Physical and biochemical characteristics of semen from rams treated with recombinant bovine somatotropin (rbST)

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ABSTRACT

To study the effect of prepubertal injection of rbST on physical and biochemical characteristics of rams' semen, a total of twenty four Rahmani lambs were used. The lambs had an initial weight 30.9±1.03 kg (5-6 months old) and fed a complete mixed ration containing 2615 Kcal ME/kg DM according to NRC (1985). The lambs were divided into two symmetric experimental groups according to rbST treatment (0, 100 mg rbST/14 days). Blood was collected fortnightly, while semen was collected twice weekly after the last injection of rbST.

The concentration of blood plasma IGF-1 was the highest (P<0.05) in rbST treated rams (578.71 *vs.* 776.00 ng/ml). However, the concentration of blood plasma testosterone was the lowest (P<0.05) in rbST treated rams (2.71 *vs.* 5.62 ng/ml). The treatment of rbST slightly improve the advanced motility (80.10 *vs.* 79.22 %), total sperm output (3.3 *vs.* 3.2 10^9/ejaculate) and total output live (2.8 *vs.* 2.7 10^9/ejaculate). It was concluded that the rbST treatment possibly improve the semen characteristics of Rahmani rams.

***Keywords*:** rbST, rams, semen characteristics, seminal plasma, dietary energy

INTRODUCTION

The number of sheep in Egypt was determined to be 5.15 million head according to FAOSTAT, (2006). Mutton in Egypt comes mainly from Rahmani and Ossimi breeds which represent the majority of the local sheep population.

The amount of the mutton depends mainly on the reproductive performance of the rams, because the reductions in the rams's fertility affect the number of the lambs born which result in reduction in the mutton yield. There are many factors affecting the reproductive performance in rams. The main factor which affects the reproductive performance is the nutritional status.

The effect of growth hormone (GH) on the reproductive performance in rams is needed to study. Now, there are some evidences suggesting that the somatotropin (ST) plays an important role in the reproductive process

(spermatogenesis and steroidogensis). The receptors of ST are found in leydig and sertoli cells, vas deference, prostate gland, epididymis and seminal vesicles (Lobie *et al*., 1990).

Therefore, the aim of this study is to evaluate the effect of recombinant bovine Somatotropin (rbST) on physical and biochemical semen characteristics and blood hormones of Rahmani lambs.

MATERIALS AND METHODS

This study was carried out at Animal Physiology Lab., Faculty of Agriculture, Cairo University, Giza, Egypt, and laboratories of Animal Production Department, National Research Centre. Dokki, Giza, Egypt. During the period from January to September 2004.

1. Experimental design.

A total of twenty four Rahmani lambs were used in this study. The lambs had an initial weight 30.9±1.03 kg (5-6 months old) and fed a complete mixed ration containing 2615 Kcal ME/kg DM (Basal energy) according to NRC (1985) Table (1). The lambs were divided into two symmetric experimental groups according to rbST treatment (0, 100 mg rbST/14 days) according to MacDonald and Deaver (1993).

Table (1): Ingredients and chemical analysis (%) of experimental diet.

Ingredients (%)	Basal energy (BE)
Yellow corn	25
Sunflower cake	15
Soya bean meal	10
Wheat bran	10
Bean straw	16
Sugar beet pulp	17
Magnapac®	3
Lime stone	2
Salt	1
Minerals and vitamins mixture	1
CP/ DM	14.0
CF/ DM	17.7
Kcal ME/kg DM	2615

The lambs allowed 15 days to be adapted to energy diet. The amount of feed was modified to cope with body weight changes. The complete mixed ration was offered in a pellet form twice daily at 8.0 am and 2.0 pm.

2. Blood samples.

Blood samples were collected fortnightly in the morning before the diet offered.

3. Semen samples.

After two months from the last dose of rbST (To evaluate the permanent effect of rbST on reproductive performance), semen samples were collected twice weekly (from July to September) for each ram for nine weeks by means of an artificial vagina. Semen volume, semen motility, abnormalities, live sperm percentage and sperm concentration were recorded. Then, semen samples were centrifuged at 5000 r.p.m. for 10 minutes at 4°C. The recovered seminal plasma fraction was centrifuged at 10.000 r.p.m. for 15 minutes at 4°C and supernatant was stored at -20°C until analysis.

