International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Zoological Research

ISSN 1811-9778
DOI: 10.3923/ijzr.2016.32.39

Research Article
Stereological Study of the Testicular Tissue of the Persian Squirrel (*Sciurus anomalus*)

[1]Bahram Ranjbaran, [2]Nader Goodarzi and [3]Majid Tavafi

[1]Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
[2]Department of Basic Science, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
[3]Department of Anatomy, Faculty of Medicine, Lorestan University of Medical Sciences, Khoram Abad, Iran

Abstract

Objective: The propose of this study was to investigate the histomorphometrical characteristics of the testicular tissue in the Persian squirrel (*Sciurus anomalus*) as the only representative of the Sciuridae family in the middle East countries using design-based and unbiased stereological tools. **Methodology:** Testes from five sexually mature animals suffered from diseases unrelated to urogenital system were used in this study. The testes were fixed in 10% buffered formaldehyde and routinely processed for histological and stereological analysis. The mean testes weight was 1.38 g leading to a 1.16 gonadosomatic index (GSI). The mean total volume of the testes was estimated as 1.01 from which 0.65 was allocated into seminiferous tubule and 0.36 was related to interstitial tissue. The average tubular diameter was 206 μm, whereas, the mean epithelium height was 30.77 μm. The Persian squirrl presents 28.62 m and an average of 20.7 m of seminiferous tubule per gram of testis. **Results:** The present data showed that despite the high GSI index in the Persian squirrel compared with other rodent species, the total volume of seminiferous tubule and seminiferous epithelium heigh are relatively small in this species. **Conclusion:** It is concluded that the Persian squirrel has a high investment of body mass allocated into the testis, which explained one of the highest GSI index compared with other rodent species.

Key words: Persian squirrel, testis, stereology, morphometry

Received: January 07, 2016 Accepted: April 30, 2016 Published: June 15, 2016

Citation: Bahram Ranjbaran, Nader Goodarzi and Majid Tavafi, 2016. Stereological study of the testicular tissue of the Persian squirrel (*Sciurus anomalus*). Int. J. Zool. Res., 12: 32-39.

Corresponding Author: Nader Goodarzi, Department of Basic Science, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
Tel: +98833832599

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Persian squirrel (*Sciurus anomalus*) is the only representative of the Sciuridae family distributed in the middle east countries including Greece, Turkey, Armenia, Georgia, Azerbaijan, Iran, Jordan, Lebanon and Syria in coniferous and mixed temperate forests (Amr *et al*., 2006). It is a herbivorous species whose diet includes mostly on pine acorns and other seeds and fruits. However, habitat fragmentation, wood cutting, deforestation, fire and habitat disturbance are the major threats facing the current population of this species in its habitats (Matsinos and Papadopoulou, 2004). Despite the wild nature of this species, it has recently been one of the most popular pets. Consequently, its referral to veterinary clinics has been increased.

The knowledge of testicular morphophysiology and histomorphometry could be an important tool in the analysis of the reproductive condition of a species in its natural habitat. These parameters can be related to environmental factors and contribute to the conservation of mammals (Kenagy and Trombulak, 1986; De Paula *et al*., 2002). Furthermore, the composition of the testicular parenchyma and relative size of the testicles can provide valuable information on the reproductive physiology and mating system of a given species, which make it possible to develop protocols for assisted reproduction (Caldeira *et al*., 2010).

Numerous studies have been conducted on morphometric features of the testis in various species including rat (Mori and Christensen, 1980; Wing and Christensen, 1982), monkey (Fouquet *et al*., 1984), hamster (Hikim *et al*., 1989), cat (Franca and Godinho, 2003) and human (Nistal *et al*., 1986). However, to the best of our knowledge, no quantitative evaluations of the testicular tissue of the sexually mature Persian squirrel are available. In order to achieve optimal reproductive performance, reliable and precise quantitative morphological data of the reproductive organs of this species are highly desirable. In this context, this study was conducted to investigate histomorphometrical characteristics of the testicular tissue in this species using design-based and unbiased stereological procedures and expand the knowledge about its reproduction in natural environments.

