

**EFFECT OF RECOMBINANT BOVINE SOMATOTROPIN (rbST) ON GROWTH PERFORMANCE AND PUBERTY INCIDENCE OF MALE AND FEMALE LAMBS BORN FROM rbST TREATED EWES**

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ABSTRACT

This experiment was a continuation for the study on crossbred ewes treated by injection with 160 mg/ewe rbST at 14-day interval during pre- and post-partum period, where lambs were injected after weaning (2 months old) by 80 mg rbST/ lamb at 14- days interval till puberty. Growth performance and puberty of male and female lambs were studied.

Live body weight (LBW) of treated male and female lambs was significantly heavier ( $P<0.05$ ) than untreated lambs in the control group.

Results showed early signs of puberty in treated male lambs, where injection of 80 mg rbST/ ram lamb reduced age ( $P<0.05$ ) at the three stages of puberty (I, II and III) comparing to control. Moreover, average LBW was significantly higher ( $P<0.05$ ) in treated ram lambs than those in control group at II and III stages of puberty. Scrotal circumference was not affected by rbST treatment at all stages of puberty. However, puberty characteristics were more pronounced during the II and III stages, where ram lambs in treated group had significantly ( $P<0.05$ ) younger age and heavier weights compared with control. Ejaculate volume, percentages of initial gross motility, sperm livability and abnormality percentage improved ( $P<0.05$ ) by injection of rbST, while sperm cell concentration was not affected. Moreover, the overall plasma testosterone concentration was higher ( $P<0.05$ ) in treated ram lambs ( $3.03 \pm 0.4$  ng/ml) compared to control group ( $1.85 \pm 0.4$  ng/ml).

In ewe lambs, injection of rbST reduced age ( $P<0.05$ ) at puberty by 60 days, while LBW at puberty in both treated and control group was almost the same. The plasma progesterone concentration during the pre-pubertal period was not affected by rbST treatment.

In conclusion, administration of 80 mg rbST/ lamb at 14-day interval starting at 2 month of age improved growth performance, puberty characteristics and semen physical characteristics of male rams. Meanwhile, slight improvement was noticed on female lambs.

*Keywords: Lambs, male, female, rbST, growth performance, puberty.*

INTRODUCTION

Improving the productive efficiency and profit are important goals for dairy farming and mutton production. The use of biotechniques such as administration of recombinant bovine somatotropin (rbST) could lower the cost of production in farms. Bovine somatotropin (bST) is a growth hormone (GH) produced by cow pituitary gland and of importance to growth, metabolism, lactation and reproduction of all animals (**Peel *et al.*, 1989** and **Lucy *et al.*, 1999**). The effects of using bST have been studied in sheep (**McDowell *et al.*, 1987** and **Sandles *et al.*, 1988**), dairy goats (**Mephram *et al.*, 1984** and **Chadio *et al.*, 2000**) and dairy cattle (**Bauman, 1992** and **Peel *et al.*, 1992**).

Many experiments have been performed to study the effects of somatotropin (GH) on the reproductive functions of cattle, but few were carried out to study its effects on sheep and goat. Somatotropin (ST) plays an important role in the reproductive process (spermatogenesis and steroidgenesis), where its receptors were found in leydig and sertoli cells, vas deference, prostate gland, epididymis and seminal vesicles (**Lobie *et al.*, 1990**). The effects of rbST on reproduction was related to rbST dose, time of starting treatment, breed and other factors such as nutritional status and milk production (**Esteban *et al.*, 1994**).

This study was performed to investigate the effect of rbSt administration during pre-

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pubertal period on growth performance and puberty of male and female lambs.

### MATERIALS AND METHODS

This study was carried out at Sakha Experimental Station- Animal Production Research Institute- Agricultural Research Center- Ministry of Agriculture, Egypt.

#### Experimental design:

##### Animals:

A total of 40 crossbred lambs (½ Finnish Landrace x ½ Rahmani), males (20) and females (20), 2 months old were used in this study. Lambs were divided into two equal groups (G1 and G2). Group 1 included 10 males and 10 females served as a control, while lambs of the 2<sup>nd</sup> group (G2) were a product of ewes previously injected with 160 mg rbST/ ewe at 14-days interval during pre- and post-partum period (**Abdel-Khalek et al., 2009**). Lambs in G2 were injected with 80 mg rbST/ lamb (Somatribove; Monsanto Europe, Brussels, Belgium) at 14-days interval from weaning (2 month) till puberty according to **Fernandez et al. (1995)**.