4. Biochemical analysis

Biochemical analyses of seminal plasma were assayed according to the following Table (2).

Table (2). Methods and kits used to quantify the different biochemical analyses of seminal plasma

Parameters	Method	Company	Reference
Hemoglobin (g/dl)	Colorimetric	Stanbio Laboratory(San Antonio, Texas,78202 USA)	Henry (1964)
Total protein (g/dl)	Colorimetric	Stanbio Laboratory(Boerne, Texas,78202 USA)	Cannon (1974)
Albumin (g/dl)	Colorimetric	Stanbio Laboratory(Boerne, Texas,78202 USA)	Dumas and Biggs (1972)
Triglycerides (g/dl)	Enzymatic-colorimetric	Stanbio Laboratory(Boerne, Texas,78202 USA)	Wahlefeld (1974)
Total Lipids (g/dl)	Colorimetric	Biodiagnostic (Egypt)	Zollner and Kirsch (1962)
Cholesterol (mg/dl)	Enzymatic-colorimetric	Stanbio Laboratory(Boerne, Texas,78202 USA)	Stein (1986)
AST&ALT (U/L)	Colorimetric	Quimica Clinica Aplicada S.A.(Amposta, Spain)	Reitman and Frankel (1957)

5. Statistical analysis

Data were analyzed using the general linear model of SAS (1998). Using the following model: -

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where;

Y_{ij} = the observation ij

μ = Overall mean

T_i =Treatment (i=1, Control and i=2, Basal energy and rbST)

E_{ij}=Experimental error associated with ij observation assumed to be randomally distributed.

Differences among means were tested using Duncan (1955). Repeated measurements were adjusting according to Neter *et al.,* (1985). Simple correlation coefficients were calculated among the relevant blood plasma hormones.

RESULTS AND DISCUSSION

Blood Hormones

Effects of rbST treatment on blood plasma hormones of Rahmani rams are presented in Table (1).

1. Testosterone

The concentration of blood plasma testosterone was lower (P<0.05) in BE-rbST rams by 51.78 % compared to the control rams (2.71 *vs.* 5.62 ng/ml).

In the present study, the plasma testosterone was negative (r= 0.11823, P= 0.3767) correlated with plasma IGF-1. Bartke (2000) reported that the excessive production of GH by adenohypophyseal adenomas in patients affected by acromegaly is often associated with reduced libido and potency.

Table (1). Effect of rbST treatment on blood plasma hormones (X^{-}±SE) of Rahmani rams

Blood hormones	Experimental groups	
	Control	BE-rbST
Testosterone (ng/ml)	5.62 ^{a} ± 0.10	2.71 ^{b} ± 0.70
IGF-1 (ng/ml)	578.71 ^{b} ± 90.63	776.00 ^{a} ± 118.30

a, b, c,…..Means having different superscript letters in the same row differ significantly (P<0.05).

The reduction in blood plasma testosterone in rbST treated rams my attributed to the biological effects of both IGF-1 and blood testosterone. All of them are sharing as anabolic factors. The IGF-1 has an anabolic effect on tissues, bones, muscles. Also, the androgens (testosterone) increasing energy, helping in maintaining erectile function and normal sex drive and in their anabolic (building) capacity are instrumental in increasing the strength of all structural tissues-the skin, bones, muscles and heart. Therefore, the injection of rbST increasing the concentration of plasma IGF-1 (mediator of GH) which acts as growth promoters in this case the body

dose not need to another growth promoter (testosterone) which result in reduction in the concentration of blood plasma testosterone (feed back mechanism).

The following Fig (1) show the interaction between blood plasma Testosterone and GH

