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Gayatri M Gadariya
M.V.Sc., College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Vishnudeo Kumar
College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Anil Sharma
College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Mahesh R Gadariya
College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Corresponding Author:
Gayatri M Gadariya
M.V.Sc., College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Investigation on micrometric traits and their association in testis of dog (*Canis familiaris*)

Gayatri M Gadariya, Vishnudeo Kumar, Anil Sharma and Mahesh R Gadariya

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Abstract
The present investigation dealt with micrometric traits and their association in stray dog testis (*Canis familiaris*). The study was conducted on the testes of 18 adult, healthy dogs captured and operated for sterilization at the Veterinary Hospital of Junagadh Municipal Corporation, Ivanagar, and Junagadh. Micrometrical observations were recorded on the H & E-stained section of the testis of the dog, using Carl Zeiss Zen (Blue edition) microscopic image analysis software. The samples were examined under a light microscope using x100 and then x400 magnification. The micrometrical parameters (µm) recorded in the study were *viz*., i) thickness of tunica albuginea ii) number of seminiferous tubules (per mm^2), iii) diameter of seminiferous tubules, iv) height of germinal epithelium, v) number of Sertoli cells/tubule, vi) nuclear diameter of Sertoli cells, vii) number of Leydig cells (per mm^2) and viii) nuclear diameter of Leydig cells. Overall mean values for corresponding traits were 528.65±53.11 µm, 14.46±1.22 per mm^2, 178.92±6.41µm, 61.88±2.88 µm,7.08±0.34/tubule,10.54±0.25 µm, 255.51±25.34 per mm^2 and 5.84±0.12 µm, respectively. The number of seminiferous tubules (per mm^2) was negatively and highly correlated (r value, -0.71 to -0.73) with their diameter (µm) and the height of epithelium (µm). The diameter of seminiferous tubules (µm) was positively and moderately correlated with its height of the epithelium (µm), No. of Sertoli cell/tubule and nuclear diameter of Sertoli cell (µm), with r value ranging from 0.44 to 0.66. Height of the seminiferous tubules' epithelium was also positively associated (R-Value 0.55 to 0.63) with the number of Sertoli cell/tubule and the nuclear diameter of Sertoli cells (µm). The number of Sertoli cells/tubule and their nuclear diameter were positively and moderately associated (R-Value, 0.44), while the number of Leydig cells/tubule and their nuclear diameter were negatively and lowly associated (R-Value, -0.277).

Keywords: *Canis familiaris*, micrometry, correlation, dog, testis

Introduction
Recent years have seen advancements in our knowledge of the processes controlling canine reproduction, particularly the maturation and development of gametes. Additionally, research has used imaging and molecular technologies to generate diagnostic ailments for reproductive abnormalities and to enhance knowledge about reproductive diseases in domestic dogs. More details are needed to comprehend the basic physiology and structure of the dog testicles. Testicular micrometric changes are essential for determining how the testes function.
Increased sperm production functionality and internal modifications are shown by higher testicular parameter observations, which are critical for canine reproductive success. A few researchers are also interested in testicular biometry parameters. (Bhagyalakshmi *et al*., 2020; Kumar *et al*., 2023; Yogesh *et al*., 2023[a]) [1, 3, 9, 10]. However, there is a dearth of documented literature on studies related to micrometric traits of dog testis. In view of this, testicular micrometric traits, including their association, were studied in non-descript dogs.

Materials and Methods
In order to have a comprehensive view of the full year, the current study was carried out on the testes of 18 adult, seemingly healthy stray dogs, six in each of April-May, September-October, and December-January.

Samples were collected from dogs that were caught and put through "neutering" surgery at the Junagadh Municipal Corporation Veterinary Hospital in Ivanagar, Junagadh (Gujarat, India). Micrometrical observations were recorded on the Hematoxylin and Eosin-stained section of the testis of the dog during three seasons of the year, using Carl Zeiss Zen (Blue edition) microscopic image analysis software. The samples were examined under a light microscope using low (x 100) and then high (x 400) magnification. The micrometrical parameters (µm) recorded in the study were *viz*., i) Thickness of tunica albuginea ii) Number of seminiferous tubules (per mm^2), iii) Diameter of seminiferous tubules, iv) Height of germinal epithelium, v) Number of Sertoli cells/tubule, vi) Nuclear diameter of Sertoli cells, vii) Number of Leydig cells (per mm^2) and viii) Nuclear diameter of Leydig cells.