MATERIALS AND METHODS

Animals and tissue preparation: Five sexually mature male Persian squirrel suffered from diseases unrelated to the urogenital system were used in this study. All procedure involving in this work was approved by ethical committee of Razi university as a part of a DVM thesis. The animals were euthanized under their owner's permission and their left testes removed. The testes were cleaned of fat and connective tissues and the epididymis were separated. The testes were then weighted and their primary volume were measured using the immersion method (Silva and Merzel, 2001). Briefly, a laboratory jar containing distilled water was placed on the scale and weighed and then the testis suspended by a thin thread was immersed in the laboratory jar so that it was fully covered by water and did not touch the bottom of the jar. The new weight in grams, minus the weight of the jar and water was the volume of the testis in cubic centimeters. The gonadosomatic index (GSI) was calculated by Eq. 1:

$$GSI = \frac{GW}{BW} \times 100 \quad (1)$$

where, GW and BW were gonadal weight and body weight respectively. Afterward, the specimens were fixed in neutral buffered formaldehyde for 7 days. The reference volume or final volume of the testis should be estimated in stereological study to prevent reference trap (Gundersen *et al*., 1988; Braendgaard and Gundersen, 1986). Cavalieri technique is the most common method for reference volume estimation. But, this method needs consecutive sections and it is time consuming. Thus, the reference volume was obtained by estimating shrinkage after tissue processing and staining without need to serial sections. Estimation of shrinkage and length of seminiferous tubules requires isotropic uniform random sections (Gundersen *et al*., 1988; Nyengaard, 1999). These sections were obtained through orientator method. A brief description of this method is presented in Fig. 1. Overall, 7-10 slabs were collected from each testis. For estimation tissue shrinkage, a circle was punched from a testis slab by a trocar (Fig. 2). The diameters of the circular piece of the testis was measured by a micrometer and the area of the circle was estimated using a usual formula for calculating the area of a circle. The cut surfaces of the all slabs and circular piece were embedded in paraffin sectioned (5 μm thicknesses) and stained by H and E method. After staining, the area of the circular piece was measured again and volume shrinkage was calculated from Eq. 2 (Kenagy and Trombulak, 1986):