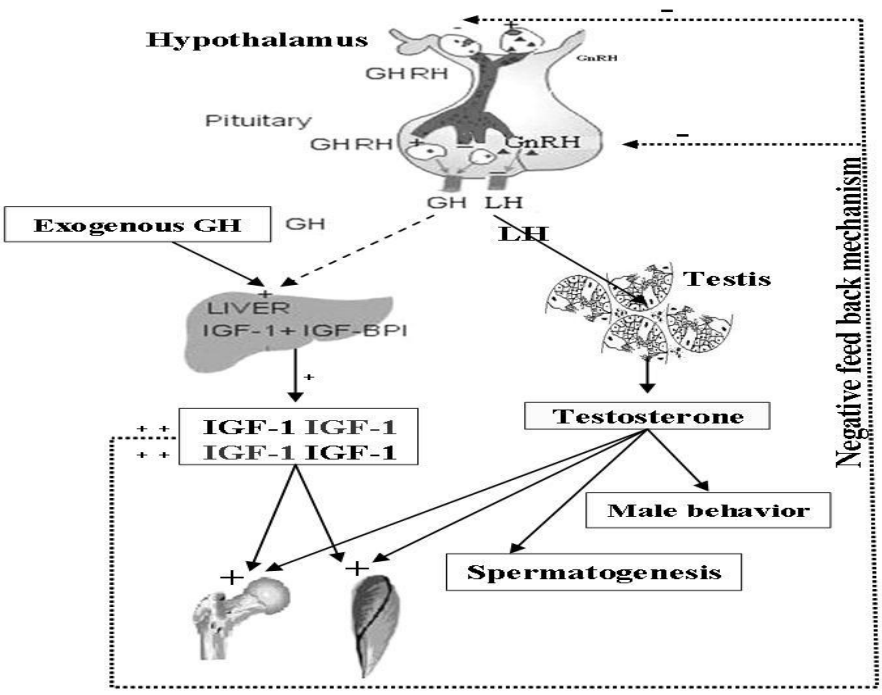

Fig. (1) The antagonistic synergism effect between exogenous GH and blood plasma Testosterone and

The effect of rbST treatment on blood plasma testosterone during the experimental period is presented in Fig. (2).

The reduction in plasma testosterone did not cause any adverse effect on sex drive or semen quality of Rahmani rams under the experimental condition. Roselli *et al.*, (2002) reported that the minimum basal concentrations of plasma testosterone required for exhibiting heterosexual courtship and copulatory behaviors was 2.2 ± 6.2 ng/ml in rams. Schanbacher and Lunstra (1976) reported that the concentration of plasma testosterone decreased gradually through the winter months and reached its lowest levels in late March (2.06 ng/ml in Finn rams and 1.01 ng/ml in Suffolk rams). Also, Borque and VaÂzquez (1999) reported that the highest plasma testosterone mean level was 7.17 ± 0.53 ng/ml in adult Manchego rams. In the present study, the concentration of plasma testosterone ranged from 2.71 to 5.62 ng/ml in Rahmani rams.

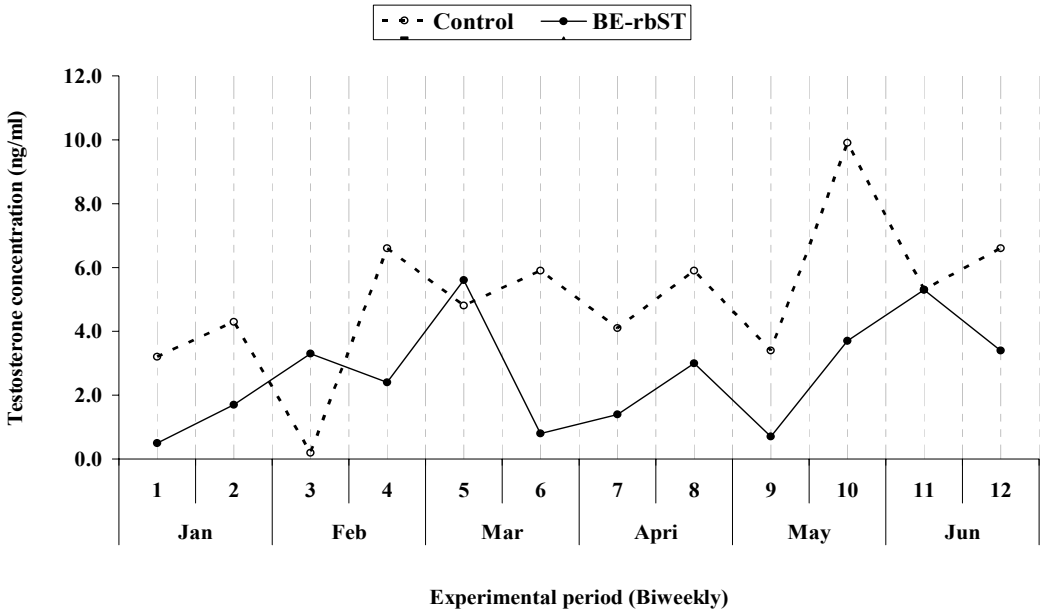

Fig. (2) Effect of rbST treatment on blood plasma testosterone during the experimental period

2. IGF-1

The concentration of blood plasma IGF-1 was higher ($P<0.05$) by 34.09 % in BE-rbST rams compared to the control rams (776.00 *vs.* 578.71 ng/ml, respectively).

