasma concentrations of insulin-like growth factor I (IGF-I), insulin and semen quality of bulls. Twenty bulls (Aberdeen Angus and Brangus) were divided by breed into two groups. Placebo group was injected with NaCl 0.9% (s.c.) and treatment group with rbST (s.c., 500 mg) at days 0 and 14 of the experiment. Immediately after semen collection, blood samples were taken on days 0, 14, 28, 42 and 56 of the experiment. Semen was also collected on day 70 of the experiment. Evaluation of sperm motility was performed at pre-freezing and post-thawing stage, whereas assessment of sperm membrane integrity was performed after freezing and thawing. Analysis of data revealed that the effect of treatment and treatment-by-collection day on plasma concentrations of IGF-I and insulin was not significant. However, mean plasma concentrations of IGF-I and insulin were affected ($p < 0.0001$) by days of blood sampling. Effect of treatment and treatment-by-collection day on motility of spermatozoa was similar ($p > 0.05$) at pre-freezing and post-thawing stage. Intactness of plasmalemma and tail membrane of spermatozoa at post-thawing stage was higher ($p < 0.05$) in rbST-treated group than in control. In conclusion, rbST did not affect plasma concentrations of IGF-I and insulin, however, it did improve post-thaw sperm membrane integrity.

Introduction

It is well known that cryopreservation compromises the quality and fertility of spermatozoa as compared with that of fresh or liquid semen. Damage caused by cryopreservation is the combination of cellular death and damage to functional capacity of live cells (Watson 2000). Cryo-resistance by cells largely varies from one individual to other, and thus it may be linked to the metabolic status of an individual (Watson 2000).

Growth hormone (GH) is a metabolic hormone that acts directly on the male gonads through its receptors and regulates the processes of steroidogenesis and spermatogenesis (Arsenijevic et al. 1989; Spiteri-Grech and Nieschlag 1990; Chandrashekar et al. 2001). The main mediator for GH action at testicular level is insulin-like growth factor I (IGF-I), a polypeptide, secreted by mitogenic cells mainly synthesized in the liver (Schams et al. 1999; Sirotkin 2004). It has been reported that exogenous injection of recombinant bovine somatotropin (rbST) increases the serum levels of IGF-I in beef cattle (Schwarz et al. 1993). Henricks et al. (1998) reported the presence of IGF-I in bovine seminal plasma; affecting the motility of spermatozoa. Furthermore, Hafez et al. (2005) reported that exogenous rbST injections improved physical characteristics of bull semen and conception rates in field trials.

Therefore, the present study was designed to study the effect of rbST on plasma concentrations of IGF-I, insulin and post-thawing quality of bull spermatozoa.

Materials and Methods

For this study, 20 adult bulls (10 Aberdeen Angus and 10 Brangus), between 54 and 60 months old, were allocated by breed into two groups. Placebo group was injected twice with NaCl 0.9% (s.c.) and treatment group with rbST (s.c., 500 mg, Lactotropin[®], Elanco, St Louis, MO, USA). The injections were administered on days 0 and 14 of the experiment.

Blood samples were collected on days 0, 14, 28, 42 and 56, immediately after semen collections, via puncture of the jugular vein into one flask containing EDTA 10%. Samples were centrifuged at $1000 \times g$ for 15 min, stored in microtubes and frozen at -80°C until analysis.

Insulin-like growth factor I concentrations were evaluated by radioimmunoassay (DSL-5600 Active[®] with extraction; DSL, Webster, TX, USA). The method includes an extraction procedure with acid-ethanol to separate IGF-I from its binding-proteins. The sensitivity of the assay was 2.2 ng/ml. The intra- and interassay coefficients of variation were 2.05% and 4.9%, respectively. Insulin was evaluated by the electrochemiluminescence immunoassay method using the Elecsys Insulin Roche[®] analyzer (Roche Diagnostics, Mannheim, Germany). The sensitivity of the assay was 0.13 ng/ml. The intra- and interassay coefficients of variation were 3.65% and 8.25%, respectively.

Semen was evaluated for motility, concentration and morphology. Semen evaluations were performed on the same day of blood collections plus an evaluation on day 70. The percentage of progressive motile cells was evaluated in a phase contrast optical microscopy under $200 \times$ magnification; sperm concentration was measured on a spectrophotometer (IMV Technologies, L'Aigle, France); and morphology evaluation was performed according to Barth and Oko (1989). After evaluations, the semen was frozen using the Bioxcell[®] extender (IMV Technologies, L'Aigle, France), and packed in 0.5 ml straws (15×10^6 spermatozoa).

