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PHYSICOCHEMICAL CHARACTERISTICS OF SEMEN FROM BUFFALO BULLS (*BUBALUS BUBALIS*) AS AFFECTED BY TREATMENT OF TWO DIFFERENT DOSES OF RECOMBINANT BOVINE SOMATOTROPIN (rbST)

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SUMMARY

A total of 15 buffalo bulls were divided into three comparable experimental groups to study the effect of two different doses of recombinant bovine somatotropin (rbST) on physical and chemical characteristics of semen during the hot months of the year (April to September). The first group (G1) was left untreated and served as control, while the 2nd (G2) and 3rd (G3) groups were injected subcutaneously either with 250 or 500 mg Somatech®/14 days/animal for eight successive injections, respectively. Bulls had an initial body weight around 350.9 kg and aged 16 to 18 months. Blood samples were collected fortnightly to determine testosterone and insulin like growth factor-I (IGF-I) in peripheral blood plasma, while semen was collected twice weekly to determine semen traits.

Treated groups with rbST had higher (P<0.05) ejaculate volume than G1 by 5 and 12.5% for G2 and G3, respectively. Sperm concentration (x 10^6/ml) was also higher (P<0.05) than G1 by ~10 and 14% for G2 and G3, respectively, which reflected an increasing (P<0.05) of sperm output by ~16 and 28% and live sperm output (P<0.05) by ~21 and 38% for G2 and G3, respectively. Sperm mass motility, advanced motility and live sperm were insignificantly higher in G2 and G3 (P>0.05), and sperm abnormalities were lower (P>0.05) in (G2) compared to (G1).

IGF-I was higher (P<0.05) in rbST treated bulls (776 and 876 ng/ml, for G2 and G3, resp.) than that in G1 (594.7 ng/ml). Similar trend was observed for testosterone concentration being lower (P<0.05) in G1 (5.62 ng/ml) compared to G2 and G3 (8.08 and 9.78 ng/ ml, resp.).

In conclusion, treated buffalo bulls with rbST either 500 mg or 250 mg /14 days/animal/ eight injections improved semen physical characteristics favorably to the 250 mg dose.

Keywords: Buffalo semen, blood plasma, rbST, IGF-I, testosterone

INTRODUCTION

Spreading genetic material (semen) of proven sires of buffaloes comes in top priorities in national breeding scheme. Conception rate in farm animals depends on many factors; one of them is semen quality. Therefore, characterization of semen traits is of importance to judge semen quality of buffalo bulls used in AI program. Conception rate in buffaloes was reported to be 43.4 and 60.8% under AI or natural mating systems, respectively (Sosa *et al*., 2003).

Egyptian buffalo bulls are accused to display poor reproductive performance during summer season (June-August), due to high ambient temperature. Freezing of semen as well is associated with poor quality semen (Barkawi *et al*., 2006). So, improving semen quality will leads to improve success of insemination ratio by about 50% (Hafez *et al.*, 2005).

Growth hormone (GH or ST) is evidenced to play an important role in the male reproductive process. It has been reviewed that ST is required for sexual differentiation, pubertal maturation, gonadal steroidogenesis and gametogenesis (Bartke, 2000 and Hull and Harvey, 2000). It is well documented that most of the actions of the GH are mediated by Insulin Like Growth Factors (IGF-I), which secreted from liver and other several tissues (Gatford *et al.*, 1996 and Hadley, 2000) to mediate the anabolic actions of GH.

Previous work indicated that the synergy of GH and FSH may, in part, be a result of increasing IGF-I production (Mauras *et al.*, 1996; Hadley, 2000 and Khalid *et al.*, 2000). Deficiency in GH leads to various reproductive defects includes; delayed puberty, subnormal development of the male reproductive system and reduced sexual behavior. Thus it has been concluded that GH signaling plays a physiological role in the control of male sexual maturation and adult reproductive functions in both human and rats (Bartke, 2000). Thereby, it is suggested that increasing GH levels in the physiological range, especially for heat stressed bulls, will increase the quality and the viability of collected semen.