$$\text{Volume shrinkage} = 1 - \left(\frac{AA}{AB}\right)^{1.5} \quad (2)$$

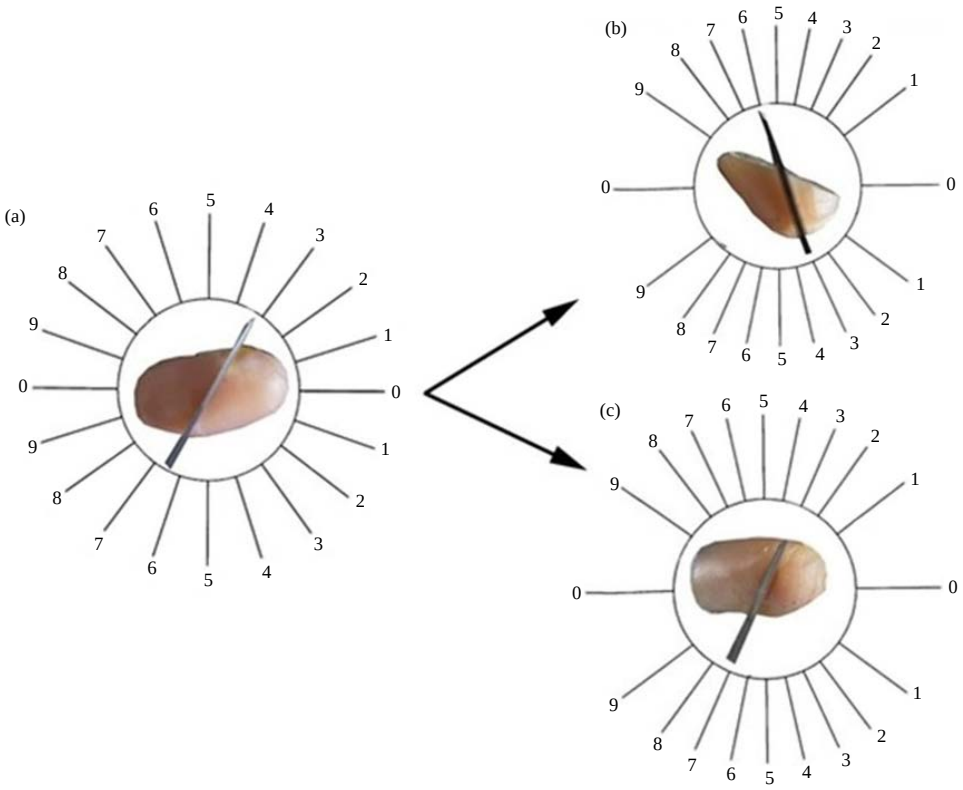

Fig. 1(a-c): Orientator method, (a) Testis was placed on the circle. such that each half of it was divided into 10 equal parts. A random number between 0 and 9 was selected. The testis was sectioned into two parts at the direction of the selected number (here 3), (b) Cut surface of one part of the testis was then placed on the 0-0 direction of the second circle with 10 unequal divisions. The circle division was done according to the cosine of the angels. Then, another random number was selected and the second cut was done (here 6). The parts were sectioned into parallel slabs and (c) Cut surface of the other part of the testis was placed vertically on the second circle. Again, a new number and direction (here 3) was selected and cut. This part was also sectioned into parallel slabs

Fig. 2: Eight to ten Isotropic Uniform Random (IUR) slabes obtained through orientator method. A circle was punched from a testis slab by a trocar. The diameter of the circular piece and the area of the circle were estimated using the usual formula for calculating the area of a circle

Then, the final volume of the testis was calculated by Eq. 3:

$$V_{final} = V_{primary} \times (1\text{-volume shrinkage}) \quad (3)$$

Estimation of the volume density: All sampled sections were analyzed by using a video microscopy system consist of a microscope (Olympus CX2, Japanese) linked to a video camera (Dinocapture ver.5, dino-lit.com 30.5 mm), a computer and a flat monitor to determine the parameters. The point probe (10×10 cm composed of 25 points) was superimposed upon the images of the tissue sections viewed on the monitor and volume density (V_V) of seminiferous tubules, germinal epithelium and interstitial tissue were obtained using a point-counting method (Fig. 3) from Eq. 4:

$$V_V = \frac{P_{structure}}{P_{reference}} \quad (4)$$

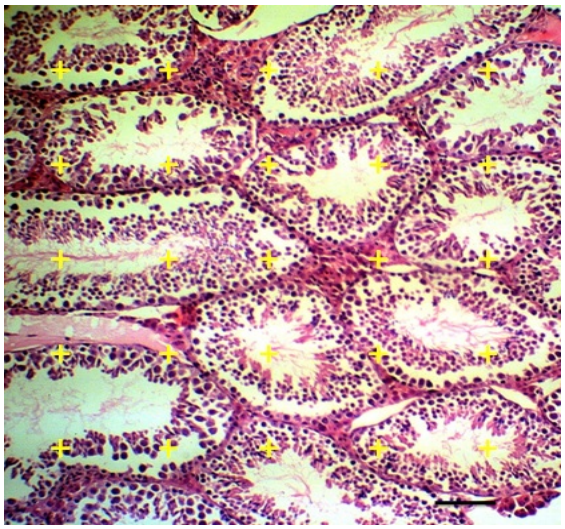

Fig. 3: Point counting method. The total number of points hitting the structure of interest is counted and divided by the total number of points hitting the reference space (×10, scale bar = 50 m)

