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# Investigation of serum testosterone level, scrotal circumference, body mass, semen characteristics, and their correlations in developing MIS lambs

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**Abstract:** The aim of this study was to investigate changes of serum testosterone, scrotal circumference, body weight, and semen characteristics in a Meat Institute Sheep (MIS) population during their developing period (between 3 and 17 months). Correlative relationships among parameters were also tested. Body weight and scrotal circumference were measured monthly, blood samples for testosterone levels were taken bimonthly (3–17 months), and semen samples were collected by an electroejaculator between the ages of 9 and 17 months. The analysis showed that the average serum testosterone levels ranged from 1.83 to 13.28 ng/mL and significantly depended on the age of the lambs ($P < 0.05$). The trend in the level of serum testosterone was characterized by pronounced variability throughout the study period. Changes in scrotal circumference and body weight were significantly dependent on age. Ejaculate volume, sperm motility, and the percentage of live spermatozoa increased with age, except for the percentage of abnormal spermatozoa, which decreased with age. Correlations among serum testosterone, scrotal circumference, and body weight were moderately to highly positive and highly significant. All parameters of semen quality were poorly correlated with testosterone and body weight, while scrotal circumference was in moderate correlation only with ejaculate volume and live sperm count ($P < 0.05$).

**Key words:** Testosterone, scrotal circumference, sperm, maturation, male lambs

## 1. Introduction

The Meat Institute Sheep (MIS) population, created at the Institute for Animal Husbandry in Belgrade, Serbia, is a meat type of sheep of strong constitution, strong carcass conformation, and good meat properties. It was obtained by using a complex combination crossing according to a precisely defined genetic procedure, using Pirot pramenka, Wuerttemberg, and Ile de France breeds (1). So far, the MIS sheep population is in the experimental stage. MIS ewes have an average body weight of 79 kg, while rams can gain from 120 to 140 kg, depending on the production stage and breeding. Body development and linear measures of MIS sheep at different ages were presented in previous research (2). MIS ewes reach sexual maturity at the age of 6 to 8 months, which puts them in the group of fast maturing sheep breeds, with an average lambing rate of 130% to 160% and the occurrence of estrous cycles throughout the year (1). However, so far, no studies have been conducted focusing on the reproductive developmental ability of male lambs in the MIS sheep population.

During the life of the ram, the body weight, scrotal circumference, and testosterone levels change under the influence of a number of internal and external factors (3). Literature data on the reproduction of rams indicate a complex relationship between the development of the neuroendocrine system, testosterone concentration, development of individual body parts, and sexual maturation (4,5).

Potent androgen testosterone, which is produced in the testes, the most important sex hormone in the ram, is responsible for the development of primary and secondary sexual characteristics, sperm production, and the regulation of sexual behavior (6,7).

Scrotal circumference in rams is considered a good indicator of their breeding ability. Sperm production is correlated with testicular measurements (8). Scrotal circumference varies with season and body weight, but is at its maximum peak during the fall breeding season (9–11).

Spermatogenesis and steroid secretion are dependent on the individual activities of two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). However, both processes are intimately linked to adequate levels of testosterone necessary for normal sperm production and maturation (12).

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Due to the wide range of effects on reproductive properties associated with sexual competitiveness (13), individual variation in testosterone production seems to be a key factor that makes a difference in male reproductive success, and therefore the understanding of how and why the variation in testosterone levels occurs is of great scientific and practical significance (5).

The purpose of this investigation was to measure the levels of serum testosterone, scrotal circumference, body mass, and semen characteristics, and the relationship between these parameters in developing male lambs of the MIS sheep population.

## 2. Materials and methods

Investigations were carried out on an experimental sheep farm of the Institute for Animal Husbandry in Belgrade. The study included 20 male lambs from the MIS sheep population. All animals used in the current study were of similar age and conditions. Prior to weaning, the male lambs were kept with their mothers; starting from the second week of age, they were fed with alfalfa hay and concentrates (160 g protein/kg plus vitamins and minerals) ad libitum. Lambs were weaned at the age of 2 months and were kept in the barn system in one group until the end of the study. The diet was based on the use of alfalfa hay and concentrates. The animals were introduced in the study at the age of 3 months and the trial was completed at the age of 17 months.