Many research works were conducted using recombinant bovine Somatotropin (rbST) to improve semen quality of cattle bulls

(Sauerwein *et al.*, 2000 and Hafez *el al.*, 2005), applying doses of 500 or 640 mg/ 14 days. Previous results indicated a significant improvement in semen quality of the treated bulls. Up to the knowledge of the authors no data are available describing the effect rbST treatment on the semen quality of buffalo bulls.

The aim of this study was to investigate the effect of two different doses of recombinant bovine Somatotropin (rbST) on semen physical characteristics in relation to testosterone and IGF-I hormones in blood of Egyptian buffalo bulls.

MATERIALS AND METHODS

This study was carried out at Animal Physiology Lab., Faculty of Agriculture, Cairo University, Giza, Egypt during the period from April to September (hot period of the year). Hormonal assay was carried out in Hormones and Immunology Lab., Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University, Giza, Egypt.

1. *Experimental design*

A total of 15 buffalo bulls with initial weight of 350.9±8.03 kg and age from 16-18 months were used in this study. Bulls were fed a total mixed ration containing 2830 Kcal ME/kg DM, 14.5% CP/kg DM and 18.1% CF /kg DM (NRC, 1996). Daily ration was offered in a pellet twice daily at 8:0 am and 2:0 pm. Bulls were kept in a semi-shaded open yards with free access to fresh water all the day.

Experimental buffalo bulls were divided into three similar (in age and weight) and equal groups (n=5). The 1st group (G1) was kept with no treatment and served as a control, while the 2nd (G2) and 3rd (G3) groups were injected subcutaneously with a doses of 250 and 500 mg Somatech®/14 days, for four months (eight successive injections) according to McDonald and Deaver (1993).

2. Blood samplings:

Blood samples were collected fortnightly in the morning (before morning feeding and watering) via jugular vein using tubes containing EDTA as anticoagulant. Blood plasma was harvested by centrifuging blood samples at 4000 rpm for 15 minutes. Harvested plasma was stored at -20°C until the time of assay.

3. *Hormonal Assay:*

Blood IGF-I was measured using immune-radiometric assay method with a sensitivity of 1 ng/dl, standard curve between 0 and 1200 ng/ml. The inter- and intra-assays coefficients of variability were 8.2 and 5.6%, respectively according to the manufacturer's instruction (Immunotech®, Bechman coulter co., France). Testosterone concentration was measured using radio-immuno assay method. According to the manufacturer's information (Biosource®, Belgium), sensitivity value was reported to be 4 ng/dl, the standard curve was between 0 and 16.4 ng/ml and the inter- and intra-assays coefficients of variability were 6.4 and 5.9 % respectively.

4. Semen collection

Semen was collected fortnightly from each bull using artificial vagina (Holland type). Volume (ml), concentration (x 10^6/ml), percentages of abnormalities and live sperm as well as motility score (0-5) were recorded as semen physical traits according to Hafez and Hafez (2000).

5. *Statistical analysis*

Data were analyzed using the general linear model of SAS (2000). Using the following model:-

$$\mathbf{Y_{ijkl} = \mu + T_i + P_j + (T_i*P_j)_k + E_{ijkl}}$$

Where:

Y_{ij} = the observation $_{ij}$

μ = Overall mean

T_i =Treatment (1, Control, 2 (250 mg rbST/ 14 days) and 3 (500 mg rbST/ 14 days)

P_j = months of treatment (6 months from April to September)

$(T_i*P_j)_k$ = interaction effect

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

Differences among means were tested using Duncan (1955). Repeated measurements (split plot in time) were adjusted according to Neter *et al.* (1985).

RESULTS AND DISCUSSION

Effect of rbST treatment:

Semen physical characteristics:

Semen volume increased (P<0.05) by 5.4 and 12.5 % in G2 and G3, respectively as compared to the control (G1). Sperm concentration (x10^6/ml), total sperm output and live sperm output /ejaculate (x 10^9/ ejaculate) were also higher ((P<0.05) in G2 and G3 compared to G1 (Table 1). However, mass motility, advanced motility, total abnormalities and live sperm were differed non-significantly (P>0.05) among the three studied groups.

