EFFECT OF RECOMBINANT BOVINE SOMATOROPIN (rbST) ON SEMEN PHYSICAL CHARACTERISTICS AND SOME BIOCHEMICAL CONSTITUENTS IN SEMINAL PLASMA OF FRIESIAN BULLS

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SUMMARY

To investigate the effects of recombinant bovine somaotropin (rbST) on semen quality and some parameters in seminal plasma of bulls, six Friesian bulls (17.5 to 21.5 month old) were divided into two groups each of three bulls. One group was injected with a dose of 500 mg of rbST every 14 days for eight injections, while the other group received saline solution (0.9% NaCl) in similar regime. Two successive semen ejaculates were collected twice weekly for 16 weeks (no= 192 ejaculates), to determine the physical characteristics, in addition to initial fructose and methylene blue reduction test. Field results of conception rate were used as an additional overall criterion of semen quality.

The obtained results revealed that rbST injection improved semen quality. Ejaculate volume was increased by 29% and mass motility was active by 34.6%, in case of rbST treated bulls. The percentage of abnormal sperm was decreased by 25% and sperm cell concentration was increased by 44% in rbST treated bulls and so increased the total number of spermatoza in ejaculates by 87%. Concentrations of AST and ALT enzymes in seminal plasma were higher in rbST treated bulls by 15.3 and 15.9 %, respectively. Semen of rbST treated bulls had lower methylene blue reduction time (10.5 vs. 20.9 min), higher initial fructose (411.6 vs. 298.8mg/100) and higher conception rate (64 vs. 36.5%). Bulls treated with rbST had higher (P<0.05) plasma testosterone (61%), which seems to parallel to improve semen quality.

The present results indicate a possible beneficial use of exogenous rbST for improving bulls semen quality.

Keywords: rbST, Friesian bulls, semen quality, seminal plasma constituents

INTRODUCTION

The importance of growth hormone (GH) for male reproductive function has initially been deduced from the observation that in humans with isolated GH deficiency, puberty is delayed and can be normalized by GH treatment (Laron, 1984). Studies in rodents had further substantiated the necessity of GH for normal spermatogenesis (Arsenijevic et al., 1989) and the development of male reproductive function (Spiteri-Grech and Nieschlag, 1990).

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A potential benefit of GH treatment on semen characteristic has been reported from GH- deficient individuals (Radicioni et al., 1994 and Ovesen et al., 1996). The effects of rbST on reproductive efficiency of Friesian bulls might be explained by investigating the endocrine status and metabolites concentration in blood and seminal plasma. Also, field measures such as non return rate of inseminated cows is one of the best measures to assess the quality of semen produced by rbST treated bulls.

The objective of this study was to examine the potency of rbST in improving seminal quality in Friesian bulls and its impact on plasma testosterone concentration.

MATERIALS AND METHODS

This study was conducted at Sakha Research Station, Kafr El-Sheikh Governorte, Animal Production Research Institute, Ministry of Agriculture during the period from September 2000 to January 2001.

1. Animals

Six young Friesian bulls aged 17.5 to 21.5 months old with an average live body weight of 295 kg were used in this study. Body weight and age of experimental bulls in control and rbST groups were nearly similar averaging 301.7±6.01 Kg, 288.3±8.02 Kg and 17.8±0.71 months, 19.3±1.01 months, respectively. Feed was formulated based on NRC allowances (NRC, 1988) and water was offered twice daily. Semen was collected from each bull twice weekly, in two consecutive ejaculates by means of artificial vagina. This routine of semen collection was continued throughout the experimental period (Septembre to January). Animals were randomly allocated to receive a subcutaneously injection of either saline solution (1.0 ml) as control or rbST (Somatech®, Elcano, USA), 500 mg per dose in the same volume of saline every 14 days for eight injections. Bulls of the two groups were kept under similar managerial conditions.

2. Blood sampling

Blood samples were collected from Jugular vein in heparinized tubes once weekly (at 8 a.m.) throughout the experimental period. The blood samples were centrifuged for 20 min at 3000 rpm for plasma separation, which kept at -20°C until analyzed.

3. Semen sampling

The semen ejaculates were evaluated with regard to volume (measured by a graduated collecting tube to the nearest 0.1 ml), mass motility of spermatozoa (checked by microscopic evaluation as a score ranging from 0 to 5, Perry, 1960). Live sperm (%) evaluation and the morphological examinations of spermatozoa in fresh ejaculate were performed according to Hancock (1951 & 1956). Semen density was determined using spectrophotometer SDM4. Total sperm output for each ejaculate was calculated according to following formula:

\[ \text{Total sperm output/ejaculate (x10^9)} = \text{sperm volume x sperm concentration/ml} \]

Methylene blue reduction time was estimated according to the method adopted by Herman and Madden (1953).

