

EFFECT OF SEASON AND SURGICAL DEVIATION OF PENIS ON SEMEN CHARACTERISTICS AND PERIPHERAL TESTOSTERONE CONCENTRATION IN AWASSI RAMS

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SUMMARY

Three surgically deviated penis, and 6 intact control Awassi rams of 3-4 years of age were used in this study. All animals were group-fed and kept under the same managerial and natural environmental conditions. During the breeding season (late spring and early summer) the control rams joined the ewes and served as sires, while the operated rams were kept separated from ewes. Semen was collected by the artificial vagina, which was followed by the venipuncture sampling of blood twice a month throughout the experimental year. Semen ejaculates were immediately evaluated, while blood serum was used for testosterone determination.

Surgical deviation of penis resulted in a decrease ($P<0.05$) in concentration, progressive motility and abnormality of sperms, while ejaculate volume and serum testosterone concentration were not significantly influenced by the treatment. These changes observed in semen quality in response to surgical treatment were probably due to the difference in the ejaculation frequency between the two groups.

Seasonal variation ($P<0.01$) was noticed for all semen characteristics studied in both groups, exhibiting the lowest semen quality during winter, and the highest quality during summer with the intermediate quality during spring. No significant differences were observed in most semen characteristics between summer and autumn in both groups. Serum testosterone level was influenced ($P<0.01$) by season, and displayed similar trends to semen quality. Additionally, serum testosterone level was positively correlated ($P<0.01$) with ejaculate volume, sperm concentration and progressive motility, while it was negatively correlated ($P<0.01$) with sperm abnormality in the two groups.

This study suggested that Awassi rams, as a fat-tailed breed in the subtropical regions, sexually respond to the long photoperiod and the moderate high ambient temperature during summer as indicated by higher volume and quality of semen accompanied with higher levels of serum testosterone. These physiological responses were almost continued during autumn. On the other hand, the present study recommended usage of the deviated penis ram as teaser in the experimental flocks, since its testis showed a normal spermatogenic and endocrine activities, besides it could be easily used in the artificial insemination.

Keywords: Awassi rams, season, surgical deviation of penis, semen characteristics, testosterone concentration.

INTRODUCTION

Reliable oestrous detection is essential in sheep flocks where artificial insemination is used. Vasectomized rams have been commonly used as teasers, but they could not be used for semen collection and may spread venereal diseases. Teaser preparation by surgical translocation or deviation of prepuce and penis has been reported previously in bulls (Roya and Biven, 1973) and rams (Ball *et al.*, 1978 and Abdalla *et al.*, 1995). Surgical deviation of penis prevents coitus and facilitates semen collection for artificial insemination. It was reported that penis deviation in rams did not influence libido or semen quality (Jochle *et al.*, 1973 and Ball *et al.*, 1978). However, effect of penis deviation on the peripheral levels of testosterone was not investigated.

On the other hand, seasonal variation in semen quality of fat-tailed sheep was studied (Hafez *et al.*, 1955; Amir and Volcani, 1965 a,b,c; El-Wishy *et al.*, 1976; El-Fouly *et al.*, 1977). Some investigators claimed that elevated ambient temperature and long photoperiod in summer are associated with inferior quality of ram semen, while others reported conflicting results under the subtropical conditions. Effect of season on the semen characteristics and testosterone levels was not tested in the deviated-penis rams, however. Therefore, this study was undertaken to investigate changes in semen quality and serum testosterone concentration in response to season and surgical deviation of penis in Awassi sheep.

MATERIALS AND METHODS

This study was carried out at the Agricultural Center for Research and Production, Jordan University of Science and Technology, Irbid-Jordan, at 35°59' E, 32°30' N and an elevation of 590 m above sea level. The annual precipitation varied between 150 and 800 mm and was distributed between December and March. Three Awassi rams were surgically operated as previously described by Abdalla *et al.* (1995) (Fig. 1) to be used as deviated-penis rams; teasers for oestrus detection in ewes and were allocated for the experimental procedure. Moreover, six intact (non-operated) Awassi rams were used to serve as control. All animals aged 3 to 4 years, weighed 65-75 kg, separated from ewes and kept under the same managerial conditions.