Blood testosterone and IGF-I concentrations:

Treating buffalo bulls with rbST in G2 and G3 increased (P<0.05) testosterone concentration in peripheral blood plasma by 43.8 and 74.0% in compare to G1, respectively (Table 2). Similar trend was observed concerning the concentration of IGF-I, which increased in G2 and G3 by 30.5 and 47.3 %, respectively compared to G1 (Table 2).

Effect of period of treatment on:

Semen physical characteristics:

Semen volume, sperm concentration, total sperm output/ejaculate and live sperm output / ejaculate increased (P<0.05) with the advancement of the experimental period compared to the first month (Table 3).

Period of the experiment indicated that ejaculate volume (ml), spermatozoa concentration (x 10^6/ ml), sperm output (10^9/ ejaculate) and total output live spermatozoa (10^9/ ejaculate) were improved (P<0.05) after two to three months of the experiment, except live sperm percentage (Table 3). Rate of improvement from the minimum to the maximum values was 46.3, 9.8, 51.9 and 54.7 % for ejaculate volume, spermatozoa concentration, total sperm output / ejaculate and live sperm output/ ejaculate, respectively.

It is interesting to point out that starting from June, almost all studied semen traits reached a plateau level, reflecting an improvement during summer months (Table 3).

Blood Testosterone and IGF-I concentrations:

Testosterone concentration in peripheral blood showed typical trend to that observed for semen characteristics. Testosterone increased gradually to reach plateau by June, and increased (P<0.05) by the end of the experiment (September) by 10% (Table 4).

IGF-I showed an opposite trend to testosterone. Starting June IGF-I showed continuous decrease before increasing non-significantly by September. Rate of decreasing in IGF-I concentration by the end of the experiment was 17% relative to the concentration of IGF-I in the beginning of the study (April) (Table 4). It is worth to point out that summer months (June – August) when the temperature is high have positive effect on testosterone concentration versus negative effect on IGF-I concentration.

Interaction between rbST and period of treatment:

Semen physical characteristics:

As shown in Table (5), there was no clear interaction between the dose of rbST treatment and period of the treatment. No interaction was observed in live spermatozoa (%), while the other traits (Ejaculate volume, spermatozoa concentration/ml, spermatozoa output per ejaculate and spermatozoa live output per ejaculate) showed a significant interaction particularly during June, July and August months.

Blood Testosterone and IGFI concentrations:

Figure 1 illustrates that during the course of the experiment concentration of testosterone was higher (P<0.05) in G2 and G3 compared to G1. Throughout the first three months of the experiment, G2 and G3 showed no significant difference in concentration of testosterone measured in peripheral blood plasma. Thereafter, testosterone concentration was higher (P<0.05) in G3 (9.3±0.23) than in G2 (8.8±0.44). In the meantime, IGF-I concentration showed similar trend in the three studied groups, however it was higher (P<0.05) in general in G2 and G3 (Figure 2). It is also so clear that summer months (mid of June till first of September) were accompanied with decreasing in IGF-I concentrations in opposition to testosterone concentration (Figure 1).

GENERAL DISCUSSION

The noticed improvement in the semen characteristics of the buffalo bulls due to rbST treatment (Table 1) agrees in trend with the results obtained by Sauerwein *et al.* (2000) who reported a significant increase in semen ejaculate volume and non-significant increase in sperm motility and sperm output in rbST treated bulls with 640 mg rbST compared to untreated ones. This trend is supported by the findings of Hafez *et al.* (2005) on their study on Friesian bulls showed an improvement (P<0.05) in ejaculate volume, live sperm percentage, sperm motility, sperm concentration and total sperm output in bulls treated with 500 mg rbST compared to the control, which is suggested to be due to the favorable partitioning of nutrients to the testicular seminiferous tubules due to rbST injection.

