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INFLUENCE OF DIETARY LASALOCID ON SOME SERUM CHARACTERSTICS AS WELL AS SEMEN QUALITY AND FREEZABILITY IN ADULT BARKI RAMS

(With 5 Tables)

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تأثير اضافة اللاسالوسيد الى الغذاء على بعض مؤشرات الدم و كفاءة السائل المنوى و قابليته للتجميد فى الكباش البرقى

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لدراسة تأثير اضافة اللاسالوسيد الى الغذاء على كل من الانسولين، الكولستيرول، الكالسيوم، الفوسفور، الماغنسيوم، الزنك، النحاس فى مصل الدم و كذلك على كفاءة السائل المنوى و قابليته للتجميد، تم أستخدام عدد ٦ من الكباش البرقي البالغة (٢٦ شهرا عمرا، ٦٤,٥ كجم وزنا) و ذلك قبل التغذية (قبل المعالجة) لمدة ٣ أسابيع متتالية و بعد التغذية (بعد المعالجة) لمدة ٩ أسابيع متتالية . كانت التغذية قبل المعالجة تتكون من ٤ كجم دراوة بالأضافة الى ٢٥٠ جم من مكعبات علف متكامل (١١٪ بروتين خام ، ٢,٣ ميجا كالورى/ طاقة ممثلة / كجم). و تم المعالجة باللاسالوسيد بأضافة ٢٢ مجم لاسالوسيد صوديوم/ رأس/ يوم الى عليقة ما قبل المعالجة. أدت التغذية على اللاسالوسيد الى أرتفاع معنوى فى مستوى كل من الانسولين (٥,٥٣-٦,١٩ نانوجرام / مللى)، الكولستيرول (٨١,٠٠ - ٨٥,٧٨ مجم / ديسيلتر)، الفوسفور (٥,١٣ - ٥,٥٢ مجم / ديسيلتر)، الماغنسيوم (٢,٩٣ - ٣,٠٣ مجم / ديسيلتر)، الزنك (١,٣٨ - ١,٤٦ مجم / لتر)، النحاس (٠,٩٩ -١,١٥ مجم / لتر) فى مصل الدم وذلك أثناء فترة ما بعد المعالجة عنها فى فترة ما قبل المعالجة (حيث كانت النتائج : ٤,٨٣ نانوجرام/مللى ، ٧٨,٥ مجم/ديسيلتر، ٤,٦٥ مجم/ ديسيلتر، ٢,٨٦ مجم/ ديسيلتر، ١,١٤ مجم/لتر، ٠,٨٦مجم/لتر على الترتيب). وعلى العكس من ذلك، كان مستوى مصل الكالسيوم أقل معنويا فى فترة المعالجة باللاسالوسيد (٩,٥٢-٩,٠٨ مجم / ديسيلتر) عنها فى فترة ما قبلها (٩,٨٨ مجم/ ديسيلتر). كان محيط الصفن أكبر فى الكباش بعد تغذيتها على اللاسالوسيد (٣٣,٩٥ - ٣٧,٨٣ سم) عنها قبل التغذية عليه (٣١,٩٢ سم) .و من ناحية أخرى لم يتاثر كل من حجم القذفة أو نسبة الحيوانات المنوية الحية بالمعالجة باللاسالوسيد. بينما تأثر كل من تركيز الحيوانات المنوية /قذفة و الحركة الفردية للحيوان المنوى بالتغذية على اللاسالوسيد حيث تحسن كل منهما فى فترة ما بعد المعالجة

(٤٢٤٣,٣ - ٦١٣٣,٣ X ١٠/ مللى لتر، ٨٥,٢٨ - ٩٠,٠٠ ٪ على الترتيب) عنها فى فترة ما قبل المعالجة (٢٤٣٤,٤ X ١٠ / مللى لتر، ٧١,٦٧٪ على الترتيب). و على العكس من ذلك أرتفع كل من التشوهات الصغرى و الكبرى باضافة اللاسالوسيد. أما بعد التجميد وأسالة السائل المنوى تم تسجيل أرتفاع معنوى فى حركة الحيوان المنوى و كذلك حيويته و ذلك بأضافة اللاسالوسيد الى الغذاء (٣٨,٣٣ - ٥٥,٨٣ ٪ ، ٤٨,٧٥ -١٠٢,٩٢ على الترتيب) عنها قبل الاضافة (٣٣,٠٦ ٪ ، ٣٨,٩١ على الترتيب) . كما تم تسجيل أرتباط معنوى بين مؤشرات الدم و بعض مكونات السائل المنوى و محيط الصفن. و يمكن التوصية بأضافة اللاسالوسيد بمعدل ٢٢ مجم/ رأس/ يوما للكباش البرقي لتحسين كفاءة السائل المنوى و قابليته للتجميد أثناء موسم التناسل و خاصة خارج موسم البرسيم.