The animals were group-fed and housed in a covered shelter with an open-air run, allowed to walk freely and given water *ad libitum*. Except on the days of semen collection, the rams were allowed to graze between 9 am and 2 pm. During the summer months, grazing was restricted to early morning and late afternoon to avoid hot weather during mid-day. Green feed was offered *ad libitum* between late winter and early spring, while wheat straw was supplied during the rest of the year. Furthermore, a concentrate ration of 0.75 kg was given to each ram daily in the afternoon throughout the experimental period.

Semen was collected in the early morning twice a month throughout each season, using an artificial vagina. Immediately after the collection, each ejaculate was evaluated for volume, percent sperm progressive motility and percentage of abnormal spermatozoa employing standard methods. The sperm cell concentration was determined using a haemocytometer. Blood samples were drawn via jugular venipuncture, immediately after the semen collection. Serum was obtained by centrifugation at 1500 X g for 20 min and kept at -20°C until thawed for assay. Serum

testosterone concentrations were determined in duplicate by radioimmunoassay kits (Kodak Clinical Diagnostics Ltd., UK). Intra- and inter-assay coefficients of variation were 8.3% and 17.6%, respectively.

The observations on each animal, which were recorded over a period of one year, were grouped under four seasons : winter (December-February), spring (March-May), summer (June-August) and autumn (September-November). Climatological information for this location during the experimental year is summarized in Table 1 (Ministry of Transport, Meteorological Department, Amman, Jordan). During the breeding season (late spring and early summer), only the six intact rams joined ewes and served as sires. The data were subjected to a split-plot design for repeated measurements using the Statistical Analysis System (SAS, 1986). Analysis of variance (ANOVA) and Duncan's test (Steel and Torrie, 1980) were used to compare performances. Pearson's correlation coefficients(r) were performed between serum testosterone levels and each of the semen characteristics.

Table 1. Climatological data during the experimental year *

Season	Month	Air temperature (°C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
Winter	December	15.6	-1.4	100	27
	January	22.2	3.0	100	28
	February	19.6	-0.4	100	20
	Mean	19.1	0.4	100	25
Spring	March	25.4	3.4	100	28
	April	37.2	3.0	98	14
	May	36.6	6.4	98	15
	Mean	33.1	4.3	99	19
Summer	June	36.0	12.0	98	15
	July	35.0	15.0	100	23
	August	40.4	15.0	100	24
	Mean	37.1	14.0	99	21
Autumn	September	39.6	14.4	100	18
	October	35.0	11.8	100	22
	November	26.8	4.6	100	39
	Mean	33.8	10.3	100	26

* Data were received from Ministry of Transport, Meteorological Department, Amman, Jordan.

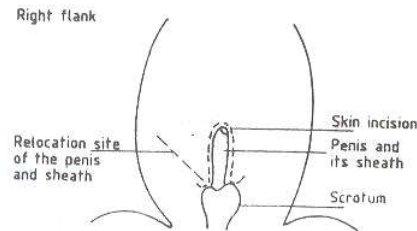


Figure1. The modified procedure for deviating the penis in rams (Abdalla *et al.*, 1995)

RESULTS AND DISCUSSION

Semen characteristics

Present study showed that surgical deviation of penis resulted in a slight decrease ($P < 0.05$) in sperm cell concentration, sperm progressive motility and sperm abnormality as shown in Table 2. These differences in semen quality between deviated-penis and intact control rams are probably due to the frequency of ejaculation and not to the surgical treatment itself, since rams with deviated penis were separated from the ewes, while the intact rams joined ewes throughout the breeding season. Semen may vary qualitatively and quantitatively with sexual activity of the animal (Mann and Lutwak-Mann, 1981). Ball *et al.* (1978) reported no significant differences between intact and deviated-penis rams with respect to ejaculate volume, sperm motility, abnormality and concentration, when both groups of rams were separated from the ewes and ejaculated twice a week.