Similar trend was observed by EL-Harairy (2000) and El-Gohary *et al.* (2011) on rams reporting improvement in semen characteristics due to rbST treatment with 80 - 100 mg/14 days rbST (4 successive injections) relative to non-treated rams.

Obtained positive effect of rbST on testosterone concentrations in peripheral blood plasma in G2 and G3 treatment compared to untreated buffalo bulls (G1) (Table 2) comes close to the previous recorded results obtained by EL-Gohary *et al.* (2011) in rams treated with 80 mg rbST /for four injections compared to untreated rams. Injection of rbST leads to increase blood plasma testosterone via either a direct effect on the axis between GH and interstitial cells (Leydig cells) (Sauerwein *et al.*, 2000), or via an indirect effect through the partitioning of glucose and other nutrients to the testis and the interstitial cells (Hull and Harvey, 2000).

The observed increase in IGF-I concentration for buffalo bulls treated with

rbST (G2 and G3, Table 2) compared to the control group (G1) is in agreement with Sauerwein *et al.* (2000) who found that the concentration of IGF-I was higher in rbST treated bulls with 640 mg compared with the control (761 vs. 438 ng/ml, respectively). Similar trend was observed in male goats by Davis *et al.* (1999) who reported that the concentration of plasma IGF-I increased significantly in rbST treated goat bucks with 100 µg rbST/BWd-1 compared to untreated control.

On the other hand, the present results of IGF-I concentration disagree with what had been reported by Vieira *et al.* (2010) who demonstrated that rbST (500 mg rbST at days 0 and 14 of the experiment) injection did not affect plasma concentrations of IGF-I compared to bulls of control. This conflicted result may be due to the short treatment with rbST, for two injections only, which might be less than the required number of doses to stimulate IGF-1secrtion.

Improving ejaculate volume in rbST treated groups (G2 and G3) compared to control group (G1) might be due to the high concentration of testosterone in the peripheral blood of these groups (Sauerwein *et al.*, 2000). Results of Sauerwein *et al.* (2000) and Hull and Harvey (2000) indicated that testosterone stimulates the secretion of accessory glands in bulls to increase ejaculate volume. On the other hand, high level of IGF-I in peripheral blood of rbST groups (G2 and G3) compared to G1 may be due to its effect on seminiferous tubules (Hull and Harvey, 2000). IGF-I as a mediator of growth hormone action (Sauerwin *et al.*, 2000) may have a direct effect on the mitotic and meiotic division of spermatogonia (Batke, 2000). This may elucidate the increase of spermatozoa concentration ($x10^6$/ ml) a total spermatozoa per ejaculate ($x10^9$/ejaculate) in G2 and G3 compared to G1 (Table 1).

Improving semen characteristics by the third month after treatment (Tables 3 and 5) most probably attributed to the effect of rbST on the bulls' spermatic waves. It is well known that the spermatic wave in bulls (differentiation of spermatogonia cells to spermatozoa) takes about 35 days (McDonald and Deaver (1993), which explains the improvement of semen traits of the treated groups by the third month after treatment.

The high concentrations of testosterone during the hot months of the year (June-August) comes in agreement with the findings of Schanbacher and Lunstra (1976) and supported by the findings of Bhosrekar *et al.* (1988), who reported that Buffaloes is adapted to hot and humid environments.

The contradicted trend between testosterone and IGF-I concentrations (Figures 1 & 2 and Table 4) may be due to the antagonism between sex hormones (testosterone) and growth hormones (IGF-I) as reported by Hadely (2000)

In conclusion, treating Egyptian buffalo bulls with rbST either by 250 or 500 mg/14 days for 8 injections improves characteristics of semen, which may have a practical application under the use of artificial insemination. Dose of 250 mg under suggested regimen is recommended due to the low cost. However, further investigation is needed to evaluate the produced semen after cryopreservation in order to determine the semen quality and viability after freezing and sawing.