From the moment of introduction of the animals in the study, body weight and scrotal circumference were measured once a month. Body weight was measured using a scale. The scrotal circumference was measured with a ribbon at the widest part of the testis, i.e. the part where the circumference was the greatest.

Blood samples for testosterone levels were collected every 2 months (at the age of 3, 5, 7, 9, 11, 13, 15, and 17 months) from the jugular vein in the morning from 0800 to 0900 hours. After 1 h, blood samples were centrifuged, and the blood serum was extracted, which was then used for the analysis. Analysis was performed by the radioimmunoassay method using a commercial kit (Immulite Total Testosterone, Siemens Healthcare Diagnostic Inc., UK) for animal use.

To analyze sperm quality, semen samples were collected by electroejaculation every 2 months between the ages of 9 and 17 months. Semen samples were collected once from each ram at each sampling to avoid reducing the number of sperm and their motility. The following sperm quality parameters were analyzed: ejaculate volume, sperm concentration, sperm motility, percentage of live sperm, and percentage of abnormal forms of spermatozoa. The ejaculate volume was read directly from a graduated collection tube. Sperm motility and sperm concentration were analyzed using computer-assisted semen analysis (CASA-ISAS Integrated system for the analysis of semen, V.1.2., Proiser, Spain). For sperm morphology and sperm live/dead ratio, the semen was stained with eosin–nigrosin and examined by microscope.

Statistical analysis of the experimental data was performed using the statistical package Statistica for Windows 7 (StatSoft Inc., USA). The equality of variances of the analyzed treatments was tested using the Leven test, and in accordance with the results, the significance of differences in the average values was tested by using parametric and nonparametric tests. One-way ANOVA was used to determine the effect of age on body weight and scrotal circumference of rams. Significant differences between means were detected using the LSD test. Testosterone levels and semen characteristics were analyzed using the Kruskal–Wallis test, and single mean comparisons were conducted using the Mann–Whitney U test. The relative dependence of the traits was determined by Pearson correlation coefficient, whose significance was tested. Analyses were performed for the significance levels of 5% and 1%, and the results are presented through the main indicators of descriptive statistics.

## 3. Results

The mean values and the variability of the significance of their differences for testosterone levels in the blood serum of tested rams depending on their ages are shown in Table 1. The average values of testosterone levels in the blood serum ranged from 1.83 ng/mL at 3 months of age to 13.28 ng/mL at the end of the test period (when rams were 17 months old), which was significant at $P < 0.01$. The data analysis showed a high variability in the testosterone level with the coefficient of variation ranging from 30.91% to 100.64%. A trend of linear increase in testosterone level was established in the period from 3 to 7 months of age, followed by a decline in values at the age of 9 to 11 months, which was then followed by distinct variations in the last three control periods with sudden leaps at 13 and 17 months, and a decline at 15 months of age.

Table 2 summarizes the development of scrotal circumference and live weight during the study. The lowest average values of the scrotal circumference (17.42 cm) and body weight (30.62 kg) were determined at 3 months of age, while the highest values were measured at 13 months of age, when they amounted to an average of 36.02 cm and 87.95 kg, respectively. There was a trend of linear increase in the values of scrotal circumference in rams between the ages of 3 and 7 months (October and November), after which a certain slight decline during the winter months was observed. At the ages of 12 and 13 months (in March and April), there was also a significant increase in the scrotal circumference values, after which the values

**Table 1.** Statistical indicators (mean ± SE and CV) for serum testosterone depending on the age of rams.

| Age of rams (in months) | Testosterone (ng/mL) | CV% | Season (months) |
|---|---|---|---|
| 3 | $1.83^{d} \pm 0.28$ | 68.30 | June |
| 5 | $6.36^{c} \pm 0.65$ | 45.75 | August |
| 7 | $9.70^{b} \pm 0.71$ | 32.58 | October |
| 9 | $6.09^{c} \pm 0.88$ | 64.37 | December |
| 11 | $4.69^{cd} \pm 1.05$ | 100.64 | February |
| 13 | $12.91^{a} \pm 0.89$ | 30.91 | April |
| 15 | $4.59^{cd} \pm 0.89$ | 86.71 | June |
| 17 | $13.28^{a} \pm 1.03$ | 34.71 | August |

$^{a,b,c,d}$: means with different superscripts differ significantly at $P < 0.01$.