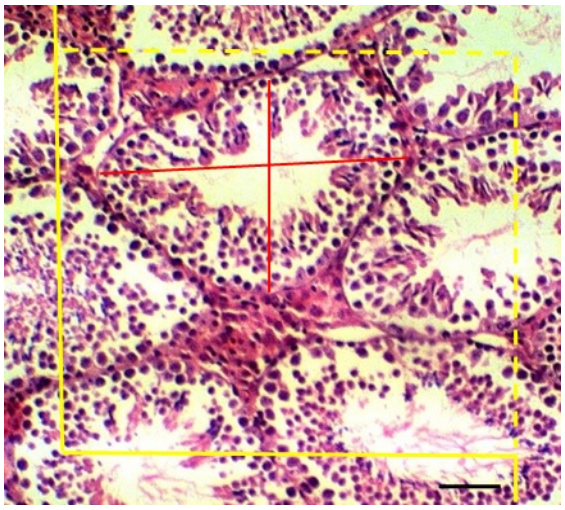

Fig. 4: Length density estimation. The counting frame with exclusion lines (the left and lower borders and their extensions) and inclusion lines (the right and upper borders) is superimposed on the images. The tubules which are either completely or partly inside the counting frame and are not touched by the exclusion lines are counted. The largest diameter orthogonal to the longest axis of the tubule and approximately touching center of the tubule is considered as its diameter (×40, scale bar = 50 m)

where, $P_{structure}$ and $P_{reference}$ are the number of test points falling on the structure's profile and on the reference space, respectively. About 8-12 microscopic fields were examined in each testis.

The absolute volume of the parameters was estimated by multiplying the fractional volume by the final volume of the kidney to prevent the reference trap (Nyengaard, 1999; Mandarim-de-Lacerda, 2003).

Estimation of the length density and diameter of tubules: The length density of the seminiferous tubules was estimated by randomly overlaying an unbiased counting frame with exclusion lines (the left and lower borders) and inclusion lines (the right and upper borders) (Nyengaard, 1999; Mandarim-de-Lacerda, 2003) with an area of 100 cm^2 on the monitor live images at final magnification of 135x. The tubule profiles completely or partly inside the counting frame but only touching the inclusion lines were counted (Fig. 4). The length density was calculated from Eq. 5:

$$L_V = 2 \times \frac{\sum Q}{a\,(\text{frame}) \times \sum \text{frame}} \quad (5)$$

where, ΣQ is the total number of the tubule profiles counted per testis, equals the area associated with a frame and Σ is the total number of frames counted. Finally, the absolute length of the seminiferous tubules were estimated by multiplying the length density by the final volume of the testis. The diameter of the tubules was measured perpendicular to the long axis where the tubule was widest (Fig. 4). An average of 100 profiles were counted per testis.

Estimation of the germinal epithelium height: The height of the germinal epithelium was estimated from Eq. 6 (Nyengaard, 1999):

$$H = \frac{V_V}{S_V} \quad (6)$$

where, V_V and S_V are the volume density and surface density of the germinal epithelium, respectively. The volume density of the germinal epithelium was obtained by point counting method. the surface density of the germinal epithelium was estimated using a linear test probe (Fig. 5).

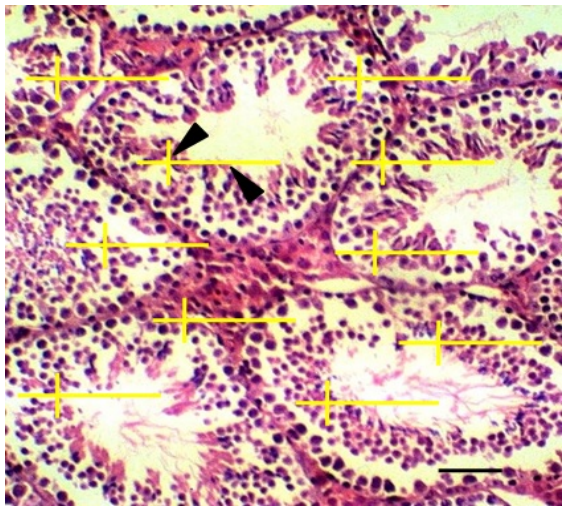

Fig. 5: A linear test probe is used. The total number of points (as upper arrowhead) superimpose on the germinal epithelium, the length of each line, number of intersections (as lower arrowhead) of linear test probe with the inner surface of the germinal epithelium are calculated (×40, scale bar = 50 m)