The data obtained have been presented as means ± standard error (SE). Pearson's correlations were worked out for testicular micrometric traits (Snedecor & Cochran, 1994) [8].

Results and Discussion

- **Micrometric Traits Dog Testis:** The testicular micrometry *viz.*, thickness of tunica albuginea (µm), number of seminiferous tubule (per mm^2), diameter of seminiferous tubule (µm), height of germinal epithelium (µm), number of Sertoli cells/tubule, nuclear diameter of Sertoli cells (x40), number of Leydig cells (per mm^2), nuclear diameter of Leydig cell (µm) have significance in reproductive behaviour and pattern of dog, hence, these were studied. The data of the above micrometrical traits have been presented in Table 1.

Table 1: Mean ± SE of the testicular micrometry of dog testis (N=18)

Micrometric traits	Mean ± SE
Thickness of tunica albuginea (µm)	528.65±53.11
Number of seminiferous tubule (per mm^2)	14.46±1.22
Diameter of seminiferous tubules (µm)	178.92±6.41
Height of germinal epithelium (µm)	61.88±2.88
Number of Sertoli cells/tubule	7.08±0.34
Nuclear diameter of Sertoli cells (µm)	10.54±0.25
Number of Leydig cells (per mm^2)	255.51±25.34
Nuclear diameter of Leydig cell (µm)	5.84±0.12

- **Thickness of tunica albuginea (µm):** The thickness of tunica albuginea in dog testis was 528.65±53.11 µm, which was slightly lower than that (725.95±4.47 µm) reported by Yogesh *et al*. (2023^b) [9, 10] in the indigenous dog of Mumbai. On the other hand, the present values were higher than those observed in two years old indigenous male goats (230±3.5 µm) by Mohammed *et al.* (2011) [4] and in large white Yorkshire pig (253.70±14.29 µm) by Sikarwar *et al.* (2018) [6]. The differences in the values of the thickness of the tunica albuginea might be due to species differences and also on account of the varying amount of collagen fibers present.
- **Number of seminiferous tubules (per mm^2):** The overall value of mean number of seminiferous tubules in each testis of the dog averaged 14.46±1.22 per mm^2. There seemed to be a dearth in documented literature for comparison and discussion of this trait. However, the number of seminiferous tubules could be negatively correlated (r value, -0.728) with the tubular diameter.
- **Diameter of seminiferous tubules (µm):** The diameter of the seminiferous tubules in dog testis was found to be 178.92±6.41 µm. As reported by Bhagyalakshmi *et al.* (2020) [1], the average diameter of the seminiferous tubule in dog averaged 176.659 µm, which was very close to the diameter of the seminiferous tubule (178.92±6.41 µm) found in the present study. The diameter of seminiferous tubules recorded in the present study (178.92±6.41 µm) was more than that reported by Naidu *et al.* (1994, as cited in Aslam, 2017) in mature indigenous pigs (159.30±0.50 µm) and by Neves *et al*. (2002) [5] in mule (127±4 µm). The smaller diameter of seminiferous tubules was reported by Neves *et al*. (2002) [5] in the donkey (222±6 µm), as compared to that in the present study. The difference in diameter of the seminiferous tubules seemed to be associated with a variation in the height of the germinal epithelium (R-Value, 0.662) and also due to species differences.
- **Height of germinal epithelium (µm):** The overall mean height of the germinal epithelium of the testis of dog was 61.88±2.88. In an earlier study, Yogesh *et al.* (2023^b) [9, 10] reported that the height of the germinal epithelium was 164.54 µm in the testes of the indigenous dog of Mumbai, which was more than twice the value observed in the present study. The height of the germinal epithelium could be directly related to the difference in spermatogenic activity and other factors like climatic and nutritional status.
- **Number of Sertoli cells/tubule:** The number of Sertoli cells/tubule of dog testis was 7.08±0.34. A paucity in the literature was a limitation for comparison and discussion of this trait. The number of Sertoli cells has been justified by the differentiation of myofibroblast cells that might penetrate the basement membrane of the seminiferous tubules to compensate for the functional demand of spermatogenesis and nourishment of the spermatozoa (Hussin *et al*., 2018) [2]. These findings were consistent with the results of Hussin *et al*. (2018) [2], who reported that in local Iraqi dogs, the number of Sertoli cells significantly increased during the active period as opposed to other sedentary periods.
- **Nuclear diameter of Sertoli cells (µm):** The mean nuclear diameter of Sertoli cells of the testis of dog was 10.54±0.25. Nuclear diameter of Sertoli cells (9.5 to 11.2 µm) observed in the present study was slightly higher than that reported by Sikarwar *et al.* (2022) [7] in the testes of the large white Yorkshire pig (*Sus scrofa*). The researchers observed that Sertoli cells were seen to be taller and longer, with an average width of 7.90±0.61 µm.
- **Number of Leydig cells (per mm^2):** In the current study, the number of Leydig cells was found to be 255.51±25.34 per mm^2. Hussin *et al*. (2018) [2] found that, in local Iraqi dogs, the number of Leydig cells significantly increased during the active period as opposed to other sedentary periods. Zayed *et al*. (1995) [11] also demonstrated that the absolute number of Leydig cells per testis in camel ranged from 3.68×10^9 cells (in fall) to 6.04×10^9 cells (in late winter and spring). The difference in the number of Leydig cells might be due to the variation in diameter of seminiferous tubules (r value, -0.370 and the number of seminiferous tubules per mm^2 (r value, 0.567), as observed in the present study, and also species difference.
- **Nuclear diameter of Leydig cells (µm):** The mean nuclear diameter of Leydig cells of each testis of the dog was 5.84±0.12 µm. The nuclear diameter of Leydig cells observed in the present study was comparable with the value (6.8 µm) reported by Zayed *et al*. (1995) [11] in camels. The nuclear diameter of Ledig cells showed a