During the experimental period, all lambs were kept under similar management condition in semi-open shaded yards. Animals were fed according to NRC (1985) allowances. Fresh water was available all times.

##### Male lambs measurements:

Male lambs in both treatment (G2) and control (G1) groups were weighed monthly after weaning up to 10 month of age. Changes in sexual behavior were tested for all lambs every 10 days from 6 months old till the onset of puberty (first successful ejaculate with motile sperm). Age, weight and scrotal circumference were determined at 1<sup>st</sup> mounting (Stage I), 1<sup>st</sup> mounting with erection (Stage II) and 1<sup>st</sup> ejaculation (Stage III, puberty).

Semen samples were collected by artificial vagina, after the 1<sup>st</sup> ejaculate, once weekly until 10 months old. Seminal volume was measured directly in milliliter to the nearest 0.1 ml using a graduated collecting tube. Percentage of initial gross motility of spermatozoa was directly examined by microscopic vision (**Melrose and Laing,**

**1970**). Live sperm (%) evaluation and the morphological examinations of spermatozoa in fresh ejaculate were performed according to **Hancock (1951 & 1956)**. Total sperm output for each ejaculate was calculated according to the formula:

Total sperm output/ ejaculate ( $\times 10^9$ ) = semen volume x sperm concentration/ ml.

##### Female lambs measurements:

In female lambs, the incident of the 1<sup>st</sup> estrus was detected using teaser rams. Lambs were subjected to teaser rams three times daily (6 and 12 a.m. and 6 p.m.) for 20 minute each starting by 4 months old. Female lambs being receptive for teaser and standing for mounting were considered in estrus. The onset of first estrus was used as indicator of the onset of puberty. Age and weight at the onset of puberty was recorded.

##### Blood samples:

Blood samples were collected biweekly from the Jugular vein of 5 randomly selected male and female lambs from each group in heparinized tubes starting from 12 weeks of age (3 month) till 40 weeks (10 month). Blood samples were centrifuged at 1500×g for 20 min for plasma separation and stored at -20 °C until analysis.

##### Plasma testosterone and progesterone concentrations (ng/ml):

Quantitative determinations of plasma testosterone and progesterone were carried out using radioimmunoassay kits (catalog No. TKTT1 and TKPG1, respectively, manufactured by Siemens- USA). The assays based on a competitive reaction (**Wilson and Foster, 1992 and Bauman, 1981**, respectively). The sensitivity of the assay during testosterone and progesterone determinations was 0.04 and 0.03 ng/ml, respectively. Plasma testosterone and progesterone concentrations were measured with the aid of a Mini-Gamma counter (LKB 1275, USA).

##### Statistical analysis:

Data were statistically analyzed by method of analysis of variance for repeated measurements using procedures of **SAS program (1998)**. Duncan Multiple Range Test (**Duncan, 1955**) was used to test the

significant differences among means at probability level 5%.

## RESULTS AND DISCUSSION

### Effect of rbST treatment on lamb's growth:

Average LBW of male and female lambs in control (G1) and treated (G2) groups are illustrated in Table (1). Results show that male and female lambs in G2 were significantly heavier ( $P < 0.05$ ) than those in G1 during post-weaning period.

The observed increase in LBW of lambs accompanied treatment with rbST during post weaning period was also detected in the same lambs during suckling period as a response to treating lactating dams with the same material (**Abdel-Khalek et al., 2009**). Moreover, results were similar to that reported by **Sallam et al. (2005)**, **Davis et al. (1999)** and **Bareille et al. (1997)** in Rahmani lambs, goats and cows, respectively. Administration of rbST improved lambs growth (**Early et al., 1990 a**), carcass composition (**Early et al., 1990b** and **McLaughlin et al., 1993**) and enhances immune competence (**Bauman, 1992**).

