

# SEMEN QUALITY AND PLASMA TESTOSTERONE CONCENTRATIONS AFTER UNILATERAL LIGATION OF TESTICULAR BLOOD VESSELS IN MALE GOATS AND RAMS

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## ABSTRACT

This study was carried out to monitor changes in the semen quality and plasma testosterone concentrations for 20 weeks following unilateral ligation of testicular vessels in three male goats and two rams. One male goat and one ram were used as untreated controls. Ejaculates from the experimental animals were collected fortnightly and evaluated for physical characteristics. Weekly blood samples were taken by jugular venepuncture and analyzed for plasma testosterone concentration by radio-immunoassay. The ejaculatory volume showed a decreasing trend in all male goats including the control, as the study advanced. Similarly, in all treated male goats and rams, total number of sperms per ejaculate decreased and remained lower than the pre-treatment values. The percentages of dead and morphologically abnormal spermatozoa were higher and those of motile spermatozoa lower in ejaculates collected during weeks 1-7 after treatment than the pretreatment values. The plasma testosterone concentrations showed a wide variation and were relatively lower in the treated than the control animals of both species. However, the number of animals used in the study was too small to draw any firm conclusions.

## INTRODUCTION

Male infertility is a major problem affecting the success of livestock industry. However, it is not always possible to get infertile animals for investigating a particular problem and the importance of experimentally induced lesions in such cases can not be overlooked. One method of inducing lesions in the testis and epididymis is to restrict the blood supply from the testicular artery.

Ligation of the testicular artery in rams induced an ischemic necrosis of the testes that simulated testicular reaction to traumatic injury (Vrzgulova, 1981). Such treatment can also affect the normal testicular functions manifested by changes in the semen quality and plasma testosterone concentration of the treated animals. Ultrasonographic and histological changes upto 30 days following unilateral ligation of the testicular artery in male goats have been reported by Eilts *et al.* (1989). However, changes in the semen quality and plasma testosterone concentration in these animals following this treatment were not described.

The aim of the study described in this paper was to monitor sequential changes in the semen quality and plasma testosterone concentrations for 20 weeks following unilateral ligation of the testicular artery and veins of the pampiniform plexus in three male goats and

two rams. Attempts were also made to study semen quality in these animals after unilateral vasectomy on the normal side.

## MATERIALS AND METHODS

### Animals

Four mature male goats (BS-152, BS-153, BS-157 and BS-158) and three rams (S-24, S-40 and S-84) with clinically normal reproductive tract and donating semen of acceptable quality were used.

### Management and Feeding

The experimental male goats and rams were housed in separate pens with free access to a grass paddock. In addition, good quality hay and clean water were provided in their pens *ad lib* and each animal received 0.3-0.5 kg of concentrate daily, depending on its body weight.

### Treatments

The testicular artery and veins of the pampiniform plexus were unilaterally ligated in three male goats (BS-152, BS-157 and BS-158) and two rams (S-24 and S-40). One male goat (BS-153) and one ram (S-84) served as untreated controls. Two ejaculates, three and one week

before treatment, were collected from all animals and evaluated for physical characteristics.

### Post-Treatment Monitoring

These animals were monitored for 16 weeks post-treatment. Beginning one week after treatment, semen samples from experimental animals were collected fortnightly with an artificial vagina using an ovariectomized and oestrogenized doe or ewe as a teaser. Immediately after collection, ejaculates were evaluated for volume, color, mass motility, individual motility, sperm cell concentration and percentages of dead and morphologically abnormal spermatozoa. The details of semen collection and evaluation have been given elsewhere (Ahmad *et al.*, 1993).