SUMMARY

To study the effect of dietary lasalocid on serum insulin, cholesterol, Ca, P, Mg, Zn and Cu as well as semen quality and freezability, a number of six mature Barki rams (about 26 months of age and 46.5kg) were used during the periods of pretreatment (before feeding for 3 sucessive weeks) and post-treatment (after feeding for 9 sucessive weeks) periods. The pretreatment diet was 4 kg green corn (Darrawa) plus 250 kg of pelleted complete diet (11% crude protein and 2.4 Mcal ME/kg). Lasalocid treatment (post-treatment) was the pretreatment diet with the addition of 22 mg lasalocid sodium\head\day. Feeding lasalocid resulted in significantly ($p<0.05$) higher serum insulin (5.53 - 6.19 ng/ml), cholesterol(81.00 - 85.78 mg/dl), P (5.13 - 5.52 mg/dl), Mg (2.93 - 3.03 mg/dl), Zn (1.38 - 1.46 mg/l) and Cu (0.99 - 1.15 mg/dl) during post-treatment period than before (pretreatment; 4.83 ng/ml, 78.5mg/dl, 4.65mg/dl, 2.86mg/dl, 1.14mg/l and 0.86mg/l, respectivily). On the contrary, serum Ca level was significantly ($p<0.05$) lower after lasalocid supplementation (9.52 - 9.08 mg/dl) than before(9.88 mg/dl). Lasalocid fed rams also exhibited a greater($p<0.01$)scrotal circumference (33.95 - 37.83 cm) than before feeding (31.92 cm). On the other hand, neither semen volume (ml) nor L/D (%) was affected by lasalocid supplementation. However, both semen concentration and individual motility were significantly ($p<0.05$) improved by lasalocid feeding (4243.3 - 6133.3 $X10^6$/ml and 85.28 - 90.00%, respectivily) than before (2434.4 x 10^6/ml and 71.67%, respectivily). On contrast, both major and minor abnormalities tended to be increased by lasalocid feeding. Meanwhile, both post-thawing motility and viability index of frozen semen were significantly ($p<0.05$) higher during post-treatment (38.33-55.83% and 48.75-102.92, respectivily) than during pretreatment (33.06% and 38.91, respectivily) period. Significant correlation coefficients were reported between some serum and semen

parameters as well as scrotal circumferance. It can be concluded that, lasalocid sodium can be used in a rate of 22mg\head\day for Barki rams for improving semen quality and freezability during breeding season.

Key words: *Barki rams - Semen quality - Dietary lasalocid.*

INTRODUCTION

The acheivement of high levels of fertility and prolificacy in sheep flocks relies not only upon the female members but also upon their male consorts. Whilist substantial literature has been developed up on the effects of nutrition in the ewe, far less attention has been given to the ram.

The common breeding season of Barki breed sheep in Egypt is June-November (Eliase, 1987). Unfortunatly, such period is found out of Berseem (Trifolium Alexandrinum) season (October-May), instead, most Egyptian farmers used to feed their animals on the available grazes or low quality roughages. Consequently, rams would suffer from undernutrition during the breeding season. However, undernutrition led mature rams to loose weight and reduce their daily sperm output, sperm concentration in the ejaculate and testicular size (Parker and Thwaites, 1972; Braden et al., 1974; Oldham et al., 1978 and Alkass et al., 1982). This may attributed to the reported decrease in testicular output of testesterone (Setchell et al., 1965), smaller seminiferous tubules (Oldham et al., 1978) or smaller pituitary gland associated with low LH content (Alkass et al., 1982) in the under fed rams.

One way to improve feed efficiency in grazing sheep and cattle is the use of feed additives. Ionophores (monensin, lasalocid and salinomycin) are feed additives that can enhance both energy, nitrogen and mineral metabolisims (Bergen and Bates, 1984). The beneficial effects of ionophores has been previously reported on age and weight at puberty in Barki-ewe-and ram-lambs (Hegazy and Eliase, 1997) as well as on reproductive performance of Barki ewes (unpublished data) but its possible effects in ram has not been reported. These performance improvements are partially due to the energetically favorable shift in ruminal fermentation, in which, ruminal acetate: propionate ratio is decreased (Hegazy and Eliase, 1997). However, the increase in propionate production alone cannot account for all improvement. Recently, ionophores has been found to influence mineral metabolism in ruminants (Greene et al., 1986). As an ionophore; lasalocid's basic effect is to facilitate the passage of ions across cell membrane and it was found to increase the apparent absorption of phosphorus, magnesium

and copper and increase retention of phosphorus, magnesium, zinc and copper (Starner et al., 1984). However, little is now concerning the nutritional interrelationship of lasalocid with minerals and fertility.