The concentration of plasma (IGF-1, ng/ml) decreased with increasing age of Rahmani rams. This finding is in agreement with the result which observed in rams (Gatford *et al.,* 1996). Time course changes in the concentration of IGF-1 in all experimental groups showed an age dependant effect. This declining trend in the concentration of IGF-1 indicated that there was possible antagonism between growth hormone and sex hormones as shown in Fig (3).

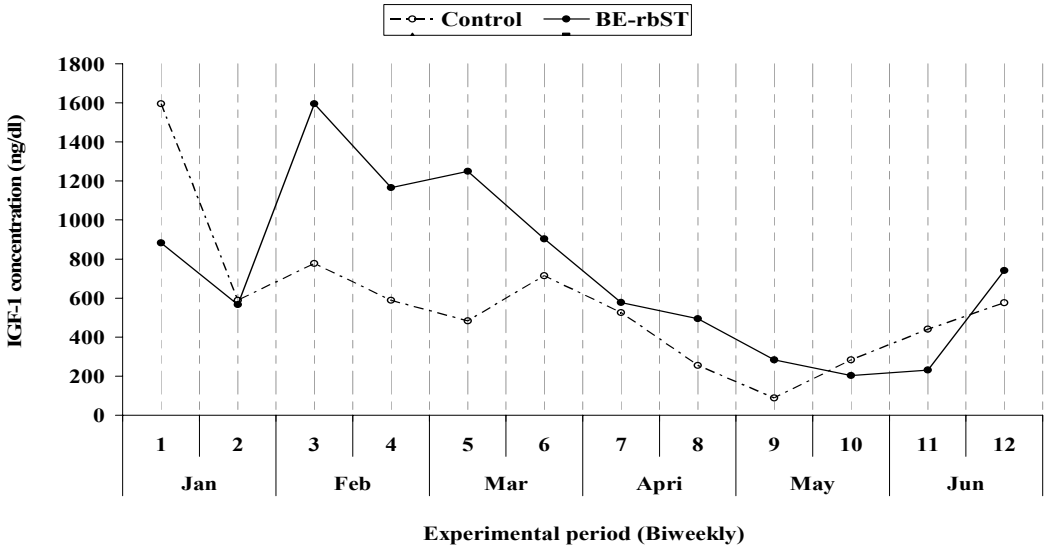

Fig. (3) Effect of rbST treatment on blood plasma IGF-1 during the experimental period

The increasing in the concentration of IGF-1 in BE-rbST rams compared to the control rams is in agreement with Davis *et al.*, (1999) who reported that the concentration of plasma IGF-I increased (P<0.05) in treated goats with rbST 100 µg rbST/BWd^{-1} compared to the control. Also, Sauerwein *et al.*, (2000) found that the concentration of IGF-1 was higher (P<0.05) in treated bulls with 640 mg compare with the control (761 *vs.* 438 ng/ml, respectively).

Semen physical characteristics

Effects of rbST treatment on semen physical characteristics of Rahmani rams are presented in Table (2).

Effect of rbST treatment on ejaculate volume, mass motility, advanced motility, live sperm, sperm output and total output live sperm was slightly higher but did not differ significantly (P>0.05) compared to the control rams. On the other hand, the sperm concentration in BE-rbST was slightly lower but did not differ significantly compared to the control rams (2.8 *vs.* 3.2 109/ml, respectively).

Table (2). Effect of rbST treatment on semen physical characteristics ($\bar{X}$±SE) of Rahmani rams.