**Table 2.** Mean ± SE scrotal circumference and body weight of rams depending on age.

| Age of rams (months) | Scrotal circumference (cm) | CV% | Body weight (kg) | CV% | Season (months) |
|---|---|---|---|---|---|
| 3 | $17.42^{h} \pm 0.49$ | 12.57 | $30.62^{h} \pm 1.07$ | 15.68 | June |
| 4 | $23.02^{g} \pm 0.60$ | 11.68 | $34.07^{h} \pm 0.98$ | 12.85 | July |
| 5 | $27.47^{f} \pm 0.54$ | 8.77 | $44.10^{g} \pm 1.03$ | 10.43 | August |
| 6 | $31.10^{e} \pm 0.49$ | 7.07 | $51.10^{f} \pm 1.23$ | 10.80 | September |
| 7 | $32.57^{cde} \pm 0.51$ | 7.06 | $60.47^{e} \pm 1.32$ | 9.74 | October |
| 8 | $32.45^{de} \pm 0.55$ | 7.61 | $62.87^{e} \pm 1.29$ | 9.21 | November |
| 9 | $31.72^{e} \pm 0.44$ | 6.27 | $69.16^{d} \pm 1.31$ | 8.49 | December |
| 10 | $30.95^{e} \pm 0.39$ | 5.69 | $71.57^{cd} \pm 1.31$ | 8.21 | January |
| 11 | $31.85^{e} \pm 0.37$ | 5.18 | $74.77^{c} \pm 1.43$ | 8.53 | February |
| 12 | $34.75^{ab} \pm 0.59$ | 7.60 | $82.95^{b} \pm 1.56$ | 8.39 | March |
| 13 | $36.02^{a} \pm 0.48$ | 5.99 | $87.95^{a} \pm 1.59$ | 8.11 | April |
| 14 | $35.00^{ab} \pm 0.40$ | 5.14 | $84.05^{ab} \pm 1.25$ | 6.66 | May |
| 15 | $34.12^{bc} \pm 0.40$ | 5.22 | $87.62^{ab} \pm 1.58$ | 8.07 | June |
| 16 | $34.67^{ab} \pm 0.43$ | 5.57 | $87.25^{ab} \pm 1.50$ | 7.69 | July |
| 17 | $35.42^{ab} \pm 0.43$ | 5.42 | $84.77^{ab} \pm 1.44$ | 7.61 | August |
| 18 | $34.40^{abc} \pm 0.48$ | 6.22 | $85.72^{ab} \pm 1.43$ | 7.47 | September |

$^{a,b,c,d,e,f,g,h}$: means with different superscripts differ significantly at $P < 0.01$.

remained relatively constant during the summer months until the end of the study at 17 months of age.

The results of the analysis of body weight during the examination period showed a linear increase of values from 3 to 13 months of age, reaching the highest average value of 88.20 kg, after which the value remained relatively constant until the end of the study, with slight fluctuations. The values of both parameters were significantly dependent on age.

Table 3 presents the mean values of semen characteristics depending on the age of the rams. As shown in Table 3, ejaculate volume, sperm motility, and

**Table 3.** Mean ± SE semen characteristics of male lambs depending on age.

| Age of rams (months) | Ejaculate volume (mL) | Sperm concentration ($\times 10^9$ mL) | Motility (%) | Live spermatozoa (%) | Abnormal sperm (%) | Season (months) |
|---|---|---|---|---|---|---|
| 9 | $1.01^{b} \pm 0.15$ | $2.04 \pm 0.26$ | $60.14 \pm 4.84$ | $68.80^{b} \pm 2.73$ | $10.60^{ab} \pm 1.38$ | December |
| 11 | $1.06^{b} \pm 0.16$ | $1.84 \pm 0.39$ | $69.45 \pm 4.28$ | $73.80^{b} \pm 2.72$ | $8.71^{b} \pm 1.42$ | February |
| 13 | $1.71^{a} \pm 0.19$ | $2.51 \pm 0.41$ | $70.88 \pm 2.78$ | $86.90^{a} \pm 2.08$ | $5.70^{b} \pm 1.17$ | April |
| 15 | $1.70^{a} \pm 0.23$ | $2.28 \pm 0.37$ | $66.19 \pm 2.29$ | $76.10^{b} \pm 3.38$ | $13.85^{a} \pm 3.04$ | June |
| 17 | $1.40^{ab} \pm 0.17$ | $2.76 \pm 0.36$ | $70.85 \pm 3.39$ | $85.10^{a} \pm 3.57$ | $9.00^{ab} \pm 1.36$ | August |