Jugular blood samples were collected every week, plasma removed and stored at  $-20^{\circ}\text{C}$ . Plasma testosterone concentrations were measured in duplicate by radioimmunoassay (Corker and Davidson, 1978). Plasma samples were extracted twice with diethyl ether and incubated with a specific antiserum raised in sheep and H3 labelled testosterone. Unbound radioactive steroid was removed with dextran-coated charcoal, followed by centrifugation. A plasma pool collected from a male goat was used as a quality control to calculate the inter-assay coefficient of variation (CV), which was 16.95%. The intra-assay CV, based on 15 duplicate determinations of a single plasma pool was 11.21%.

Sixteen weeks after ligation, animals in which testicular blood vessels had been ligated were unilaterally vasectomized on the normal side. Semen collection and evaluation were continued for another four weeks. Following this, they were killed, their genital organs removed and examined for gross and histological changes, using hematoxylin and eosin staining procedure (Bacha and Wood, 1990).

## RESULTS

### Semen Characteristics

#### Male Goats

The physical characteristics of semen quality before and after ligation of testicular blood vessels in male goats are shown in Fig.1. The ejaculatory volume showed a decreasing trend in all male goats including the control, as the study advanced. Similarly, in all treated bucks, the total sperms per ejaculate decreased and remained lower than the pre-treatment values. A similar trend was seen for sperm cell concentration in all treated

male goats except BS-152, in which the sperm cell concentration fluctuated and was comparable to pretreatment values. Such changes were not seen in the control buck BS-153

In BS-152, the percentages of dead spermatozoa was high between 1-7 weeks after treatment and declined later. A relatively high percentage of abnormal spermatozoa was only observed in ejaculates collected on the 3rd week after treatment. Later, these values decreased but remained higher than the pretreatment values.

In BS-157, relatively low sperm motility and a high percentage of dead and abnormal spermatozoa were noted in ejaculates collected five weeks after treatment; this remained so until the study was concluded.

In BS-158, a relatively high percentage of dead and abnormal spermatozoa and low sperm motility were noted in the ejaculate collected on the 5th and the 9th week after treatment.

Ejaculates collected from all treated animals after unilateral vasectomy of the normal side were thin and watery in consistency and yellowish in appearance. These ejaculates had a few spermatozoa, all of which were dead and thus immotile, and a majority of them appeared to have degenerated heads. A few epithelial cells were also identified in these ejaculates. In BS-157, some degenerated and detached sperm heads were also seen.

#### Rams

A decrease in the ejaculatory volume observed in male goats was not seen in treated or control rams. In both rams of the treatment group, the sperm cell concentration and total sperms per ejaculate showed wide variations but were, in general lower than the pretreatment values (Fig.2).

In S-24, an increase in the percentages of dead and abnormal spermatozoa, with a corresponding decrease in sperm motility, was observed between three and seven weeks after treatment. Later, these characteristics improved but could not reach the pretreatment values.

In S-40, relatively high percentages of dead and abnormal spermatozoa, and low motile sperm, were noted in ejaculates collected between one and five weeks after treatment. Later, these parameters improved though not to the extent of pretreatment levels.

Ejaculates collected after vasectomy of the normal testis were watery and opaque in appearance, and showed similar characteristics as those described for male goats.

### Plasma Testosterone Concentrations

The plasma testosterone concentrations at different time intervals after ligation of testicular vessels in male goats and rams are presented in Fig. 1, 2. These varied widely with the mean testosterone concentrations in BS-152, BS-157 and BS-158 were  $1.33 \pm 0.21$ ,  $2.91 \pm 0.49$  and  $2.31 \pm 0.36$  ng/ml, respectively, versus  $3.19 \pm 0.47$  ng/ml in the control male goat BS-153. In the two treated rams S-24 and S-40, these values were  $4.49 \pm 0.79$  and  $5.67 \pm 1.02$  ng/ml, respectively, versus  $6.89 \pm 0.97$  ng/ml in the control ram S-84.

