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Scrotal–testicular biometry, sperm quality and quantity in rams (*Ovis aries*)

Divya V¹, Girish Kumar V¹, Nandi S^{2*}, Ramchandra SG³, William Rasican Surin⁴¹Department of Veterinary Biochemistry, Veterinary College, KVAFSU, Hebbal, Bangalore 560 024, India²National Institute of Animal Nutrition and Physiology (NIANP) Adugodi, Bangalore–560030, India³Central Animal Facility, Indian Institute of Science, Bangalore 560 012, India⁴Department of Microbiology, Indian Institute of Science, Bangalore 560 012, India

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ABSTRACT

Objective: To estimate scrotal circumference, testicular (along with epididymis) weight, sperm concentration, live and dead sperm percentage in different age groups of rams. **Methods:** In view of assessing the most reliable technique that could be used for the evaluation of spermatogenesis and the applicability of the same in rams the present study was conducted, 24 normal rams were divided into four groups, i.e., group I (< 6 months of age), group II (6 months to 1 year), group III (1 to 2 year) and group–IV (2–3 year) and wethers were grouped as group V. **Results:** the mean sperm concentration in group II was found to be significantly lower when compared with group III and group IV. The comparison of the mean scrotal circumference between the groups revealed significant difference between group I, group II, group III and group IV. The comparison of live and dead sperms within the groups revealed no significant difference in group I, group III, group IV and group V. **Conclusion:** The analysis of various physiological attributes like, sperm concentration, scrotal circumference, testis weight and live and dead sperms percentage in all the groups shows higher levels in group III and group IV rams.

1. Introduction

Spermatogenesis is a complex physiological process of conversion of the spermatogonial cells to elongated spermatozoa by a process of spermatocytogenesis, meiosis and spermiogenesis [1]. Factors such as epigenetics, hormones, temperature, drugs, radiations nutrition, and toxic chemicals are said to influence spermatogenesis. Evaluation of spermatogenesis is done by conventional methods like breeding soundness examination (physical examination, scrotal circumference measurement), semen evaluation (sperm concentration, sperm motility, sperm morphology analysis) and histometric analysis of the testis. A positive correlation between SC and testes weight, percentage of seminiferous tubules exhibiting the presence of elongated spermatids as well as testis score, where as they observed for negative correlation between epididymal weight and SC, testes weight, percentage of tubules with

elongated spermatids as well as testis score [2]. The males should be examined for a number of different tests to assess the fertility or performance of crossbred rams like scrotal measurement, semen examination, libido testing, hormonal profile and the other examinations [3]. The present study was undertaken to estimate scrotal circumference, testicular (along with epididymis) weight, sperm concentration, live and dead sperm percentage in different age groups of rams.

2. Materials and methods

A total of thirty rams comprising 24 normal rams of different age group and six wethers were used for this study. The twenty four normal rams were divided into four groups, i.e., group I, group II, group III and group IV. The rams of less than six months of age were grouped as group I, rams between six months to one year of age were grouped as group II, rams between one to two year of age were grouped as group III and rams between two to three years of age were grouped as group IV. The age of the rams which were belonging to group I and group II (i.e., below one year of

*Corresponding author: William Rasican Surin, Department of Microbiology, Indian Institute of Science, Bangalore 560 012, India.
E-mail: snandi71@yahoo.co.in

age) was ascertained by looking for presence or absence of sperm in the epididymis and absence of first pair of permanent incisors tooth, while the age of rams belonging to group III and group IV (i.e., above one year of age) were ascertained by their dentition. On the other hand, all the six wethers were grouped as group V. The age of all the wethers was ascertained from the farmer who had bought it for the slaughtering and among them four wethers were less than one year age (i.e., absence of permanent incisors), while the other two wethers were between one to two years of age (i.e., rams with one pair of permanent incisors). Testis (including the epididymis) and blood sample were collected from all the rams at the time of their slaughter. The testis along with epididymis was bought to the laboratory in the ice pack, weighed and immediately subjected for assay of physiological attributes viz., epididymal sperm count^[4], weight of testis as well as epididymal live and dead sperm count. However before slaughter, the scrotal circumferences of all the rams were recorded. The epididymis from the entire thirty testes was used for assay of total sperm count as well as live and dead sperm count. The processing of the epididymis was as per the method of Luna^[5]. Several longitudinal incision were made on the caudal part and was placed in petridish containing one milli liter of PBS (pH 7.4) and allowed for ten minutes for the sperms to swim out and additional one milli liter of PBS was used to rinse the sperms from the incised part of epididymis. Finally total sperm concentration in this total two milli liter of PBS was calculated using haemocytometer^[6]. Likewise, the epididymal live and dead sperm count was assayed according to the method described earlier^[7]. The scrotal circumferences of all the thirty rams were measured before the slaughter as per the method described earlier^[8,9]. The weight of the testis along with its epididymis was also recorded. Mean values and standard error of mean were

calculated and all the values were expressed as Mean±SEM by using Graph Pad Prism 5 Software, 2007. The variations within the group and between the groups were tested by one way ANOVA, using Tukey's test. The *P* value <0.05 was considered as significant.