### Puberty characteristics of male lambs:

Puberty characteristics including age, LBW and scrotal circumference of ram lambs at different puberty stages are shown in Table (2). Injection of 80 mg rbST/ lamb reduced age gap ( $P < 0.05$ ) to the three stages of puberty (I, II and III) comparing to control group. Average LBW was significantly higher ( $P < 0.05$ ) in treated than control group at II and III stages of puberty. Meanwhile, scrotal circumference was not affected by rbST treatment at all stages of puberty.

Results of the present study show early signs of puberty in treated ram lambs. Treated lambs showed 1<sup>st</sup> mounting earlier than lambs in the control group. However, puberty characteristics were more pronounced during the II and III stages, where ram lambs in treated group had significantly ( $P < 0.05$ )

younger age and heavier weights as compared to those in the control group. **Kumi-Diaka and Hallford (1985)** reported that ram lambs managed under intensive system compared to those under extensive under sub-tropical conditions showed earlier age of puberty and wider scrotal circumference. In addition, **Castrillejo et al. (1995)** found that onset of puberty of Corriedale ram lambs was attained earlier at 180-216 days of age with lower scrotal circumference (23 cm) than that obtained in the present study (26.8 and 28.2 cm, Table 2). The gradual increase in scrotal circumference observed in the present study, at all stages of puberty in treated (G2) and control (G1) ram lambs was similar to that obtained by **Ali and El-Saidy (2003)** and **El-Saidy (2004)**.

### Semen physical characteristics:

Semen physical characteristics of ram lambs in control and treated groups during different stages of puberty are presented in Table (3). Ejaculate volume, percentages of initial gross motility, sperm livability and abnormality percentage improved ( $P < 0.05$ ) by injection of 80 mg rbST. On the other hand, sperm cell concentration was not affected by rbST treatment. Results agree with **EL-Harairy (2000)** who reported an increase ( $P < 0.05$ ) of semen ejaculate volume, percentage of live sperm and total sperm output and decrease ( $P < 0.05$ ) in abnormal spermatozoa in mature rams injected with 100 mg rbST five times with 14 days gap. **Sauerwein et al. (2000)** reported a decrease ( $P < 0.05$ ) in sperm abnormalities percentage and an increase of sperm output from Simmental sires injected with 640 mg rbST seven times every 14 days gap. Moreover, injection with rbST (5 injections, 100 mg/ male at 14 day-intervals) improved semen quality and ejaculate volume of rams (**El-Harairy et al., 2002**) and goats (**El-Saidy, 2002**). **Hafez et al., (2005)** reported that all physical characteristics of Friesian bull semen improved ( $P < 0.05$ ) by rbST treatment.

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**Table (1): LBW (LSM± SE) of male and female lambs in control and treated groups at different ages during post- weaning period.**

Sex	Group	Post-weaning age (week)							
		12	16	20	24	28	32	36	40
Ram lambs (Kg)	G1	16.1	20.5	24.1	28.5	32.8	35.6	37.9	40.5
	(n= 10)	±0.22	±0.28	±0.40	±0.41	±0.45	±0.41	±0.41	±0.42
	G2	20.3	26.6	32.5	38.7	44.6	48.8	51.8	54.8
	(n= 10)	±0.15	±0.15	±0.18	±0.25	±0.28	±0.28	±0.36	±0.34
Significance		*	*	*	*	*	*	*	*
Ewe lambs (Kg)	G1	15.4	19.5	22.1	24.5	27.4	30.5	33.8	36.7
	(n= 10)	±0.16	±0.24	±0.40	±0.33	±0.15	±0.41	±0.40	±0.53
	G2	18.5	21.8	24.2	27.7	31.4	36.5	40.8	43.6
	(n= 10)	±0.17	±0.14	±0.19	±0.27	±0.21	±0.20	±0.17	±0.22
Significance		*	*	*	*	*	*	*	*

G1= Male and female lambs served as control.

G2= Male and female lambs injected with rbST.

\* = Significant at P<0.05.