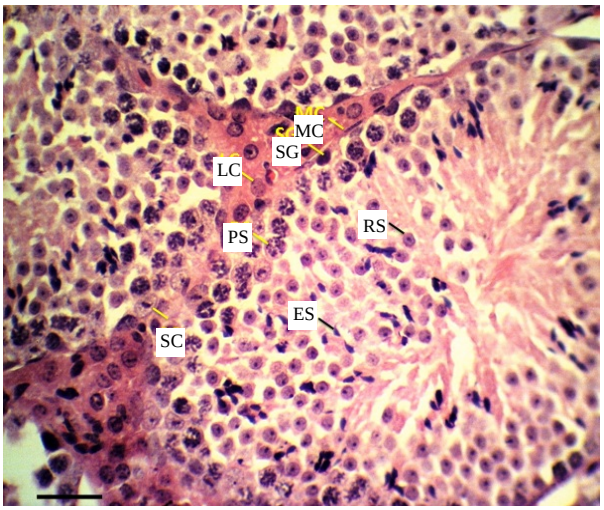

Fig. 6: Testicular parenchyma of the Persian squirrel, LC: Leydig cell, SG: Spermatogonia, PS: Primary spermatocyte, RS: Round spermatid, ES: Elomgated spermatid, SC: Sertoli cell, MC: Myoid cell (×40, scale bar = 50 m)

RESULTS

The testes of the Persian squirrel were paired organs located in the scrotum and surrounded by a thick capsule of connective tissue (testicular albuginea). The testicular parenchyma was divided into two compartments, namely tubular and interstitial. The tubular compartment consists of the seminiferous tubules, which comprises the tunica propria, the seminiferous epithelium and lumen. The tunica propria has a monolayer of myoid cells; the seminiferous epithelium was composed of spermatogenic cells in various stages of development and sertoli cells with characteristic irregular nucleus and prominent nucleoli. The interstitial compartment was constituted of leydig cells, cells and fibers of connective tissue, blood vessels and lymphatic space (Fig. 6).

The obtained values for the morphometric and stereological parameters of testes in the Persian squirrel are presented as Mean±SD in Table 1 and 2 respectively. The mean length, width and thickness of the testes were 1.52±0.53, 0.92±0.13 and 0.83±0.1 respectively. The mean testis weight was found to be 1.38±0.01 g leading to a 1.16±0.14 % GSI. The mean absolute volume of the testis was estimated as 1.01±0.13 with 0.65±0.12 seminiferous tubules and 0.36±0.14 interstitial tissue. About 0.5±0.14 of the seminiferous tubules were belonged to the germinal epithelium and 0.15±0.01 of that was related to the lumen. The mean total length of the seminiferous tubules was 28.60±2.57 m and the mean tubular diameter and germinal epithelium height were estimated as 206±4.24 and 30.77±4.77 µm, respectively.

Table 1: Mean±SD of the body weight and morphometric parameters of the testis in the Persian squirrel (n = 5)

Parameters	Mean±SD
Body weight (g)	240±28.2
Testis weight (g)	1.38±0.01
Length (cm)	1.52±0.53
Width (cm)	0.92±0.13
Thickness (cm)	0.83±0.1
Gonadosomatic index (%)	1.16±0.14

Table 2: Mean±SD of the absolute volume and total length (m) of testis and its subcomponents in the Persian squirrel (n = 5)

Parameters	Mean±SD
Testis volume (cm^3)	1.09±0.39
Seminiferous tubules volume (cm^3)	0.65±0.24
Germinal epithelium volume (cm^3)	0.50±0.14
Luminal volume (cm^3)	0.15±0.01
Interstitial tissue volume (cm^3)	0.36±0.04
Epithelial height (µm)	30.78±4.77
Tubular diameter (µm)	206.00±4.24
Tubular length (m)	28.60±2.57
Tubular length per gram/testis	20.70±1.70

DISCUSSION

To our knowledge, this is the first study to use stereological approach to provide a comprehensive morphometric and histomorphmetric data set of the testis in the Persian squirrel. Although, there are many different measuring methods such as compass (Love *et al*., 1991), orchidometer (Sakamoto *et al*., 2007; Schiff *et al*., 2004; Karaman *et al*., 2005) and ultrasonography (Eilts *et al*., 1993; Al Salim *et al*., 1995) for estimating testicular volume, all of these procedures are practically used on live animals and human beings. Hence, in the present study, design-based stereological techniques were followed to estimate the realistic and unbiased values for histomorphometric features of the testes in the Persian squirrel. Although, stereological method is not applicable in practice but it has been one of the preferred method used in volumetric evaluation of materials study by pathologists and anatomists and obtained results are most reliable both for human and veterinary usage.