3. Results

The mean sperm concentration, mean scrotal circumference, mean testis weight and mean percentage of live/dead sperms is presented in Table 1. The comparison of the mean sperm concentration between the groups revealed no significant difference between group I and group V so also between group III and group IV. However the mean sperm concentration in group II was found to be significantly lower when compared with group III and group IV. The comparison of the mean scrotal circumference between the groups revealed significant difference between group I, group II, group III and group IV, however there was no significant difference in the mean scrotal circumference between group III and group IV. Further, the mean scrotal circumference in group I was found to be significantly lower (*P*<0.05) when compared with all other groups and the mean scrotal circumference of group II was found to be significantly lower when compared with group III and group IV. The comparison of the means between the groups revealed no significant difference between group I and group V as well as between group III and group IV. However, the mean testis weight in group II was found to be significantly lower when compared with group III and group IV. The comparison of live and dead sperms within the groups revealed no significant difference in group I, group III, group IV and group V. However only in group II the percentage of dead sperms was found to be significantly higher when compared to the live sperms percentage.

Table 1

Scrotal–testicular biometry, sperm quality and quantity in rams.

Groups	Sperm concentration (millions/mL)	Scrotal circumference (cm)	Testis weight along with epididymis (g)	Percent live and dead sperms	
				Live sperms	Dead sperms
Group I	0.00 ^a	12.19±0.56 ^a	6.68±0.83 ^a	0.00 ^{Aa}	0.00 ^{Aa}
Group II	196.70±20.11 ^b	22.65±0.50 ^b	86.23±9.20 ^b	38.04±7.44 ^{Ab}	61.95±7.44 ^{Bb}
Group III	316.70±19.44 ^c	26.29±0.53 ^c	142.30±5.20 ^c	56.32±4.78 ^{Ab}	43.68±4.78 ^{Ab}
Group IV	413.30±50.31 ^c	26.46±0.51 ^c	120.70±10.31 ^c	54.23±4.47 ^{Ab}	45.77±4.47 ^{Ab}
Group V	0.00 ^a	–	5.50±0.31 ^a	0.00 ^{Aa}	0.00 ^{Aa}

Superscripts bearing different letters between the groups differ significantly (*P*<0.05). Superscripts bearing different capital letters (with respect to live and dead sperms percentage only) within a group differ significantly (*P*<0.05).

4. Discussion

The ejaculated sperm concentration was significantly lower in the one year old rams when compared with two, three and four year old rams^[10]. Further others have proposed that the rate of spermiogenesis in the younger monkeys was lower due to relatively lower serum FSH: Testosterone ratio^[4]. Hence the observation of significantly low level of mean sperm concentration in group II when compared with group III and group IV could be either due to the influence of age and/or

the influence of hormonal in the animal^[10,11]. There exists a significant positive correlation between sperm concentration and scrotal circumference^[10]. Hence the observation of significantly lower scrotal circumference in group I when compared with all the other groups and in group II when compared with group III and group IV could be due to the age of the animal and/or the hormonal status of the animal^[11] and/or due to the positive correlation between the scrotal circumference and sperm production^[10]. There exists a positive correlation between scrotal circumference and testicular weight^[2]. Further earlier study shows that there exists a significant positive correlation between sperm

concentration and scrotal circumference^[10] in their study has put forth that. Hence the observation of significantly lower mean testis weight in group II when compared with group III and group IV could be due to the age of the animal and/or due to the hormonal profile^[2, 10,11].

The significantly higher percentage of dead sperms in the epididymis of the pubertal animals could be due to the failure of attainment of the desired concentration of glyceryl phosphoryl choline (GPC) as GPC is the major constituent of epididymal secretions required for the maintenance of sperm membrane stability^[12]. Comparison of mean live and dead sperms percentages between the groups revealed no significant difference between group I and group V rams as well as when observed between group II, group III and group IV rams. The study on the evaluation of semen characteristics in 11–25 months old bucks has reported that there was no significant difference in the semen characteristics between different age groups^[13]. Hence the observation of significantly no difference in the live and dead sperm percentage between group II, group III and group IV rams could be due to the absence of influence of age on the semen characteristics^[12].

In conclusions, the analysis of various physiological attributes like, sperm concentration, scrotal circumference, testis weight and live and dad sperms percentage in all the groups revealed for higher levels in group III and group IV rams.

Conflict of interest statement

We declare that we have no conflict of interest.

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