The aim of this work was to study the effect of dietary lasalocid on some serum constituents and semen quality and freezability of adult Barki rams.

MATERIALS and METHODS

Animals:

This study was undertaken at the experimental farm of Animal Reproduction Research Institute (Giza, Egypt) and involving six mature clinically healthy Barki rams (about 26 months of age and 46.5kg).

Feeding:

Rams were group fed in an open shed and had free a ccess to water and mineral blocks. At the start of experiment (early June, 1996), rams were fed (pretreatment period) on the farm diet (basal diet) containing about 4kg of green corn (Darrawa) plus 250g of pelleted complete diet (containing 11% crude protein and 2.3 MCal/ME/kg). Three weeks later, rams were fed on the same basal diet supplemented with 22mg/head/day of lasalocid sodium (Avatec® supplied by Hoffman La Roche Co.). The first 3 weeks after supplementation were conisedered as (adaptation period). After this period and for 9 weeks feeding lasalocid was continued (post-treatment period) until the end of the experiment.

Serum analysis:

Four blood samples were taken by veinpuncture from each ram for estimation of serum insulin, cholesterol, calcium (Ca), inorganic phosphorus (P), magnesium (Mg), zinc (Zn) and copper (Cu). The first sample was taken one week before lasalocid supplementation (pretreatment). The other 3 samples (post-treatment) were taken at the day of first semen sample during post-treatment period, then every 28 days until the end of exeperiment. Serum insulin was determined by Radioimmunoassay according to Taylor (1976) using diagnostic reagents of Sorin Biomedica, Italy. Serum cholesterol, Ca, P and Mg was determined by enzymatic methods using a commercial diagnostic kits purchased from Bio Merieux, France. Serum Zn and Cu were estimated by Atomic Absorption Spectrophotometer (Mod.3300,Perkin Elmer, USA).

Scrotal circumference:

Testicular mass based on scrotal circumference was assessed at the same day of blood sampling (i.e. 4 times during experiment) with the animals

in a standing postion, by retaining both testes in the base of the scrotum and measuring the combined circumferance of scrotal tissues plus the two testes by using tape-measaure.

Semen analysis and freezability:

Semen was collected from each ram using an artificial vagina, where the first and second ejaculates of each collection were pooled together. During pretreatment period, semen was collected weekly from all rams and for 3 sucessive weeks. The results were considered as control (pretreatment). Meanwhile, during adaptation period (3 weeks), semen was collected twice weekly to stabilize extragonadal sperm reserves. After that, and for 9 weeks, semen was harvested weekly (post-treatment). Immediatly after collection, semen samples were subjected to the convential methods of evaluation (Salisbury and Van Demark, 1961) including volume (ml), sperm concentration ($x10^6$ \ml), individual motility (%), live spermatozoa (%) (Compbell et al., 1956) and abnormal spermatozoa (Blom, 1983).

After collection, semen samples were extended in tris-glucose-citric acid egg yolk diluent which prepared as two portions according to Abdel Malk (1994) where post-thawing motility (%) and viability index were recorded (Milovanov, 1962).

Statstical analysis:

All data were statistically analysed using PCSTAT computer program, (c) copyright 1985, Univ. Georgia, USA. Two ways analysis of variance (single observation) without interaction was used in analysis. The first factor was the individuality (to remove the effects of individual variations between rams) while, the second factor was sampling time. The obtained results of semen quality during pretreatment periods (3 samples) were averaged and introduced into analysis as one sample. Simple correlation coefficients were performed between scrotal circumference, semen and serum parameters according to Snedecor and Cochran (1982).

RESULTS

Scrotal circumference:

As shown in Table 1, scrotal circumference (cm) was significantly ($p\angle 0.001$) increased after lasalocid supplementation (post-treatment) than before (pretreatment) .