### Postmortem Findings

In all male goats and rams, the ligated testes were atrophied. The three segments of the epididymis were atrophied, hard and dry, as if fibrosed. Histologically, the ligated testis showed diffuse coagulative necrosis with mineralization and fibrous tissue reaction at the periphery. In the three segments of the epididymis, the normal tubules were not seen, the specimen was composed of well organized fibrous connective tissue moderately infiltrated by macrophages. Occasionally, a small number of thin and empty ducts, lymphatics and vessels within an extensive fibrous tissue mass could be seen.

In one male goat (BS-157), however, the epididymal body and the tail were enlarged and were of hard consistency but were dry in texture due to the absence of secretions. Histologically, the epididymal body and the tail showed chronic epididymitis with cystic dilatation of the tubules.

## DISCUSSION

The main aim of this study was to monitor changes in the plasma testosterone concentrations and semen quality after an experimentally-induced ischaemia of the testis and the epididymis in male goats and rams. Unfortunately, the number of animals used was too small to make any firm conclusions. However, in general, the total number of sperms per ejaculate decreased after treatment and remained lower than the pre-treatment values. The percentage of dead spermatozoa was relatively higher during weeks 1-7 after treatment than the pre-treatment values, and decreased later.

It is apparent that the ischaemia and necrosis of the testis and epididymis ultimately results in cessation of spermatogenesis and sperm maturation. The decrease in the total number of sperms in the ejaculate as early as one week after ligation suggests that there was an early effect upon sperm production and release. However,

interpretation of this is complicated because of the possible ejaculation of spermatozoa from the ampulla of the vas deferens of the ligated side (Perera, 1974) and also from the epididymis and ampulla of the normal control side.

Sperms in the ejaculates collected after unilateral vasectomy of the normal side probably originated from the ampulla of the normal control vasectomized side, as has been reported following vasectomy in rams (Dunlop *et al.*, 1963; Perera, 1974), since it is very unlikely that by sixteen weeks after ligation, dead sperms would have remained physically intact in the epididymis. Histological appearance at slaughter also showed that by this stage most of the epididymal tissue had been replaced by fibrous tissue.

The decrease in the dead and abnormal spermatozoa in the ejaculates collected during the later stages of the study was not observed in one male goat (BS-157). Obviously no spermatozoa were produced

in the atrophied and necrotic testis in this buck. Perhaps the dead and abnormal spermatozoa were contributed from the pool of spermatozoa trapped in the epididymal tail which was swollen and showed chronic inflammation with cystic dilatation of its tubules.

An increasing trend was observed in the number of spermatozoa in ejaculates collected during the later part of the study, before vasectomy of the normal side. Since the sperm cell concentration was determined by the absorbency of the ejaculates using a spectrophotometer, the presence of degenerated epithelial cells or their products in the ejaculates might have affected the results. The authors are unable to locate any experimental evidence regarding the compensatory hypertrophy of the contralateral normal testis after necrosis and atrophy of one testis.

The ejaculatory volume showed a decreasing trend in treated male goats as the study advanced. A similar trend was seen in control animals indicating that it was not due to the treatment, rather it was due to some other factors e.g. season.

In the present study, relatively lower plasma testosterone concentrations were recorded in the treated than the control animals of both the species. However, the data could not be analyzed statistically. Furthermore, it is well known that testosterone is secreted episodically in male goats (Muduuli *et al.*, 1979) and rams (Scanbacher and Ford, 1976). It seems that the frequency of blood sampling used in the present study (once every week) was not adequate enough to draw any conclusion. Further studies involving higher number of animals with more frequent blood sampling protocol may be carried out for explaining these results.