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Table 1. Effect of dose of rbST on semen physical characteristics ($\overline{X}$±SE) of buffalo bulls kept with no treatment as a control (G1) or treated either with 250 mg (G2) or 500 mg (G3) for eight injections at 14 days interval

Semen characteristics	Experimental groups		
	G1	G2	G3
Ejaculate volume (ml)	2.80[a]±0.12	2.95[b]±0.08	3.15[b] ±0.02
Mass motility (score; 0-5)	2.08±0.04	2.24±0.03	2.44±0.03
Advanced motility (%)	79.22±1.51	80.10±1.34	82.80±2.31
Live sperm (%)	79.24±1.81	82.30±1.15	85.10±2.15
Total abnormalities (%)	14.20±0.99	13.32±0.98	14.32±0.98
Sperm concentration (10^6/ml)	1671[a]±170	1846[b] ±89	1899 [b]±120
Sperm output (10^9/ejaculate)*	4.68 [a]±0.18	5.44 [b]±0.09	5.98[b]±0.06
Total output live (10^9 /ejaculate)**	3.70 [a]±0.28	4.46 [b]±0.26	5.09 [b]±0.26

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

* Sperm output (10^9/ejaculate) = Ejaculate volume (ml) x Sperm concentration (10^6/ml)

** Total output live (10^9 /ejaculate) = Sperm output (10^9/ejaculate) x Live sperm (%)

Table 2. Concentrations ($\overline{X}$±SE) of testosterone and IGF-1 hormones in peripheral blood plasma of buffalo bulls kept with no treatment as control (G1) or treated either with 250 mg (G2) or 500 mg (G3) for eight injections at 14 days interval

Blood hormones	Experimental groups		
	G1	G2	G3
Testosterone (ng/ml)	5.62^{a} ±0.10	8.08^{b}±0.70	9.78^{b}±1.90
IGF-1 (ng/ml)	594.71^{a}±80.43	776.00^{b}±81.30	876.00^{b}±99.10

a, b, Means having different superscripts in the same row differ significantly (P<0.05)
G1: control, G2: injected with 250 mg rbST/ 14 days for 8 injections and G3: injected with 500 mg rbST/ 14 days for 8 injections.

Table 3. Average monthly semen physical characteristics ($\overline{X}$±SE) of buffalo bulls throughout the experimental period

Semen characteristics	Months of the experiment					
	April	May	June	July	August	September
Ejaculate volume (ml)	2.03^{a}±0.14	2.83^{b}±0.07	2.97^{b}±0.08	2.85^{b}±0.06	2.83^{b}±0.09	2.93^{b}±0.09
Live sperm (%)	80.04±0.81	81.67±1.37	81.55±1.45	79.67±1.41	83.04±0.81	80.60±0.52
Sperm concentration (10^{6}/ml)	1704^{a}±120	1655^{a}±110	1772^{b}±90	1786^{b}±88	1817^{b} ±120	1806^{b}±99
Sperm output/ ejac. 10^{9}	3.47^{a}±0.08	4.69^{b}±0.18	5.26^{b}±0.08	5.08^{b}±0.11	4.99^{b}±0.13	5.27^{b}±0.08
Live sperms output / ejac. (10^{9})	2.78^{a}±0.24	3.84^{b}±0.24	4.29^{b}±0.24	4.16^{b}±0.13	4.10^{b}±0.08	4.30^{b}±0.04

a, b Means having different superscript small letters within the same column differ significantly (P<0.05).

Table 4. Average monthly concentration ($\overline{X}$±SE, ng/ml) of testosterone and IGF-I hormones in peripheral blood plasma of buffalo bulls throughout the experimental period

Blood hormones	Months of the experiment					
	April	May	June	July	August	September
Testosterone	7.57^{a}±0.09	7.83^{a}±0.4	8.33^{b}±1.3	8.34^{b}±0.13	8.29^{b}±0.70	8.29^{b}±0.47
IGF-1	805.0^{b}±7.4	825.8^{b}±71.3	794.2^{b}±79.1	680.8^{a}±84.4	668.3^{a}±71.3	705.2^{a}±60.1

a, b Means having different superscript small letters within the same column differ significantly (P<0.05).