Serum analysis:

Table 1 also presented the effect of lasalocid feeding on both serum insulin and cholesterol levels during the pre-and post-treatment periods in

Barki rams. When compared with pretreatment samples (control), both serum insulin and cholesterol were significantly ($p<0.01$) elevated by lasalocid supplementation. While, such effect was delayed until sample 2 (post-treatment) in cholesterol, it persisted the rest of the experiment in both parameters.

Table 1: Effects of dietary lasalocid on scrotal circumference as well as serum insulin and cholesterol levels in Barki rams:

Sampling time	SC* (cm)	Insulin (ng\ml)	Cholesterol (mg\dl)
pre-treatment	31.916±0.62^a	4.83±0.08^a	78.50±0.52^a
post-treatment:**			
1	33.950±0.56^b	5.53±0.104^b	81.00±0.33^a
2	35.917±0.59^c	6.19±0.051^c	83.42±0.53^b
3	37.833±0.72^d	6.04±0.050^c	85.78±0.64^c

Within column, means with different superscripts are significant on at least ($p<0.05$). *Scrotal circuference ** With 28-day interval.

Table 2 illustrats the effect of lasalocid supplementation on serum mineral profile in Barki rams. It was found that, each of serum P,Mg,Cu and Zn was significantly ($p<0.05$) increased after lasalocid treatment. The differences between pre and post-treatment samples were noticed until the end of the experiment. On contrast, significant ($p<0.01$) lower serum Ca concentrations were reported after lasalocid supplementation.

Data gathering in table 3 showed the effect of diatery lasalocid on semen characterestics in Barki rams. Their analysis revealed that, neither volume nor L/D percentage was significantly varied by supplementation. However, both semen concentrations ($X10^6$ /ml) and individual motility % were significantly ($p<0.05$) improved by lasalocid treatment. These improvements were noticed from the first week during the post-treatment period and persisted the rest of the experiment. On the contrary, both minor and major abonormalities (%) were significantly ($p<0.05$) elevated by lasolacid treatment. This effect was noticed after the second week during post-treatment period.

Concerning freezability parameters, tables 4 revealed that both viability index (%) and post-thawing motility were significantly ($p<0.01$) enhanced in those samples that frozen after lasalocid supplementation than those before one.

Correlation between serum and semen characterstics :

Table 5 presented the correlation coefficients between scrotal circumference, serum and semen parameters. It was noted that, scrotal circumference was positively correlated with viability index, major abnormalities, semen concentration and post-thawing motility. Meanwhile, each of insulin, P, Mg, Zn and Cu (table 5) was significantly positively correlated with semen concentration, viability index and post-thawing motility. However, cholesterol was positively correlated with both post-thawing motility and viability index only. On the other hand, Ca level was significantly negative with semen concentration, post-thawing motility and viability index.

DISCUSSION

The reported increase in testicular mass (as measured by scrotal circumference) in response to lasalocid feeding had been reported by Neuendorff et al. (1985) in bull and Hegazy and Eliase (1997) in Barki ram-lambs. The present work has also indicated a strong association between lasalocid feeding, testis size and testis functions (taking into account the significant positive correaltion that reported between scrotal circumferance and semen characterstics). This was in line with Tegegne et al. (1992) who clarified that such increase in testis size was associated with increases in the proportion, length and diameter of seminiferious tubules in the testis as well as in the number of germ cells in this tubules (Curtis and Amann, 1981 and Amann, 1983).

The effect of ionophores feeding on insulin levels was previously reported by Hegazy (1997) in buffaloes. The reported increase may be attributed to the shift in propionate concentrations in the rumen associated with lasalocid feeding (Hegazy and Eliase, 1997). Meanwhile, Bergman and Wolff (1971) found that I/V administration of propionate (at a physiological level) was accompanied by a significant increase in insulin level. However, Armstrong and Spears (1988) suggested a non-rumenal way by which ionophores affect insulin level through either direct cellular mechanism (by increasing the intracellular calcium) or indirect mechanisms including B-adrenergic stimulation and Catecholamine release. In the present study, serum cholesterol level was also significantly elevated after lasalocid supplementation. Such elevation may due to inceased bacterial lipid synthesis as well as increased ruminal fatty acids resulted in increasing the availability of lipids for absorption (O'Kelly and Spiers 1988&1990). Meanwhile,