Semen characteristics	Experimental groups	
	Control	BE-rbST
Ejaculate volume (ml)	1.00 ^{ab} ± 0.04	1.18 ^{a} ± 0.07
Mass motility (score; 0-5)	4.88 ± 0.06	4.94 ± 0.03
Advanced motility (%)	79.22 ± 1.51	80.10 ± 1.34
Live sperm (%)	84.14 ± 1.31	84.30 ± 1.15
Total abnormalities (%)	16.20 ± 0.99	16.32 ± 0.98
Sperm concentration (10^9/ml)	3.2 ^{a} ± 0.17	2.8 ^{ab} ± 0.17
Sperm output (10^9/ejaculate)	3.2 ^{ab} ± 0.28	3.3 ^{a} ± 0.28
Total output live (10^9 /ejaculate)	2.7 ± 0.26	2.8 ± 0.26

a, b, c…..Means having different superscript letters within the same row differ significantly (P<0.05).

These findings are in agreement with the results obtained by EL-Harairy (2000) who found that the sperm motility and sperm concentration did not differ significantly in Rahmani x Finn rams treated with 100 mg rbST compared to the control rams. Also, Sauerwein *et al.*, (2000) found that total sperm output was higher, but did not differ significantly in bulls treated with 640 mg rbST compared to the control. MacDonald and Deaver (1993) reported that daily administration with 0.2 mg rbST/kg BW$^{0.75}$ did not cause any significant effect on the integrity of spermatozoal chromatin structure; these observations suggest that rbST does not affect mitosis of

undifferentiated Sertoli cells in bull calves. In addition, did not seem an effect of rbST on the integrity of spermatogenesis in developing bulls compared to control.

On the other hand, our results disagree with EL-Harairy (2000) found that the semen ejaculate volume, live sperm percentage and total sperm output were increased ($P<0.05$), but the percentage of abnormal sperm was decreased ($P<0.05$) in rbST treated rams compared to the control. Also, Hafez *et al.,* (2005) reported that the ejaculate volume, live sperm percentage, sperm motility, sperm concentration and total sperm output were increased ($P<0.05$) in bulls treated with 500 mg rbST compared to the control. This difference in rbST results may be due to the difference in the dose of rbST.

Seminal plasma constituents

Table (3) shown the effect of rbST treatment on seminal plasma parameters. There were not significant differences due to the rbST treatment on seminal plasma constituents compared to the control rams.

Table (3). Effect of rbST treatment on seminal plasma characteristics (X^{-}±SE) of Rahmani rams.

Biochemical seminal plasma	Experimental groups	
	Control	BE-rbST
Total protein (g/dl)	6.64± 0.24	6.45 ± 0.24
Albumin (g/dl)	3.18 ^{ab} ± 0.09	3.37 ^{a} ± 0.09
Globulin (g/dl)	3.46 ± 0.23	3.09 ± 0.23
A/G ratio	1.15 ± 0.10	1.19 ± 0.10
Total lipids (g/dl)	0.87 ^{ab} ± 0.03	0.92 ^{a} ± 0.03
Triglycerides (mg/dl)	235.50 ± 8.36	232.76 ±7.33
Cholesterol (mg/dl)	202.20 ^{ab} ± 6.08	214.47 ^{a} ± 6.16
AST (RFU/L)	76.64 ±4.97	73.75 ± 6.12
ALT (RFU/L)	64.79 ^{ab} ± 8.10	58.31 ^{b} ± 3.92
AST/ALT ratio	1.40±0.17	1.33 ± 0.11

a, b, c…..Means having different superscript letters within the same row differ significantly ($P<0.05$).

Our results disagree with Hafez *et al*., (2005) who reported that the activity of AST in seminal plasma was higher ($P<0.05$) in bulls treated with 500 mg rbST than the control bulls (114.1 *vs.* 99.0 RFU/ml, respectively). However, the activity of ALT was lower ($P<0.05$) in treated bulls than the control (33.5 *vs.* 28.9 RFU/ml, respectively). This difference might be attributed to using high dose of rbST (500 mg) which might be increased the activity of the spermatozoa.

CONCLUSION

Use of rbST slightly improves the semen characteristics of Rahmani rams.

ACKNOWLEDGEMENT

The authors would like to thank the National Research Center, Dokki, Giza, Egypt for the financing this work throughout the project of **"Some nutritional and physiological studies to enhance the productive performance of Baladi Goats under newly reclaimed land condition"**, Project number 1/34/6. This work has been conducted at Animal Physiology Lab., Faculty of Agriculture, Cairo University and Animal Production Dept., National Research Center.

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