After freezing, two semen straws of each bull were thawed at 37°C for 30 s. Evaluation of sperm motility and morphology was performed as previously described. Tail membrane integrity was evaluated through the hyposmotic swelling test. For this, two solutions were used; one with 100 mOsm/l and another with 290 mOsm/l (Vazquez et al. 1997). First, 1 ml of semen was diluted in 10 ml of each solution and incubated for

1 h. Then, 200 spermatozoa were counted under 1000 × magnification. The percentage of spermatozoa that presented a curled tail, indicating intactness of tail membrane, was recorded. Plasmalemma integrity was evaluated by fluorescent probes in an epifluorescence microscope. The semen was incubated with carboxyfluorescein diacetate (C 5041; Sigma, St Louis, MO, USA) and propidium iodide probes (P 4170; Sigma, St Louis, MO, USA). After incubation, 200 spermatozoa were counted and classified as having intact (green fluorescence) or damaged plasmalemma (red fluorescence),

through stimulation in WU filter (America Inc., Center Valley, PA, USA) at 400 × magnification (Harrison and Vickers 1990).

The results are presented as mean ± SE of the mean. All the statistical analyses were performed on SAS (SAS Institute Inc., Cary, NC, USA). Data were analyzed as repeated measures within bull using the unstructured adjustment. When the effect of treatment, collection day or treatment-by-day interaction was significant ($p < 0.05$). Pair-wise comparisons were carried out with Tukey–Kramer test.