cholesterol is considered as a precursor in synthesis of steroid hormones (Singh et al., 1974) which may clarify the reported correlation between serum cholesterol level and semen traits. The present study has also demonstrated that serum Ca level was significantly lowered by lasalocid feeding. A result that could be supported by the work of Starner et al. (1984) who found lower soluble Ca in ruminal fluid with ionophore feeding. However, Hochman and Perlman (1976) and Pressman and Fahim (1982) suggested that this decrease may occur through the effect of lasalocid on the extracellular Ca by bringing it into the cell via an exchange diffusion carrier. Regarding serum P,Mg and Zn concentrations, the reported elevation in these parameters by lasalocid could be supported by the work of many authors who found increases in apparent absorption and retention of P (Starner et al. 1984 and Kirk et al.1985), Mg (Kirk et al.1994 and Starner et al., 1984) and Zn (Costa et al., 1985 and Kirk et al.,1985) lasalocid or monensin fed sheep or steers. From the present study, lasalocid may enhance Cu status of rams. A result that previously observed by Starner et al. (1984) and Reffett-Stabl et al. (1989) in lasalocid fed steers. The later added that, ionophores may increase Cu bio-availability by reducing ruminal protozoal number or by reducing ruminal production of sulfid from sulfer containing amino acids which may reduce its absorption.

The present results have also revealed a profound effect of lasalocid supplementation on semen quality and freezability. Similar nutritional influences on semen traits have also been reported in bulls (Rekwot et al., 1988 and Tegegne et al., 1992). Concerning the effect of nutritional status on testicular functions, Martin and Walkden-Brown (1995) developed a concept that both GnRH- independent and dependent pathway are involved in nutritional effects on spermatogenesis. For the GnRH -dependent pathway, the stimulatory effect of intracerbral infusion of insulin on LH secretion in mature rams (Miller et al., 1995) implies a role for insulin in conveying information on nutritional status to the GnRH neurons (an effect that was noted in the present study). Moreover, previous results also provide evidence that lasalocid can alter release of LH hormone from the pituitary and secretion of testesterone (Rutter et al., 1991). It was found that, increasing intracellular Ca (that suggested to be occured in our study) with divelent ionophore (lasalocid) may altered several events on the gonadotropic critical to LH release such as receptor recycling (King, 1984 and Stein et al., 1984) and post receptor events mediated by Ca (Conn et al.,1987). Meanwhile, alterations of extracellular concentrations of minerals such as Mg , Cu and Zn (as occured in the present work) may have altered GnRH release or the

affinity of GnRH for its receptors (Armstrong and Spears, 1988). Equilibrium saturation binding studies indicated that, Mg increased FSH binding by increasing the apparent numbers of binding sites (Ford and LaBarbera, 1987). Moreover, the involvement of P in phospholipid and cAMP synthesis may be a key to its effect on reproduction (Hurley and Doane, 1989). Regarding the significant positive correlations between serum Zn level and some semen traits (sperm concentration, viability index and post-thawing motility)can be supported by (Chesters, 1978 and Apger, 1985). Zn is known to be essential for spermatogenesis and for testosterone biosynthesis (Leathem,1966) who clarified that, zinc's function in regulating androgen metabolism may be at the testicular level (indepenedent-GnRH pathway),

On the other hand, the reported elevation in percentage of major and minor abnomalities could be supported by the work of Tegegne et al. (1994) who found that, bull fed high nutrition had about 10% higher abnormal sperm cells than bull in low nutrtion. These differences may be associated with higher levels of scrotal fat deposition which could affect testicular heat change, spermatogenesis and spermatozoal morphology as shown by Wildeus and Entwistle (1986) after scrotal insulation. Such effect may explain the significant positive correlation between scrotal circumferance and major abnormalities.In spite of the recorded elevation in both major and minor abnormalties (total abnormalities) by lasalocid treatment which reached its highest count at the 5th week (18.67%), such conut still present within the normal range recorded for semen used for A.I. (25%) by Bearden and Fuquay (1984).

From this study, it can be concluded that, lasalocid can be fed on a rate of 22 mg\head \day to Barki rams to enhance semen quality and freezability during breeding season especially out of berseem season.

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Table 2: Effects of dietary lasalocid on serum Ca, P, Mg, Cu and Zn levels in Barki rams:

Sampling time	Ca (mg\dl)	P (mg\dl)	Mg (mg\dl)	Cu (mg\l)	Zn (mg\l)
pre-treatment	9.88±0.079^{a}	4.65±0.039^{a}	2.86±0.025^{a}	0.865±0.017^{a}	1.145±0.04^{a}
post-treatment:*					
1	9.52±0.059^{b}	5.13±0.03^{b}	2.93±0.02^{b}	0.99±0.024^{b}	1.38±0.025^{b}
2	9.08±0.03^{c}	5.33±0.03^{c}	2.95±0.03^{b}	1.106±0.04^{c}	1.29±0.028^{b}
3	9.23±0.06^{c}	5.52±0.028^{d}	3.03±0.77^{c}	1.150±0.029^{d}	1.457±0.02^{b}

Within column, means with different superscripts are significant on at least ($p\angle 0.05$).
*With 28-day interval.