$^{a,b}$: means with different superscripts differ significantly at $P < 0.05$.

percentage of live spermatozoa in the ejaculate increased with age. Linear increase was observed from the 9th to the 13th month of age, followed by a pause or decrease at the age of 15 months, only to increase again at the age of 17 months. Abnormal forms of spermatozoa in the ejaculate decreased with age, also linearly, from 9 to 13 months, and then varied in the last 2 months of testing.

The correlations between the level of testosterone, scrotal circumference, and body weight of rams are presented in Table 4. A moderately positive correlation was found between the testosterone level and scrotal circumference ($r = 0.52$; $P < 0.01$). Moreover, there was a moderately positive correlation between the serum testosterone levels and the body weight of rams ($r = 0.40$) and a highly positive correlation between body weight and scrotal circumference ($r = 0.81$; $P < 0.01$).

All parameters of semen quality were poorly correlated with testosterone concentration and body mass ($P > 0.05$) except live spermatozoa ($r = 0.26$; $P < 0.05$). Moderate correlations were found between ejaculate volume and scrotal circumference ($r = 0.34$; $P < 0.01$) and between live sperm count and scrotal circumference ($r = 0.31$; $P < 0.01$); the other sperm parameters were poorly correlated with scrotal circumference ($P > 0.05$).

## 4. Discussion

Testosterone is a key mediator in the expression of numerous morphological and behavioral traits in mammals, but the factors underlying individual variation in circulating testosterone levels are poorly understood (5). The level of testosterone in the blood circulation of rams varies by breed, age, nutrition, season, and manifestations of estrus in ewes (14,15). The present study showed a significant correlation between the testosterone level in the blood serum of rams and their age, with a high variability in the testosterone level (CV of 30.91% to 100.64%). The variable character of testosterone levels in the blood of rams and the significant effect of age between 2 and 14 months were established in research conducted by Elmaz et al. (11), with the lowest values recorded between the ages of 80 and 100 days, and the highest values between the ages of 260 and 300 days. Moreover, the obtained results were confirmed in earlier studies by Wilson and Lapwood (16) and Ungerfeld and Gonzalez-Pensado (17), showing an increase in the testosterone level with age as well as fluctuations in the determined values. Preston et al. (5) reported that the production of testosterone changed during the life of rams, with increasing levels of hormones from birth until they reach full sexual maturity and a decrease thereafter.

**Table 4.** Coefficients of correlations (r) between testosterone levels, scrotal circumference, body weight of rams, and semen characteristics.

| Parameters | T | SC | BW | EV | C | MOT | AS | LS |
|---|---|---|---|---|---|---|---|---|
| T | | 0.52** | 0.40** | $0.03^{ns}$ | $0.15^{ns}$ | $0.19^{ns}$ | $-0.15^{ns}$ | 0.26* |
| SC | | | 0.81** | 0.34** | $0.14^{ns}$ | $0.11^{ns}$ | $-0.01^{ns}$ | 0.31** |
| BW | | | | 0.24* | $-0.05^{ns}$ | $-0.16^{ns}$ | $0.13^{ns}$ | 0.24* |

*$P < 0.05$; **$P < 0.01$; ns – not significant.
T – testosterone; SC – scrotal circumference; BW – body weight; EV – ejaculate volume; C – sperm concentration; MOT – motility; AS – abnormal sperm; LS – live sperm.

Testicular size is often used to assess the fertility of rams and as the basis of livestock selection for breeding. Generally, morphometric properties of testes are readily measurable, both in young and adult animals. Our results showed a significant effect of age on scrotal circumference, which were in accordance with results obtained by Salhab et al. (18) and Elmaz et al. (11). The scrotal circumference in rams may be influenced by many factors, and is usually highest during the natural breeding season, which is associated with the impact of the length of the photoperiod on neurohormonal complex mechanisms that regulate reproductive function. Although the present study did not show pronounced seasonal fluctuations, the recorded reduction of the scrotal circumference during the winter months was in line with the findings of Gastel et al. (9) and Kafi et al. (10) on the smallest value of scrotal circumference of rams during the winter.