4. Conception rate

The seminal ejaculate was divided into two halves; one half for artificial insemination purposes which was diluted by egg yolk sodium citrate extender to
20x10^6 spermatozoa per 0.25 ml, aliquated in straws, frozen and stored in liquid nitrogen. The other half was frozen at -20°C for biochemical assay. The fertility of the semen samples was obtained from the results of pregnancy diagnosis through rectal palpation two months after the first service using 50 clinically normal cows from the same farm (25 cows per group). All cows received one insemination by the same inseminator.

5. **Seminal plasma assays**

5.1. **Fructose concentration (mg/100 ml)**

Initial fructose concentration was determined calorimetrically throughout the experimental period in seminal plasma according the method of Mann (1964).

5.2. **Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentration in seminal plasma**

The concentration of AST and ALT (RFU/ml) were determined colorimetrically in seminal plasma according to the method described by Retiman and Frankel (1957). Results were expressed as Retiman-Frankel units (R.F.U/ml).

6. **Blood plasma testosterone concentration (ng/ml)**

Assessment of plasma testosterone concentration was performed using a single antibody radioimmunoassay (RIA) technique (DSL- 4000 kits, USA). According to the manufacturer information, the sensitivity of the test was reported to be 0.08 ng/ml. The cross reactivities of the testosterone antiserum are 5.8, 4.2 and 2.3 % for 5α-dihydrotestosterone, 11-oxotestosterone and androstene-dione, respectively. The intra and inter assay coefficients of variability was 8.1 % and 9.1%, respectively.

7- **Statistical analysis**

Data were analysed using the general linear model of SAS (1998) using the following model:

\[ Y_{ij} = u + T_i + E_{ij} \]

where:

- \( Y_{ij} \): The observation \( i \) \( j \)
- \( u \): overall mean
- \( T_i \): treatment (1 for control and 2 for treatment)
- \( E_{ij} \): Experimental error associated with \( i^{th} \) and \( j^{th} \) observations assumed to be randomly distributed.

**RESULTS AND DISCUSSION**

1. **Semem physical characteristics**

All physical characteristics of Friesian semen improved (P<0.05) by rbST treatment (Table 1). This trend is in agreement with the findings of EL-Harairy (2000) who reported an increase (P<0.05) of semen ejaculate volume, percentage of live sperm and total sperm output and decrease (P<0.05) of abnormal spermatozoa in mature rams treated by 100 mg rbST every 14 days for five injections. The decrease (P<0.05) of sperm abnormalities percentage and the increase of sperm output in bulls treated with rbST are in accordance with those of Sauerwein et al. (2000) on Simmental sires treated with 640 mg rbST every 14 days for 7 injections, this
treatment decreased percentage of deformed spermatozoa by 15.3% and increased sperm cell concentration by 30%.

**Table 1. Semen physical characteristics (Means±S.D) as affected by rbST treatment**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>rbST group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejaculate volume (ml)</td>
<td>3.1±0.77</td>
<td>4.0±1.19</td>
</tr>
<tr>
<td>Mass motility (score; 0-5)</td>
<td>2.6±0.64</td>
<td>3.5±0.67</td>
</tr>
<tr>
<td>Live sperm (%)</td>
<td>69.1±10.68</td>
<td>77.2±8.62</td>
</tr>
<tr>
<td>Abnormal sperm (%)</td>
<td>22.5±4.22</td>
<td>16.8±5.83</td>
</tr>
<tr>
<td>Sperm concentration (x10^9/ml)</td>
<td>0.890±0.437</td>
<td>1.287±0.464</td>
</tr>
<tr>
<td>Sperm output (x 10^9/ ejaculate)</td>
<td>2.926±1.941</td>
<td>5.484±3.060</td>
</tr>
</tbody>
</table>

Means within the same row having different superscripts differ significantly at 5%.

2. **Seminal plasma constituents**

It is clear from the present results (Table 2) that the reduction of methylene blue was faster in semen collected from rbST treated bulls than control ones by 10 minutes (10.5 vs 20.9 min, respectively). Moreover, the treatment with rbST resulted in an increase (P<0.05) in fructose and AST and ALT concentrations in seminal plasma. Hignett (1957) stated that methylene blue reduction time was less than 15 minutes in case of good semen samples in mature Friesian bulls.

**Table 2. Relevant seminal plasma constituents concentrations (Means±SD) as affected by rbST treatment**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>rbST group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene blue reduction time (min.)</td>
<td>20.94±16.67</td>
<td>11.55±7.76</td>
</tr>
<tr>
<td>Fructose (mg/100)</td>
<td>289.8±79.9</td>
<td>411.6±121.8</td>
</tr>
<tr>
<td>Asparate aminotransferase (RFU/ml)</td>
<td>99.0±28.5</td>
<td>114.1±27.9</td>
</tr>
<tr>
<td>Alanine aminotransféras (RFU/ml)</td>
<td>28.9±9.5</td>
<td>33.5±11.6</td>
</tr>
</tbody>
</table>

Means within the same row having different superscripts differ significantly at 5%.