**Table (2): Puberty characteristics of treated and non treated ram lambs during different stages of puberty.**

Characteristics	G1	G2	Sig.
Stage I (1 <sup>st</sup> mounting):			
Age (day)	210.3±0.26	150.2±0.31	*
Body weight (kg)	32.8±0.45	32.5±0.18	NS
Scrotal circumference (cm)	18.0±0.19	19.2±0.21	NS
Stage II (1 <sup>st</sup> mounting with erection):			
Age (day)	240.5±0.51	180.6±0.12	*
Body weight (kg)	35.6±0.41	38.7±0.25	*
Scrotal circumference (cm)	22.2±0.14	24.5±0.27	NS
Stage III (1 <sup>st</sup> ejaculation, puberty):			
Age (day)	300.4±0.28	210.6±0.21	*
Body weight (kg)	40.5±0.42	44.6±0.28	*
Scrotal circumference (cm)	26.8±0.23	28.2±0.15	NS

\*= Significant at P<0.05      NS= non significant.

**Table (3): Semen characteristics of first ejaculate containing spermatozoa of ram lambs in rbST and control groups.**

Characteristics	G1	G2	Sign.
Ejaculate volume (ml)	0.26±0.12	0.32±0.11	*
Initial gross motility (%)	43.6±0.13	50.6±0.10	*
Live sperm (%)	41.3±0.12	50.2±0.12	*
Abnormal sperm (%)	17.2±0.12	10.6±0.10	*
Sperm concentration (x10 <sup>9</sup> /ml)	1.23±0.12	1.42±0.12	NS

\* Significant at P<0.05. NS: non significant.

**Table (4): Live body weight (kg) and age at puberty of ewe lambs in treated and control groups.**

Item	G1	G2	Sign.
LBW at puberty (kg)	36.7±0.53	36.5±0.20	NS
Age at puberty (day)	300.3±0.23	240.6±0.15	*

\* Significant at P<0.05. NS: Not significant.

### Blood plasma testosterone

Plasma testosterone concentrations during the experimental period are shown in Figure (1). Ram lambs treated with rbST had higher (P<0.05) plasma testosterone concentration (3.03± 0.4 ng/ml) than lambs in the control group (1.85± 0.4 ng/ml). This result agrees with **El- Harairy (2000)**, who reported higher blood plasma testosterone concentration for rams treated with rbST compared to untreated rams (2.65 vs 2.52 ng/ml). Moreover, **Hafez et al., (2005)** reported that bulls treated with rbST had 61% higher (P<0.05) plasma testosterone compared to untreated bulls.

The improvement of physical characteristics of semen observed in the present work may be attributed to the effect of rbST on Leydig cell function (**Carani et al., 1999**) via increasing LH secretion (**Sauerwein et al., 2000 and Chandrasheker and Barttke, 1998**). The increase of LH level might be the cause of the increase in testosterone concentration observed with treated rams (**Sauerwein et al., 2000**). **Sauerwein et al. (2000)** also reported that rbST act directly on the spermatogenic surface of the testicular tubules or indirectly on elevating IGF-1 concentration in plasma. They suggested that poor semen quality in bovine could be restored by treatment with growth hormone. Moreover, **Henault et al. (1995)** and **Sauerwein et al. (2000)** showed that the mode of action of growth hormone on fertilization efficiency is through improving the components of the ejaculate and increase of fructose (mg/ 100 ml) concentration in treated sires

### Puberty characteristics of female lambs:

Injection of rbST reduced ewe lambs age (P<0.05) at puberty by 60 days in treated comparing to control lambs (Table 4). Meanwhile, LBW of ewe lambs at puberty in both treated and control group was almost the same.

**Radcliff et al. (1997)** reported that treatment with rbST (25 µg/ kg of body weight) reduced age at puberty up to 24 days and increased daily body weight gain of Holstein heifers. Moreover, injection of rbST (25 µg/ kg of body weight) at a high plane of nutrition reduced age at puberty in Holstein heifers by 90 days (**Radcliff et al., 2000**). In contrast, **Murphy et al. (1991)** reported that administration of rbST for 120 days before puberty did not affect pre- pubertal LBW gain, age at first puberty and age at first estrus. **Hall et al. (1994)** and **Bhatti et al. (2007)** reported that rbST had no effect on age and weight at maturity, where it only altered the intermediary metabolism in a manner that increased lean tissue and decreased fat deposition.