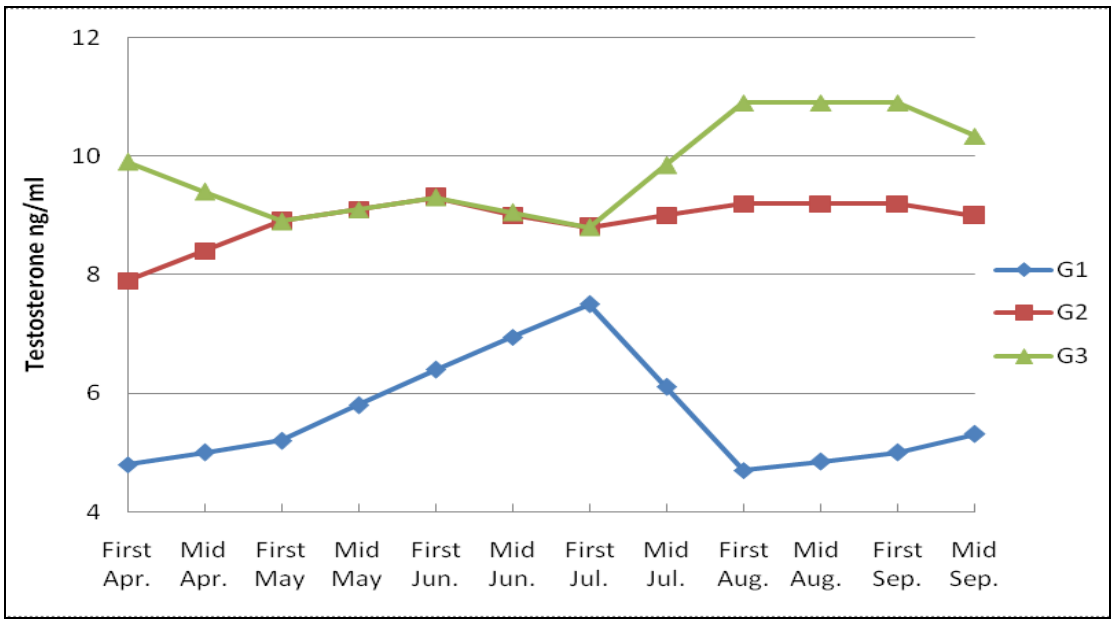

Fig. 1. Blood plasma testosterone during the experimental period in buffalo bulls groups injected fortnightly with placebo (G1), 250 mg rbST (G2) and 500 mg rbST (G3) throughout the experimental period

Table 5. Average monthly semen physical characteristics ($\overline{X}$±SE) of buffalo bulls groups injected fortnightly with placebo (G1), 250 mg rbST (G2) and 500 mg rbST (G3) throughout the experimental period