negative and low association (R-Value, -0.277) with no. of Leydig cells/tubule. The nuclear diameter of Leydig cells might be correlated with the activity of Leydig cells to produce androgen, especially during the active period. Zayed *et al*. (1995) [11] found that in camel, the larger Leydig cell nucleus size was seen during the post-rutting period. Similarly, also found in the camel of the Algerian extreme arid region the post-rutting season was coincided to larger Leydig cell nucleus size.

Association among testicular micrometric traits in dog

Pearson's correlations were worked out for micrometric traits of dog and the correlation matrix of the analysis is furnished in Table-2.

The number of seminiferous tubules (per mm^2) was negatively and highly correlated (r value, -0.71 to -0.73) with their diameter (µm) and the height of epithelium (µm). The diameter of seminiferous tubules (µm) was positively and moderately correlated with its height of the epithelium (µm), No of Sertoli cell/tubule and nuclear diameter of Sertoli cell (µm), with r value ranging from 0.44 to 0.66. Height of the seminiferous tubules' epithelium was also positively associated (R-Value 0.55 to 0.63) with the number of Sertoli cells/tubule and nuclear diameter of Sertoli cell (µm). No. of Sertoli cells/tubule and their nuclear diameter were positively and moderately associated (R-Value, 0.44), while the number of Leydig cells/tubule and their nuclear diameter were negatively and lowly associated (R-Value, -0.277).

Table 2: Correlation matrix for micrometric traits of dog testis

Micrometric trait	Thick-ness of TA	No of ST	Diameter of ST	Height of ST epithet Lium	No of Sertoli cells	Nuclear dia of Sertoli cell	No of Leydig cells	Nuclear dia of Leydig cell
Thickness of tunica albuginea (TA), µm	1.000							
No of Seminiferous Tubule (ST), per mm^2	-0.032	1.000						
Diameter of ST Tubule, µm	0.074	-0.728	1.000					
Height of ST epithelium, µm	0.012	-0.710	0.662	1.000				
No. of Sertoli cell/tubule	0.061	-0.249	0.443	0.554	1.000			
Nuclear diameter of Sertoli cell, µm	0.279	-0.323	0.595	0.628	0.439	1.000		
No. of Leydig cells, per mm^2	0.107	0.567	-0.370	-0.320	0.161	0.122	1.000	
Nuclear diameter of Leydig cell, µm	0.041	0.047	0.143	0.391	0.083	0.362	-0.277	1.000

Conflict of Interest

Not available

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Not available

Reference

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