Season exerted significant ($P < 0.01$) effects on all semen characteristics studied. The lowest semen quality was obtained during winter and the highest during summer (Table 2). Similar results were reported in Egypt for native sheep breeds; Ossimi rams (El-Wishy *et al.*, 1976), and Ossimi and Rahmani rams (El-Fouly *et al.*, 1977). The latter authors showed that the best quality semen was that of the summer and the poorest quality semen was that of the winter. Present results also indicate that semen quality was similar in summer and autumn except sperm cell concentration which was lower ($P < 0.01$) in autumn than in summer. Semen quality during spring in all parameters studied (Table 2) was found to be intermediate between winter and summer values. Similarly, El-Fouly *et al.* (1977) reported that sperm output of rams during autumn and spring was significantly lower than that of summer but excelled that of winter. Moreover, El-Wishy *et al.* (1976) found that sperm density of Ossimi rams was the highest in summer.

In the present study, ejaculate volume reached its peak during summer and autumn. The same trend was observed by El-Wishy *et al.* (1976) in Ossimi rams, and Amir and Volcani (1965 b) in Awassi rams. The lowest sperm motility observed in the present study during winter agreed with those reported by Hafez *et al.* (1955), Symington (1961) and Amir and Volcani (1965,b) in tropical and subtropical conditions. In Jordan, mean ambient temperature during winter ranged between 0.4 and 19.1°C (Table 1). Amir and Volcani (1965, c) claimed that a minimum air temperature under 10°C during the day of collection, or an air temperature lower than 15°C during the collection time are detrimental to sperm motility. This might indicate that high relative humidity coupled with a low winter temperature (Table 1) exerted detrimental effects on the testis and / or the epididymal function of rams resulting in decreased sperm motility.

The highest quality of semen obtained in this study during summer and autumn is probably due to the long photoperiod during summer and its carryover effect during autumn. Amir and Volcani (1965, c) suggested that the spermatogenic activity of fat-tailed rams in the subtropics responds to the influence of increasing daylight with a lagged effect of two months, due to the spermatogenic cycle and the transport of sperms through epididymis. It seems therefore that in rams the secretion of FSH influencing the process of spermatogenesis increases and decreases with the length of daylight. Emmens (1959) reported that the fundamental factor which influences the breeding ability of the rams is the photoperiod. Analysis of variance showed that

there was a significant interaction between season and treatment with respect to sperm cell concentration ($P<0.05$) and abnormality ($P<0.01$), Table 2.

Serum testosterone concentration

Surgical deviation of penis did not influence serum testosterone concentration, while season exerted a significant ($P<0.01$) effect, and there was a significant ($P<0.01$) interaction between treatment and season. Serum testosterone concentration displayed the same trend as semen characteristics, being significantly ($P<0.01$) the highest during summer and autumn, the lowest during winter, and intermediate during spring. In agreement with these results, Gomes and Joyce (1975) in the USA showed that testosterone concentrations of different breeds of rams were the lowest in December, increased gradually through April and May, and reached a peak in July, then decreased in August and September. These authors suggested that light is the major stimulus to testicular steroidogenesis where summer temperatures are moderate, but in hotter climates thermal factors may override photoperiodic effects.

Table 2. Overall mean and least square means for some semen characteristics and serum testosterone concentrations in deviated-penis rams (DPR) and intact rams (IR) during different seasons