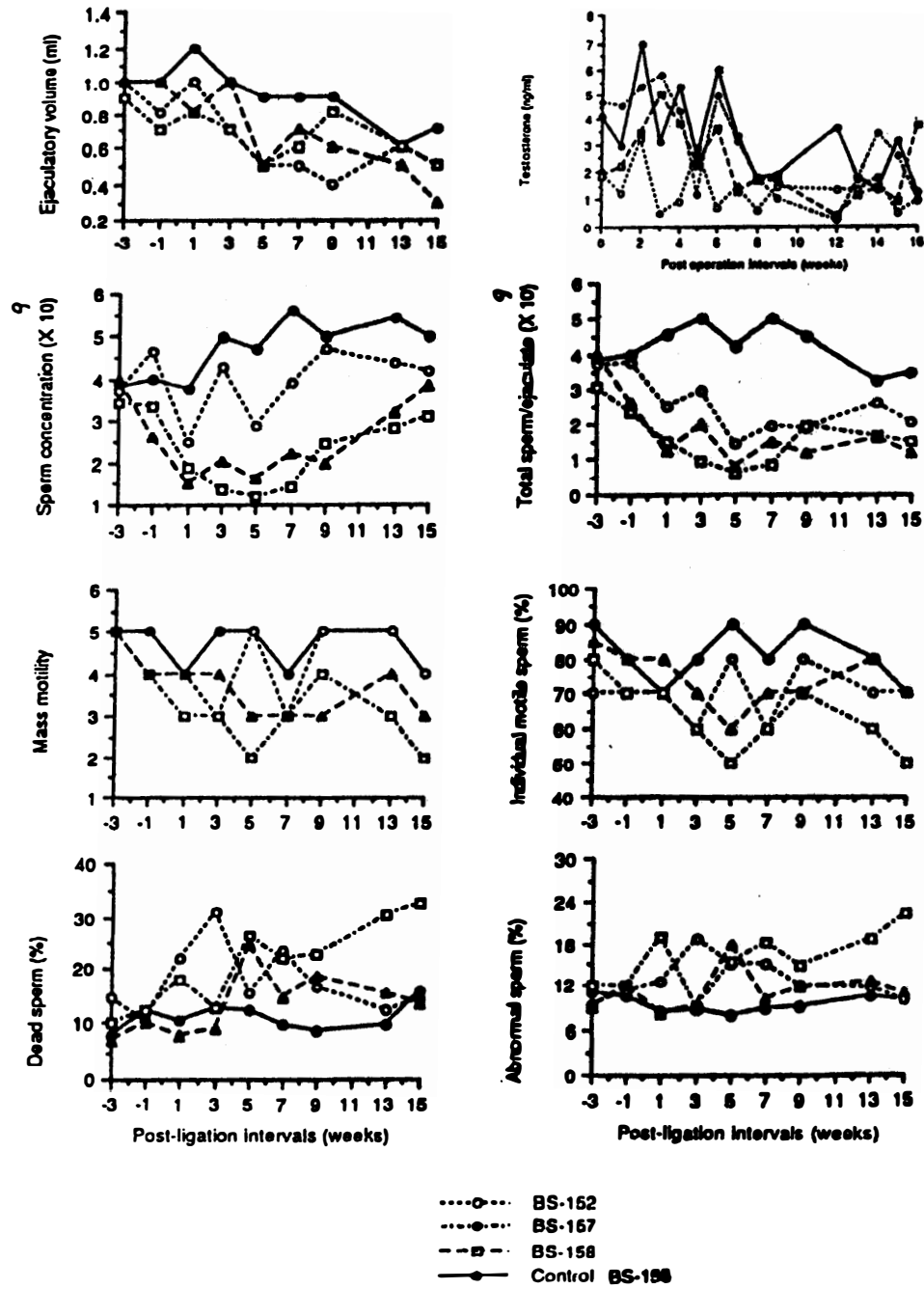


Fig.1 Physical characteristics of semen quality and plasma testosterone concentration in control (solid lines) and treated male goats (broken lines) at different time intervals after ligation. Negative signs indicate values before treatment.