The gonadosomatic index (GSI) indicates the investment in gonads relative to body weight and is an important reproductive parameter (Franca and Russell, 1998). The GSI observed in the adult Persian squirrel was 1.16% which is a higher value than those were reported for gerbil (0.72%) (Segatelli *et al*., 2004), spiny rat (0.93%) (Cordeiro-Junior *et al*., 2010), rat (0.76%), mice (0.74%) and chinchilla (0.88) (Rocha *et al*., 1999; Leal and Franca, 2009). Costa *et al*. (2010) in a study reported this parameter for dasycropta leporine and agouti paca (with 2.5 and 5.4 kg b.wt.) as two large rodent species 0.33 and 0.22% respectively. Because the testicular size is not proportional to body size, the somatic investment in gonadal mass is greater in small animals compared to larger body size animals. Hence, it corroborates the well-established assumption that smaller mammal's species have higher allocation and energy expenditure in testicular mass, which improves their reproductive success (Kenagy and Trombulak, 1986).

The testicular parenchyma consist of two compartments namely seminiferous tubules and interstitial tissue (Amann and Schanbacher, 1983; Russell *et al*., 1990a). Seminiferous tubules comprise the main compartment of the testis and occupy from 70-90% of testis parenchyma in most mammals species (Russell *et al*., 1990b). The intertubular compartment consists of leydig cells, blood and lymph vessels, nerves and connective tissue cells populations namely fibroblasts, macrophages and mast cells (Russell, 1996). In contrast to the high value of GSI, seminiferous tubules occupied approximately 65% (0.65 ± 0.12 cm^3) of the testicular parenchyma in the Persian squirrel which is out of the range of other mammals. This value have been reported to be 0.89 and 0.1 in rat (Mehranjani *et al*., 2009) and mice (Noorafshan *et al*., 2011) testis respectively. Accordingly, the mean volume of the interstitial tissue in the Persian squirrels testis (0.36 ± 0.14) was higher than those reported by Mehranjani *et al*. (2009) 0.23 and Chang *et al*. (2011) 0.19 for rat testis. This difference suggest that the number of leydig cells in the testis of the Persian squirrel probably would be higher than that in rat testis.

Tubular measurement could be used as an indicator for spermatogenic activity in studies related to testicular function (De Souza *et al*., 2005; Da Silva *et al*., 2006). Although, the tubule diameter can reach up to 550 μm in some species of marsupials, mean tubular diameter varies from 180-350 μm in most mammals (Roosen-Runge, 1977; Setchell *et al*., 1994). The Persian squirrel shows mean tubular diameter of 206 μm which is in the above range but is lower than those found in rat (Mehranjani *et al*., 2009; Chang *et al*., 2011) and hamster (275) (Hikim *et al*., 1989) and higher than those described for spiny rat (172) (Cordeiro-Junior *et al*., 2010), agouti (193) and paca (185) (Costa *et al*., 2010). Along with tubular diameter, the height of the seminiferous epithelium is a more effective criteria for evaluating spermatogenic activity (Wing and Christensen, 1982). The height of the germinal epithelium of the seminiferous tubules in our subjects (30.77 μm) was lower than those found for most rodent species (Mehranjani *et al*., 2009; Chang *et al*., 2011; Cordeiro-Junior *et al*., 2010; Rocha *et al*., 1999; Leal and Franca, 2009; Costa *et al*., 2010).