Source of variation	EV (ml)	SC, $\times 10^9$ /ml	SM (%)	SA (%)	TC (ng/ml)
Overall mean \pm SD	1.13 \pm 0.30	2.72 \pm 0.52	87.0 \pm 7.81	8.61 \pm 3.43	2.53 \pm 0.82
Treatment (T)					
DPR	1.17 \pm 0.11 ^a	2.50 \pm 0.10 ^a	84.0 \pm 1.17 ^a	7.17 \pm 0.16 ^a	2.25 \pm 0.27 ^a
IR	1.11 \pm 0.08 ^a	2.83 \pm 0.07 ^b	88.5 \pm 0.82 ^b	9.33 \pm 0.11 ^b	2.67 \pm 0.19 ^a
Significance	NS	$P<0.05$	$P<0.05$	$P<0.01$	NS
Season (S)					
Winter	0.87 ^a	1.99 ^a	76.7 ^a	12.70 ^a	1.85 ^a
Spring	1.14 ^b	2.93 ^b	86.9 ^b	7.90 ^b	2.21 ^b
Summer	1.30 ^c	3.09 ^c	90.1 ^c	6.31 ^c	2.85 ^c
Autumn	1.25 ^c	2.65 ^d	91.3 ^c	6.07 ^c	2.95 ^c
SE	± 0.03	± 0.03	± 0.67	± 0.25	± 0.07
Significance	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
Interaction (T x S)					
(DPR) Winter	0.87	1.87 ^a	74.4	11.28 ^a	1.71 ^a
Spring	1.19	2.79 ^b	83.6	6.28 ^b	2.16 ^b
Summer	1.35	2.93 ^c	88.6	5.50 ^b	2.54 ^c
Autumn	1.27	2.41 ^d	89.4	5.61 ^b	2.61 ^c
SE	± 0.04	± 0.05	± 1.10	± 0.40	± 0.11
(IR) Winter	0.87	2.11 ^a	78.9	14.14 ^a	1.99 ^a
Spring	1.10	3.07 ^b	90.2	9.53 ^b	2.26 ^b
Summer	1.25	3.26 ^c	91.7	7.11 ^c	3.15 ^c
Autumn	1.24	2.88 ^d	93.2	6.53 ^c	3.29 ^c
SE	± 0.03	± 0.03	± 0.77	± 0.28	± 0.08
Significance, T x S	NS	$P<0.05$	NS	$P<0.01$	$P<0.01$

EV, ejaculate volume; SC, sperm concentration; SM, sperm progressive motility; SA, sperm abnormality; TC, testosterone concentration.

a,b,c,d Means in columns within treatment (T), season (S) or (T x S) with unlike superscripts differ ($P<0.05$, $P<0.01$).

In the present study, in both groups of Awassi rams, serum testosterone concentration was positively correlated ($P<0.01$) with ejaculate volume, sperm cell concentration and sperm progressive motility, and negatively correlated ($P<0.01$) with sperm abnormality (Table 3). In addition, ejaculate volume was the only characteristic more correlated with blood level of testosterone. This study clearly indicates that there is a seasonal variation in semen quantity and quality of Awassi rams under the climatic conditions of Jordan. These seasonal changes in semen characteristics observed in this study were close to those obtained in the fat-tailed sheep in Egypt (El-Fouly *et al.*, 1977). On the other hand, fluctuations in semen quality during different seasons paralleled seasonal changes in blood testosterone level, and correlations between both were significant ($P<0.01$).

Table 3. Effect of season or treatment (deviated-penis rams; DPR versus intact rams; IR) on Pearson's correlation coefficients (r) between serum testosterone levels and each of semen characteristics

Source of variation	Ejaculate volume (ml)	Sperm cell concentration ($\times 10^9$ /ml)	Sperm progressive motility (%)	Sperm abnormality (%)
Treatment				
DPR	0.67**	0.38**	0.38**	-0.31**
IR	0.50**	0.35**	0.37**	-0.47**
Season				
Winter	-0.09	0.47**	-0.20	0.14
Spring	0.12	0.22	0.01	0.31*
Summer	0.52**	-0.09	0.15	0.45**
Autumn	0.46**	0.20	0.38**	0.19

* $P<0.05$

** $P<0.01$

This study suggested that long photoperiod and moderate hot ambient temperature during summer are necessary to activate spermatogenic and endocrine functions of the testis, and this continued during autumn season. While in the temperate regions, the ram like the ewe, shows seasonal changes in gonadal endocrine activity, being less active during summer, although some degree of spermatogenesis continues throughout the whole year (Katongole *et al.*, 1974). On the other hand, present results also indicate that surgical deviation of penis is preferable in ram teasers, since their semen characteristics and endocrine activity of testes were maintained normal. Moreover, rams with deviated penises could successfully serve to detect oestrus (Abdalla, 1996) and to supply semen for artificial insemination purposes (Ball *et al.*, 1978). Thus, the presence of such teasers is highly recommended in the experimental flocks of sheep.