Body weight is one of the common production indicators that are regularly measured in order to monitor and assess the various aspects of production. It is also one of the most commonly used selection criteria; it is considered that reaching sexual maturity in rams is more conditioned by body development than by chronological age (19,20). There are no literature data on the physical development of MIS rams during the examination period, but a similar linear trend of increasing body weight of rams with age, as found in our study, was reported by Elmaz et al. (11) in the study of the development of rams from 2 to 14 months of age.

Salhab et al. (21) and Kridli et al. (14) earlier pointed out that semen characteristics improved with age. However, other studies such as that of Tabaa et al. (22) showed that age is not a reliable predictor of sperm production. In our study, it was observed that the volume of ejaculate, sperm motility, and the percentage of live spermatozoa in the ejaculate increased with age, while abnormal forms of spermatozoa in the ejaculate decreased. A similar trend in the values of sperm parameters was found by Elmaz et al. (11).

A moderately positive correlation was determined between the testosterone level and scrotal circumference ($r = 0.52$; $P < 0.01$). A moderate and significant correlation ($r = 0.45$) between these parameters in rams aged 2 to 14 months was confirmed in a study by Elmaz et al. (11). Fourie et al. (23) established a weak correlation between scrotal circumference and testosterone levels in rams aged 10 to 12 months ($r = 0.23$), but stated that the established relationship was significant. Preston et al. (5) found that the variation in the size of the testicles is a powerful predictor of testosterone levels during the mating season. It is possible that bigger testes contain more androgen producing tissues (Leydig cells) or higher testosterone levels stimulate the growth and development of spermatogenic tissues, which affect the size of the testes. In contrast to these findings and the values determined in our study, Schoeman et al. (24) and Greyling and Taylor (25) failed to establish a significant correlation between these parameters.

Our study showed a significant, moderately positive correlation between the serum testosterone levels and the body weight of rams ($r = 0.40$). This value is identical to the value of the correlation coefficient determined for the same properties by Elmaz et al. (11) ($r = 0.40$). A moderately positive correlation between these parameters ($r = 0.30$; $P < 0.05$) was reported in a study by Greyling et al. (26). A much stronger relationship with a correlation coefficient of up to 0.95 ($P < 0.0001$) was found by Zarkawi and Salhab (27) in male lambs during the first 10 months of life. In contrast to these results, Fourie et al. (23) failed to establish any relationship between the body weight of rams and their testosterone levels ($r = –0.05$).

Various studies have suggested that the scrotal circumference is strongly positively correlated with the body weight of animals at different ages (11,28). In the present study, a highly positive correlation between body weight and scrotal circumference of rams was determined ($P < 0.01$). The correlation coefficient value was 0.81. The results of the present study clearly show a strong positive relationship between body weight and scrotal circumference; our results are in contrast with reports by Gastel et al. (9) and Kafi et al. (10), who found no relation between these traits.

Many authors have presented observations regarding sperm production and its quality in terms of correlations with testicular size and plasma testosterone levels (8,29,30). However, in our study all parameters of semen quality were poorly correlated with testosterone concentration and body weight, while scrotal circumference was in moderate correlation only with ejaculate volume and live sperm count. Although there is a structural and functional integration of testicular production of testosterone and sperm that causes the testosterone-dependent nature of spermatogenesis, there are still open questions concerning the precise level of testosterone required for the maintenance of normal spermatogenesis.

In conclusion, these findings show the variable character of serum testosterone caused by various internal and external factors. There is an indisputable relationship between serum testosterone level, scrotal circumference, and body weight, while semen characteristics were poorly to moderately correlated with these parameters. Therefore, scrotal measurements and body weight cannot be used as reliable predictors of sperm production and the link between testosterone and sperm quality should be investigated from the viewpoint of the minimum levels of androgen required for normal spermatogenesis. Based on the results of the present study, we can conclude that the

rams of the MIS sheep population are completely mature at the age of 12–13 months and can be used for breeding and conservation purposes.

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