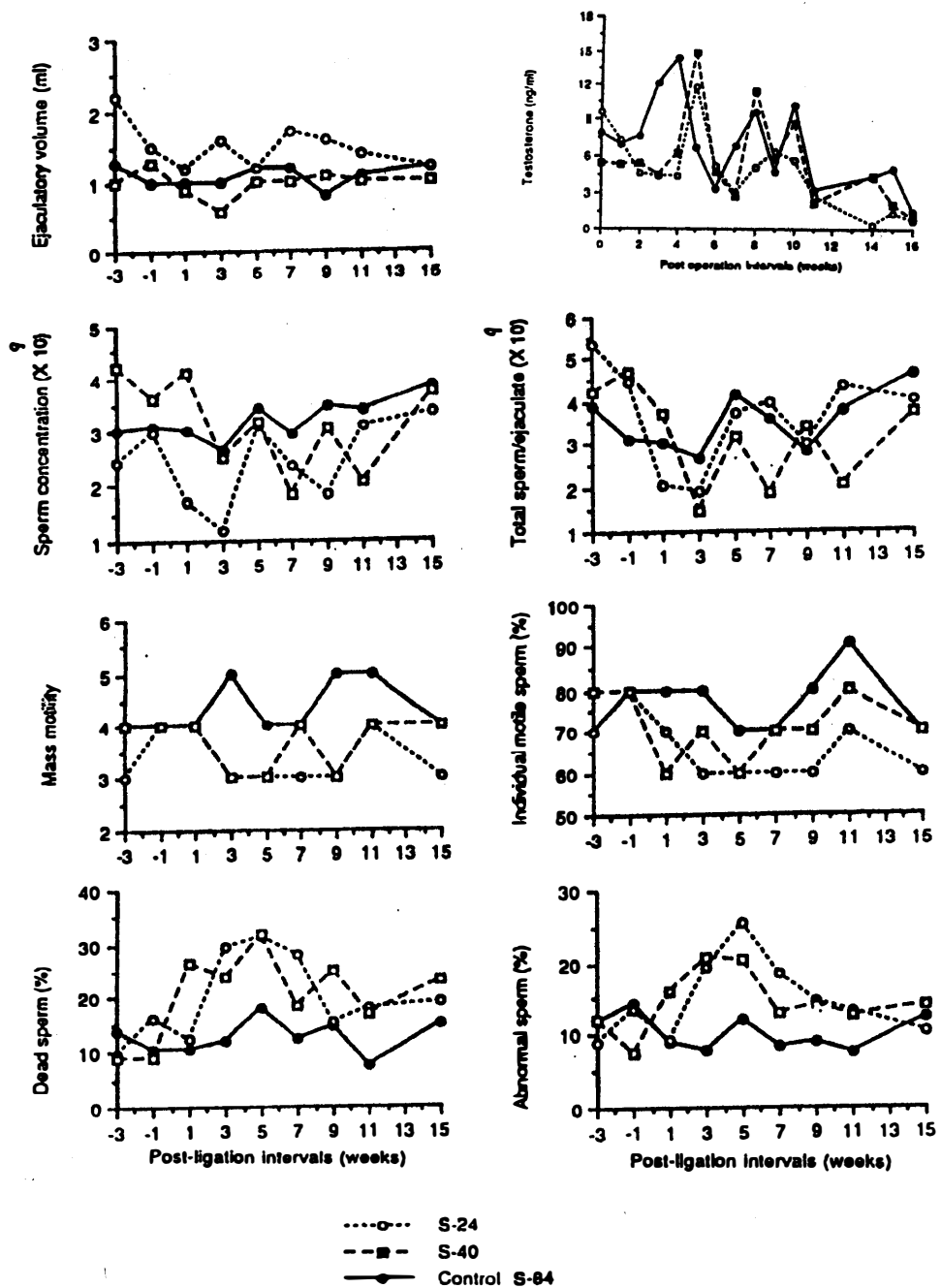


Fig.2 Physical characteristics of semen quality and plasma testosterone concentration in control (solid lines) and treated rams (broken lines) at different time intervals after ligation. Negative signs indicate values before treatment.

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