Semen characteristics	Months of the experiment					
	April	May	June	July	August	September
Ejaculate volume (ml)						
G1	1.90^{Aa}±0.15	2.40^{Ba}±0.12	2.80^{Ba}±0.15	2.41^{Ba}±0.12	2.55^{Ba}±0.17	2.60^{Ba}±0.19
G2	2.05^{Aa}±0.24	2.95^{Bb}±0.08	2.95^{Bb}±0.08	2.99^{Bb}±0.08	2.95^{Bb}±0.08	2.98^{Bb}±0.08
G3	2.15^{Aa}±0.02	3.14^{Bb}±0.02	3.15^{Bb}±0.02	3.15^{Bb}±0.02	3.00^{Bb}±0.02	3.15^{Bb}±0.02
Live sperm (%)						
G1	79.04±0.91	79.24±1.81	79.24±1.81	74.24±1.81	80.24±1.81	76.24±0.92
G2	80.30 ±0.10	81.30 ±0.15	82.30 ±1.15	81.38±1.15	82.30 ±1.15	82.30 ±1.15
G3	81.10 ±0.75	85.10 ±2.15	83.10 ±2.15	84.80±2.15	85.10 ±2.15	84.10 ±2.15
Spermatozoa concentration (x 10^6/ml)						
G1	1671^{a}±170	1620^{a}±170	1671^{a}±170	1561^{a}±99	1502^{a}±170	1671^{a}±170
G2	1746^{a} ±79	1756^{b} ±77	1846^{b} ±89	1898^{b} ±89	1846^{b} ±89	1846^{b} ±89
G3	1699^{a}±80	1591^{b}±110	1799^{b}±120	1899^{b}±120	1857^{b} ±120	1901^{b}±77
Spermatozoa output per ejaculate (x 10^9)						
G1	3.20^{a}±0.18	3.89^{a} ±0.19	4.68^{a}±0.18	3.76^{a}±0.21	3.83^{a} ±0.33	4.34^{a} ±0.18
G2	3.58^{a}±0.09	5.18^{b} ±0.19	5.45^{b}±0.09	5.68^{b}±0.25	5.45^{b} ±0.09	5.50^{b} ±0.09
G3	3.65^{a}±0.06	5.00^{b}±0.26	5.67^{b}±0.06	5.98^{b}±0.35	5.57^{b}±0.16	5.99^{b}±0.44
Total output live per ejaculate (x 10^9)						
G1	2.52^{Ab}±0.28	3.08^{Aa}±0.28	3.70^{Aa}±0.28	2.79^{Aa}±0.33	3.07^{Aa}±0.28	3.31^{Aa}±0.08
G2	2.86^{Aa}±0.26	4.20^{Bb}±0.26	4.46^{Bb}±0.26	4.62^{Bb}±0.29	4.48^{Bb}±0.26	4.53^{Bb}±0.26
G3	2.96^{Aa}±0.26	4.25^{Bb}±0.76	4.71^{Bb}±0.26	5.07^{Bb}±0.26	4.74^{Bb}±0.26	5.04^{Bb}±0.26

a, b Means having different superscript small letters within the same column differ significantly (P<0.05).
A,B Means having different superscript capital letters within the same row differ significantly (P<0.05).

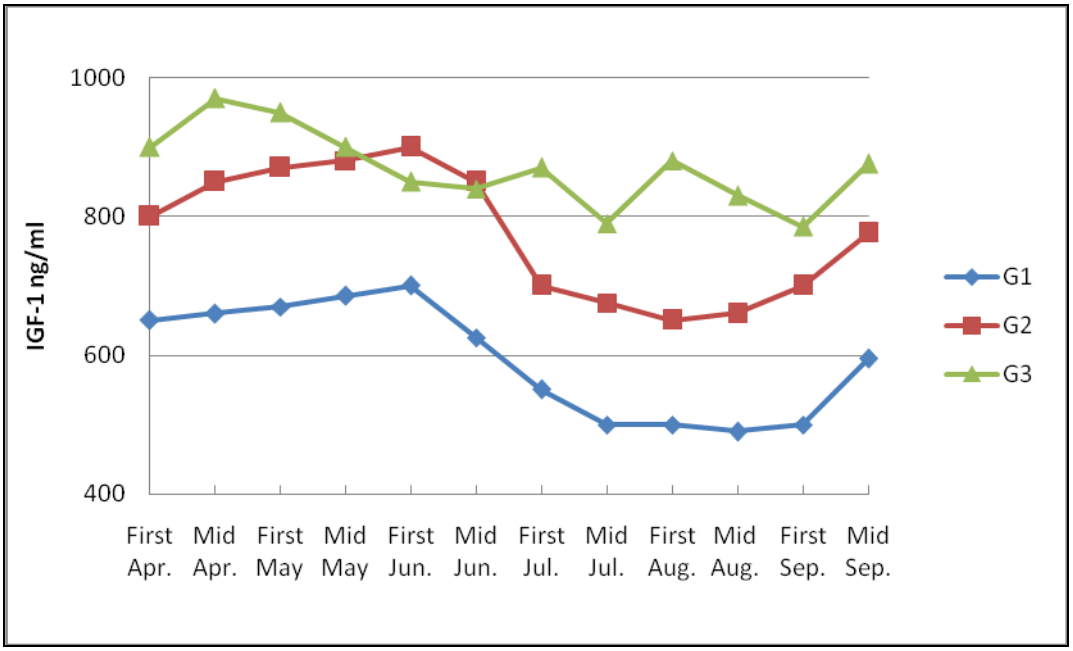

Fig. 2. Blood plasma IGF-1 throughout the experimental period in buffalo bulls groups injected fortnightly with placebo (G1), 250 mg rbST (G2) and 500 mg rbST (G3) throughout the experimental period