The total length of seminiferous tubules could be affected by testis size, volume of seminiferous tubules and tubular diameter. The total length of seminiferous tubules in the Persian squirrel was 28.62 m. When considering the differences in testis size among species, the conversion of the total tubular length to total length of the seminiferous tubules per gram of testis, makes the measurement independent of the animal size. The Persian squirrel presented 20.7 m of tubule per gram of testis, which is within the pattern value observed in other rodents (Costa *et al*., 2010). The total length of tubules in the Persian squirrel was lower than that of spiny rat (38) (De Paula *et al*., 2002) and higher than those of gerbil (10) (Segatelli *et al*., 2004) and hamster (25.92) (Hikim *et al*., 1989). In general, 10-15 m of seminiferous tubules are found per gram of testis parenchyma (Wing and Christensen, 1982). Due to the lower values for seminiferous tubules volume and tubular diameter, it is expected that tubular length in the Persian squirrel to be higher compared

with rodents species with similar body weight and testis weight. The greatest values recorded for the tubular length per gram of testis have been found in rodents such as the agouti andpaca at 32 and 35 m respectively (Costa *et al*., 2010). These findings indicates a significant investment in spermatic production in these and confirm the diversity in the reproductive strategies of the different species.

It should be considered that the cited studies calculated the total length of the seminiferous tubules dividing total volume of the seminiferous tubules by their mean tubular diameter. but we used a different stereological method has been described in materials and methods. Also, the used technique for estimating the height of the germinal epithelium in the present study was different from that was used in the previous reports.

It concluded that the Persian squirrel has a high investment of body mass allocated into the testis, which explained one of the highest GSI index compared with other rodent species. However, the values for seminiferous tubule volume and seminiferous epithelium height in the present study are relatively small in the sexually Persian squirrel. This results could be served as a foundation for future studies involving the reproductive biology in this species.

ACKNOWLEDGMENT

The authors express their thank to Mr. M. Azarhoosh and Miss Khedmatgozar for their valuable assistance in photograph preparation.

REFERENCES

Al Salim, A., P.J.T. Murchison, A. Rana, R.A. Elton and T.B. Hargreave, 1995. Evaluation of testicular volume by three orchidometers compared with ultrasonographic measurements. Br. J. Urol., 76: 632-635.

Amann, R.P. and B.D. Schanbacher, 1983. Physiology of male reproduction. J. Anim. Sci. Suppl., 57: 380-403.

Amr, Z.S., E. Eid, M.A. Qarqaz and M. Abu Baker, 2006. The status and distribution of the persian squirrel, *Sciurus anomalus* (Mammalia: Rodentia: Sciuridae), in dibbeen nature reserve, Jordan. Zool. Abhandlungen, 55: 199-207.

Braendgaard, H. and H.J.G. Gundersen, 1986. The impact of recent stereological advances on quantitative studies of the nervous system. J. Neurosci. Methods, 18: 39-78.

Caldeira, B.C., T.A.R. de Paula, S.L.P. da Matta, M.K. Balarini and P.K.A. Campos, 2010. Morphometry of testis and seminiferous tubules of the adult crab-eating fox (*Cerdocyon thous*, Linnaeus, 1766). Revista Ceres, 57: 569-575.

Chang, Q., Z. Liu, W.Z. Ma, C.C. He, X.S. Shen, X.J. Qian and Z.L. Xu, 2011. Drug synergistic antifertility effect of combined administration of low-dose gossypol with steroid hormones in rats. Chin. Med. J., 124: 1678-1682.

Cordeiro-Junior, D.A., G.M.J. Costa, S.A. Talamoni and L.R. Franca, 2010. Spermatogenic efficiency in the spiny rat, *Trinomys moojeni* (Rodentia: Echimyidae). Anim. Reproduc. Sci., 119: 97-105.

Costa, G.M.J., M.C. Leal, C.S. Ferreira, D.A. Guimaraes and L.R. Franca, 2010. Duration of spermatogenesis and spermatogenic efficiency in 2 large neotropical rodent species: The agouti (*Dasyprocta leporina*) and Paca (*Agouti paca*). J. Androl., 31: 489-499.

Da Silva, Jr. V.A., A.C.S. Vieira, C.F. Pinto, T.A.R. de Paula and M.B. Palma *et al*., 2006. Neonatal treatment with naloxone increases the population of Sertoli cells and sperm production in adult rats. Reprod. Nutr. Dev., 46: 157-166.