Semen quality and freezability :

Table 3: Effects of dietary lasalocid on semen characterstics of Barki rams:

Sampling time	Volume (ml)	Concentration (x10^\ml)	Ind. Mot. (%)	Major Ab. (%)	Minor Ab. (%)	L\D (%)
pre-treatment	1.49±0.1^{a}	2434.4±135.1^{a}	71.67±4.8^{a}	2.37±0.83^{a}	2.11±0.68^{a}	86.28±0.7^{a}
post-treatment:						
week: 1	1.58±0.15^{a}	5770.0±381.6^{bcd}	90.00±1.2^{b}	0.83±0.28^{a}	2.33±1.31^{a}	89.00±1.6^{a}
2	1.42±0.27^{a}	4243.3±430.5^{b}	85.00±1.1^{b}	±0.38^{b}	6.00±4.71^{bc}	88.33±0.6^{a}
3	1.17±0.23^{a}	5463.3±710.3^{bcd}	90.00±1.0^{b}	8.00±0.47^{b}	6.00±0.78^{bc}	89.67±0.2^{a}
4	1.40±0.14^{a}	6133.3±509.7^{cd}	90.00±1.1^{b}	8.83±1.01^{b}	5.33±0.81^{b}	90.00±1.3^{a}
5	1.45±0.11^{a}	6041.3±1052.9^{d}	86.67±1.5^{b}	10.0±1.34^{b}	8.67±1.01^{c}	89.00±0.7^{a}
6	1.25±0.06^{a}	4793.3±307.58^{b}	87.50±1.6^{b}	10.0±4.08^{b}	5.67±1.12^{b}	91.83±1.0^{a}
7	1.46±0.07^{a}	4743.3±645.8^{b}	86.67±0.9^{b}	9.16±1.03^{b}	7.50±1.50^{bc}	86.17±3.0^{a}
8	1.55±0.27^{a}	5750.0±196.1^{bcd}	88.33±1.5^{b}	6.00±0.58^{c}	5.33±0.65^{b}	85.82±0.9^{a}
9	1.21±0.15^{a}	4273.3±540.1^{b}	85.28±0.6^{b}	7.16±0.79^{c}	5.17±0.89^{b}	88.00±1.5^{a}

Within column, means with different superscripts are significant at ($p\angle 0.05$).

Table 4: Effects of dietary lasalocid on sperm freezability of Barki rams:

Sampling time	Post-thaw motility(%).	Viability Index
pre-treatment	33.06±3.29^{a}	38.91±5.01^{a}
post-treatment:		
Week: 1	38.33±1.92^{ab}	49.17±6.37^{ab}
2	38.33±0.96^{ab}	51.42±3.41^{b}
3	40.00±1.17^{b}	48.75±5.43^{ab}
4	43.33±0.96^{bc}	72.92±4.06^{cd}
5	44.17±1.40^{bc}	75.00±3.12^{cde}
6	47.50±3.28^{c}	83.33±7.40^{ce}
7	44.17±4.92b^{c}	66.67±5.19^{d}
8	55.83±2.74^{d}	86.25±5.97^{e}
9	55.00±1.67^{d}	102.92±2.9^{f}

Within column, means with different superscripts are significant on at least ($p\angle 0.05$).

Table 5: Correlation coefficients between scrotal circumferance, serum and semen parameters in Barki rams :

	Volume	concent-ration	Ind.Mot.	Major ab.	Minor ab.	L\D %	Viability Index	Post-thwing Motility
Insulin	—	0.41*	—	—	—	—	0.64**	0.69**
Choles terol	—	—	—	—	—	—	0.43*	0.70**
Ca	—	-0.44 *	—	—	—	—	-0.57**	-0.62**
P	—	0.37*	—	—	—	—	0.67**	0.70**
Mg	—	0.61**	—	—	—	—	0.67**	0.64**
Cu	—	0.40*	—	—	—	—	0.66**	0.77**
Zn	—	.044*	—	—	—	—	0.63**	0.61**
SC#	—	0.50*	—	0.50*	—	—	0.60**	0.68**

*p<0.05 **p<0.01 #Scrotal circumferance.