الخصائص الطبيعية والكيميائية للسائل المنوي لفحول الجاموس المحقونة بجرعتين مختلفتين من هرمون النمو البقرى المخلق وراثياً (rbST)

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تم تقسيم عدد ١٥ فحل جاموسي إلى ثلاث مجموعات متماثلة لدراسة تأثير جرعتين مختلفتين من هرمون النمو البقرى المخلق وراثياً على الخصائص الطبيعية والكيميائية للسائل المنوي خلال الأشهر الحارة من السنة (إبريل- سبتمبر). عوملت المجموعة الأولى (G1) كمجموعة ضابطة أما الثانية (G2) والثالثة (G3) تم حقنها تحت الجلد - ٢٥٠ أو ٥٠٠ ملليجم Somatech® كل ١٤ يوم/ حيوان لعدد ثمانية حقن متتالية على الترتيب. كان الوزن الأولى للذكور ٣٥٠.٩ كجم والعمر يتراوح بين ١٦- ١٨ شهر. تم جمع عينات الدم مرتين شهرياً لتقدير هرمون التستستيرون وعامل النمو رقم ١ (IGF-1) ببلازما الدم، بينما تم جمع السائل المنوي مرتين أسبوعياً لدراسة خصائصه المختلفة.

أشارت النتائج إلى زيادة حجم القذفة الناتجة من الحيوانات المعاملة بهرمون النمو البقرى المخلق وراثيا عن المجموعة الضابطة بـ٥ و١٢.٥% للمجموعة الثانية والثالثة على الترتيب. أظهر تركيز الحيوانات المنوية ($x\ 10^6$/ ملليتر) ارتفاعاً بمقدار ١٠ و١٤% للمجموعة الثانية والثالثة على الترتيب عن المجموعة الضابطة، والتي تعكس زيادة عدد الحيوانات المنوية للقذفة بمقدار ١٦ و٢٨% وكذلك عدد الحيوانات المنوية الحية بالقذفة بمقدار ٢١ و٣٨% للمجموعة الثانية والثالثة على الترتيب. مالت الحركة الجماعية، الحركة التقدمية للحيوانات المنوية والحيوانات المنوية الحية إلى الارتفاع معنوياً في المجموعة الثانية والثالثة، بينما مالت نسبة الشواذ الكلية إلى الانخفاض في حيوانات المجموعة الثانية مقارنة بالمجموعة الضابطة.

ارتفع تركيز عامل النمو رقم١ (IGF-1) ببلازما الدم معنوياً في الفحول الجمسى المعاملة بهرمون النمو البقرى المخلق وراثياً (٧٧٦ و٨٧٦ نانو جرام/ ملليتر، للمجموعة الثانية والثالثة على الترتيب) بالمقارنة بالمجموعة الضابطة (٥٩٤.٧ نانو جرام/ ملليتر). أما تركيز هرمون التستستيرون ببلازما الدم كان منخفضاً في المجموعة الضابطة (٥.٦٢ نانو جرام/ ملليتر) بالمقارنة بالمجموعة الثانية والثالثة (٨.٠٨ و٩.٧٨ نانو جرام/ ملليتر على الترتيب).

نستنتج من النتائج أن حقن فحول الجاموس بهرمون النمو البقرى المخلق وراثياً سواء ٥٠٠ أو ٢٥٠ ملليجم/ ١٤ يوم/ حيوان بمعدل ثمانية حقن أدت إلى تحسن الخصائص الطبيعية للسائل المنوي وكانت الأفضلية للجرعة ٢٥٠ ملليجم).