4. **Blood plasma testosterone**

Bulls treated with rbST had higher (P<0.05) plasma testosterone concentration compared to control group averaging 2.9±2.5 and 1.8±1.3 ng/ml, respectively. This result agrees with El- Harairy (2000), reporting higher blood plasma testosterone concentration in rams treated with rbST compared to the untreated rams (2.65 vs 2.52 ng/ml).

5. **Conception rate**

As shown in figure (1) conception rate was higher (64 %) in rbST treated sires compared to the control one (36%). This may be due to the effect of rbST in improving semen characteristics (Table 1). This is in agreement with the findings of, Sauerwein *et al.* (2000) who reported that rbST treatment had improved fertilization rates (66.8 to 73.2%) of breeding bulls used for artificial insemination.
The present results indicated that treatment with rbST improved significantly the physical characteristics of semen of Friesian bulls (Table 1). This is most probably attributed to the effect of rbST on Leydig cell function (Carani et al., 1999) via increase of LH secretion (Sauerwein et al., 2000 and Chandrasheker and Bartke, 1998). The increase of LH might cause the observed increase of testosterone concentration in treated bulls which is in accordance with the findings of Sauerwein et al. (2000).

Besides the alteration of testosterone, rbST act directly on the spermatogenic surface of the testicular tubules or indirectly by elevating IGF-1 plasma concentration (Sauerwein et al., 2000). The present findings are supported by the results of Lee et al. (1995) and Breier et al. (1998) in men and Schallenberger et al. (1993) and Sauerwein et al. (2000) in bovine indicating that poor semen quality could be restored by treatment with growth hormone. Henault et al. (1995) showed that the mode of action of growth hormone on fertilization efficiency is by improving the components of the ejaculate and increase of fructose (mg/100ml) concentration in treated sires (Sauerwein et al., 2000). The increase of AST and ALT concentration in seminal plasma was positively correlated with live sperm percentage (Roussel and Stallcup, 1966). Also, Pareek et al. (1981) claimed that AST release was positively correlated with ram sperm motility. There is substantial evidence that growth hormone does affect testicular function by modulating gonadal steroid synthesis and gametogenesis (Zachman, 1992).

Moreover, injection of rbST in bulls causes a kind of nutrient partitioning to improve the semen quality of the bulls (Sauerwein et al., 2000).
In conclusion, injection of rbST in Friesian bulls improves physical and biochemical characteristics of semen and the fertilizing ability of the treated bulls.

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تأثير الحقن بهرمون النمو المخلق وراثياً على الصفات الطبيعية للسائل المنوي وبعض المكونات البيوكيميائية في بلازما السائل المنوي في عوائل الفريزيان

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استخدمت هذه الدراسة عدد 6 عوائل فريزيان (ترارح أعمارها بين 17.5 - 21.5 شهر) تم تقسيمها إلى مجموعتين كل مجموعة 3 عوائل أحد المجموعتين حقن أجهزة مندوبين 500 ميالجرام/ 14 يوم من هرمون النمو المخلق (rhST) لعدد 8 جرعات متساوية. بينما المجموعة الأخرى حقن بمعالجة فسيولوجية من كلوريد الصوديوم (0.9%) بنفس معدل الحقن. تم تجميع فئتين متساويتين من السائل المنوي مرتبطين كل أسبوع لمدة 16 أسبوع (192 فئنة منوية) حيث يتم تدقيق الصفات الطبيعية للسائل المنوي واختبار أزرق البيثيلين وكذلك النتائج الحقلية لعملية الخصوبة لاختبار كفاءة السائل المنوي.

وقد أشرت النتائج على أن المعاملة بهرمون النمو المخلق (rhST) كان لها تأثيراً مهماً على تحسين الصفات الطبيعية للسائل المنوي، حيث تم زيادة حجم الفئنة (29%). كما حدث زيادة في نشاط حركة الحيوانات المنوية (34.6%) ونسبة الحيوانات النشطة (12%) وتركيز الحيوانات النشطة في الفئنة (44%). بينما خفضت النسبة المنوية للحيوانات الحية (25%) و الزمن لاندلاع ارتفاع القيتال (10 دقيقة). كما حدث ارتفاع في نسبة الفئنة للخصوبة (معدل الحمل حيث وصلت إلى 64% بينما كانت 36% في المجموعة المقارنة. حاول أيضاً زيادة معنوية في مستوى سكر الفرانتوز (42%) وأيضاً في تركيز كل من إنزيمات الكبد المتقدمة. هناك زيادة معنوية في مستوى هرمون التستوسترون في بلازما السائل المنوي في عوائل الفريزيان بدرجة معينة من البكالوروس (15.3) ALT

الخالية: تشير النتائج الحالية إلى إمكانية الاستفادة من استخدام هرمون النمو المخلق (rhST) في تحسين

خصائص السائل المنوي لعوائل الفريزيان.