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تأثير الموسم وإنحراف القضيب جراحيا عن موضعه على صفات السائل المنوي ومستوى هرمون التستسترون في الكباش العواسى

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أستخدمت فى هذه الدراسة ثلاثة كباش منحرفة القضيب جراحيا وستة كباش طبيعية للمقارنة وذلك من كباش العواسى فى عمر 3-4 سنوات . غذيت كل الحيوانات بنظام التغذية الجماعية وربيت تحت نفس الظروف الطبيعية من مناخ ورعاية . خلال موسم التناسل (نهاية الربيع وأوائل الصيف) أستخدمت الكباش الطبيعية لتلقيح النعاج بينما بقيت الكباش المعاملة جراحيا منفصلة عن النعاج . تم جمع السائل المنوي بالمهبل الصناعى تبعه جمع عينات الدم من وريد الرقبة بمعدل مرتين فى الشهر خلال أشهر السنة . ثم قيمت عينات السائل المنوي مباشرة بعد الجمع بينما حفظت عينات سيرم الدم لتقدير هرمون التستسترون . نتج عن الإنحراف الجراحى للقضيب نقص معنوى (إحتمال خطأ أقل من 0.05) فى التركيز ، الحركة التقدمية ونسبة الشواذ للحيوانات المنوية ، بينما لم يتأثر معنويا بالمعاملة كل من حجم القنفة وتركيز هرمون التستسترون فى مصل الدم . ومن المحتمل أن ترجع هذه التغيرات الملاحظة فى صفات السائل المنوي الى الاختلافات فى تكرار القذف بين المجموعتين الذى يحدث أكثر فى الكباش الطبيعية المصاحبة للإناث خلال موسم التناسل .

لوحظ تباين موسمي (إحتمال خطأ أقل من 0.01) لكل صفات السائل المنوي التى درست فى كلا المجموعتين ، حيث أظهرت صفات السائل المنوي أدنى نوعية خلال الشتاء وأعلى نوعية خلال الصيف ونوعية متوسطة خلال الربيع . لم يلاحظ إختلافات معنوية فى أغلب صفات السائل المنوي بين موسمي الصيف والخريف فى كلا المجموعتين . ولقد تأثر مستوى التستسترون فى مصل الدم معنويا (إحتمال خطأ أقل من 0.01) بفصل السنة ، وأظهر تغيرات موسمية مماثلة لتلك التغيرات فى صفات السائل المنوي . بالإضافة الى ذلك لوحظ وجود ارتباط موجب معنوى (إحتمال خطأ أقل من 0.01) بين مستوى التستسترون وكل من حجم القنفة وتركيز الحيوانات المنوية والحركة التقدمية للحيوانات المنوية ، بينما كان مرتبطا ارتباطا سالب معنوى (إحتمال خطأ أقل من 0.01) مع نسبة الشواذ فى الحيوانات المنوية فى المجموعتين . تقترح هذه الدراسة أن الكباش العواسى فى المناطق تحت الإستوائية تستجيب جنسيا لفترات الضوء الطويلة والحرارة البيئية المتوسطة الإرتفاع خلال الصيف كما وضع من تحسن حجم وصفات السائل المنوي المصحوبة بزيادة مستوى التستسترون خلال الصيف . وهذه الإستجابات الفيزيولوجية فى الصيف استمرت غالبا خلال الخريف . ومن جهة أخرى أوصت هذه الدراسة بإستخدام الكباش المنحرف القضيب جراحيا ككاشف للشياخ فى القطعان التجريبية حيث أن خصبة أظهرت نشاط طبيعى لإنتاج المنى والهرمونات الجنسية بجانب سهولة إستخدامه فى جمع السائل المنوي .