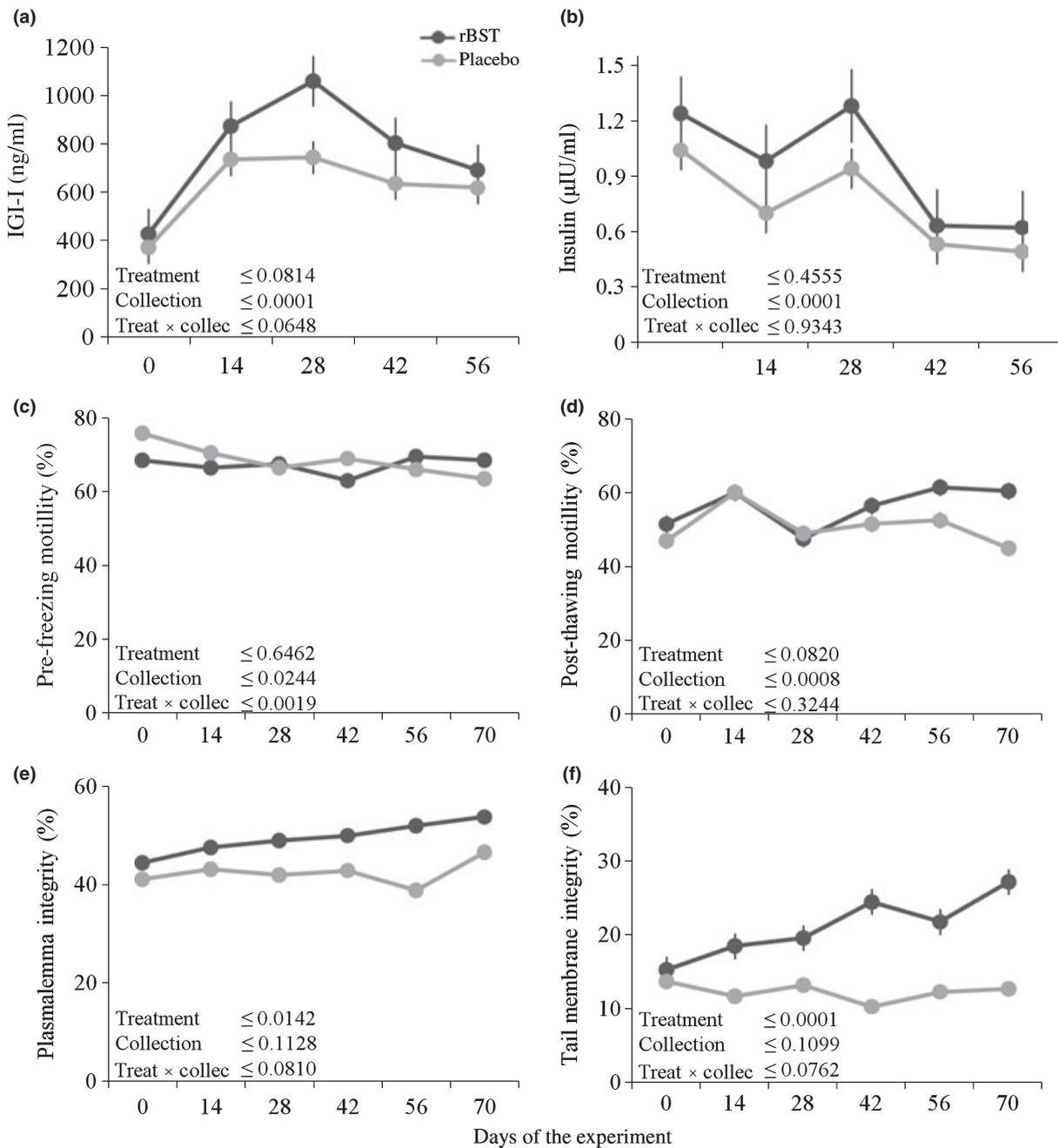


Fig. 1. Parameters evaluated in bulls treated with exogenous recombinant bovine somatotropin (500 mg) or placebo on days 0 and 14 of the experiment. (a) Plasma concentration of IGF-I (ng/ml) and (b) insulin (μ IU/ml); (c) percentage of progressive motile cells at pre-freezing and (d) post-thawing stage; (e) percentage of cells with intact plasmalemma and (f) tail membrane at post-thawing stage

Results

Effect of treatment and treatment-by-collection day on plasma concentrations of IGF-I and insulin was not significant. However, mean plasma concentrations of IGF-I and insulin were affected ($p < 0.0001$) by days of blood sampling. Effect of treatment and treatment-by-collection day on motility of spermatozoa was similar ($p > 0.05$) at pre-freezing and post-thawing stage. Intactness of plasmalemma and tail membrane of spermatozoa at post-thawing stage was higher ($p < 0.05$) in rbST-treated group than in control (Fig. 1). Effect of treatment and treatment-by-collection on sperm morphology was similar ($p > 0.05$) in both groups; $81.86 \pm 0.84\%$ and $79.09 \pm 0.78\%$ of normal cells at pre-freezing and post-thawing, respectively.

Discussion

Recombinant bovine somatotropin-treated bulls had improved sperm membrane integrity. One possible explanation is that rbST increased seminal plasma IGF-I concentrations (Ovesen et al. 1996), which improved membrane integrity (Selvaraju et al. 2009). Supporting this, the presence of the IGF receptor in sperm is associated with improved capacitation (Sánchez-Luengo et al. 2005); a process dependent on membrane integrity. Another possibility is that improved membrane integrity was due to increased Leydig cell function (Carani et al. 1999), as rbST enhances testosterone and luteinizing hormone secretion (Hafez et al. 2005).

The rbST administration did not improve IGF-I concentrations in this study, in agreement with observations from Holzer et al. (1999), but contrasting with Santos et al. (1999) and Sauerwein et al. (2000). In addition, insulin concentrations were not improved by rbST injection, contrasting with previous studies (Holzer et al. 1999; Sauerwein et al. 2000). As high body condition is associated to high IGF-I blood concentrations (Caldeira et al. 2007), this lack of rbST effect could be attributed to the high body condition of the bulls used in this work.

The pre-freezing sperm motility was not affected by rbST administration, although there was a tendency for improved post-thaw motility. Sauerwein et al. (2000) observed increased post-thaw motility in rbST-treated bulls and attributed this to increased local IGF-I and IGFBP3 production. However, in the present work numeric differences were observed only after 42 days of the first rbST injection.

Exogenous GH did not increase the proportion of normal cells in the present work. In contrast, Sauerwein et al. (2000) and Hafez et al. (2005) observed decreased abnormalities and deformed spermatozoa in rbST-treated bulls. However, no alterations in testicular morphometric parameters or cell apoptosis at different stages of sperm development were observed in rbST-treated bulls (Santos et al. 1999).

In conclusion, rbST did not affect plasma concentrations of IGF-I and insulin; however, it did improve post-thaw membrane integrity of spermatozoa.

Author contributions

MB Vieira is the first author and responsible for the execution of the experiment and preparation of the manuscript. I Bianchi, EM Madeira, CA Oliveira and PV Furtado collaborated in data collection. VFB Roll, collaborated in data analysis. A Schneider collaborated in the analysis and preparation of the manuscript. MN Corrêa, I Pivato, NC Severo and FAB Del Pino collaborated in the research design and data interpretation.

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Author's address (for correspondence): Marcio Nunes Corrêa, Departamento de Clínicas Veterinária, Campus Universitário s/n, Universidade Federal de Pelotas, CEP 96010-900, Pelotas, Rio Grande do Sul, Brazil. E-mail: marcio.nunescorrea@pesquisador.cnpq.br