The early incidence of puberty in treated ewe lambs may attribute to that rbST may affect through direct actions of ST where its receptors are found within granulosa cells and oocytes (**Lucy et al., 1991 and 2000**). They also, reported that the actions of ST may be indirectly mediated by increase IGF-I and (or) nutrient partitioning that occurs after rbST injection. The IGF stimulate ovarian function by acting synergistically with gonadotropins to promote growth and steroidogenesis of ovarian cells. The direct action of ST may also causes an increase in the number of small follicles (6-15 mm) in lactating cows and the largest ovarian follicles in both lactating and non lactating cows (**De-La Sota et al., 1993**). **Gallo and Block (1991)** found that treatment with exogenous rbST increased the number of ovarian follicle with a distinct class of follicular diameter. Moreover, **Lucy et al, (1993)** reported that ovary is the most likely target organ of rbST as a direct effect on a variety of reproductive cells and m-RNA for ST receptor within reproductive tissues such as, hypothalamus, pituitary gland, and ovarian follicles.

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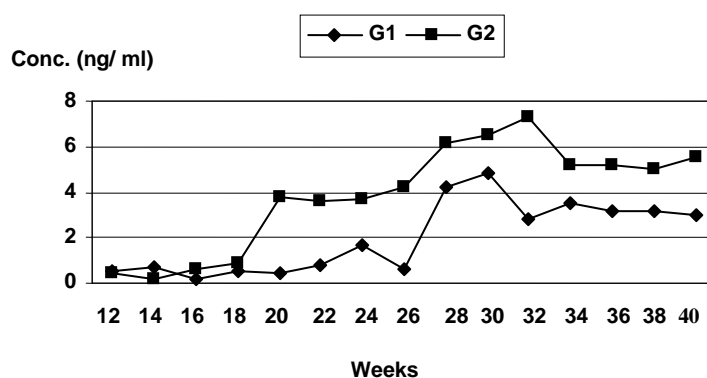
### Blood plasma progesterone

The overall plasma progesterone concentrations during the experimental period was not affected by rbST treatment (Fig 2) being the same in treated ( $0.41 \pm 0.08$  ng/ml) and non treated ewe lambs ( $0.35 \pm 0.08$  ng/ml).

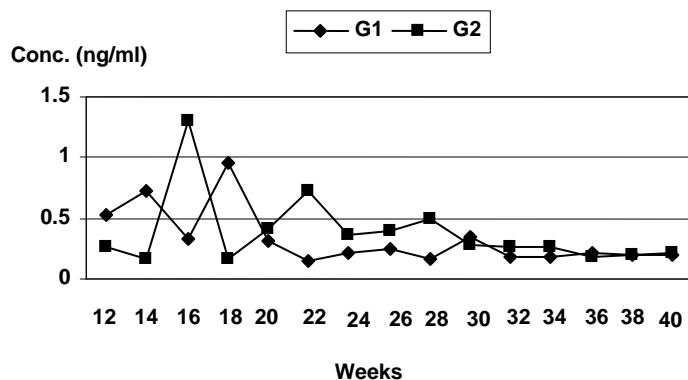
In contrast, **Barreca *et al.* (1993)** reported that ST treatment in human and pig stimulate estradiol synthesis. **Chadio *et al.* (2002)** observed that rbST treatment resulted in a tendency for higher progesterone levels in

treated ewes (**Brozos *et al.*, 1999**) and heifers (**Gong *et al.*, 1991**).

The current study concluded that administration of 80 mg rbST/ lamb, at 14-days interval starting at 2 month of age for both male and female lambs produced from crossbreed ewes ( $\frac{1}{2}$  Finnish Landrace x  $\frac{1}{2}$  Rahmani) previously treated with 160 mg rbST/ewe, improved growth performance and some puberty characteristics in male lambs and slightly improved age at puberty in ewe lambs.



**Figure 1: Testosterone levels (ng/ ml) pre- puberty and during different stages of puberty in control and treated ram lamb.**



**Figure 2: Progesterone concentration (ng/ ml) pre- puberty period and puberty in control and treated ewe lamb.**

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