De Paula, T.A.R., D.S. Costa and S.L.P. da Matta, 2002. [Quantitative histologic evaluation of adult caybara (*Hydrochoerus hydrochaeris*) adultas]. Bio. Sci. J., 18: 121-136.

De Souza, P.C., T.A.R. de Paula, A.J. Natali, S.L.P. da Matta, D.S. Costa, C.C. Fonseca and P. Sarti, 2005. Efeito do exercicio cronico voluntario e do sedentarismo, com e sem o uso do esteroide anabolico nandrolona, sobre os componentes do parenquima testicular de ratos adultos. Revista Ceres, 53: 305-316.

Eilts, B.E., D.B. Williams and E.B. Moser, 1993. Ultrasonic measurement of canine testes. Theriogenology, 40: 819-828.

Fouquet, J.P., N. Meusy-Dessolle and D.C. Dang, 1984. Relationships between leydig cell morphometry and plasma testosterone during postnatal development of the monkey, *Macaca fascicularis*. Reprod. Nutr. Dev., 24: 281-296.

Franca, L.R. and C.L. Godinho, 2003. Testis morphometry, seminiferous epithelium cycle length and daily sperm production in domestic cats (*Felis catus*). Biol. Reprod., 68: 1554-1561.

Franca, L.R. and L.D. Russell, 1998. The Testis of Domestic Animals. In: Male Reproduction: A Multidisciplinary Overview, Regadera, J. and F. Martinez-Garcia (Eds.). Churchill Livingstone, Madrid, Spain, pp: 197-219.

Gundersen, H.J.G., T.F. Bendtsen, L. Korbo, N. Marcussen and A. Moller *et al*., 1988. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS., 96: 379-394.

Hikim, A.P.S., A.G. Amador, A. Bartke and L.D. Russell, 1989. Structure/function relationships in active and inactive hamster leydig cells: A correlative morphometric and endocrine study. Endocrinology, 125: 1844-1856.

Karaman, M.I., C. Kaya, T. Caskurlu, S. Guney, E. Ergenekon, 2005. Measurement of pediatric testicular volume with Prader orchidometer: Comparison of different hands. Pediatr. Surg. Int., 21: 517-520.

Kenagy, G.J. and S.C. Trombulak, 1986. Size and function of mammalian testes in relation to body size. J. Mammal., 67: 1-22.

Leal, M.C. and L.R. Franca, 2009. Slow increase of Sertoli cell efficiency and daily sperm production causes delayed establishment of full sexual maturity in the rodent *Chinchilla lanigera*. Theriogenology, 71: 509-518.

Love, C.C., M.C. Garcia, F.R. Riera and R.M. Kenney, 1991. Evaluation of measures taken by ultrasonography and caliper to estimate testicular volume and predict daily sperm output in the stallion. J. Reprod. Fertil. Suppl., 44: 99-105.

Mandarim-de-Lacerda, C.A., 2003. Stereological tools in biomedical research. Acad. Bras Cienc., 75: 469-486.

Matsinos, Y.G. and E. Papadopoulou, 2004. Investigating the viability of squirrel populations; A modeling approach for the Island of Lesvos, Greece. Nat. Resour. Mod., 17: 423-444.

Mehranjani, M.S., A. Noorafshan, H.R. Momeni, M.H. Abnos, M. Mahmoodi, M. Anvari and S.M. Hoseini, 2009. Stereological study of the effects of vitamin E on testis structure in rats treated with para-nonylphenol. Asian J. Androl., 11: 508-516.

Mori, H. and A.K. Christensen, 1980. Morphometric analysis of leydig cells in the normal rat testis. J. Cell Biol., 84: 340-354.

Nistal, M., R. Paniagua, J. Regadera, L. Santamaria and P. Amat, 1986. A quantitative morphological study of human Leydig cells from birth to adulthood. Cell Tissue Res., 246: 229-236.

Noorafshan, A., S. Karbalai doust, A. Valizadeh and E. Aliabadi, 2011. Ameliorative effects of curcumin on the structural parameters of seminiferous tubules and Leydig cells in metronidazole-treated mice: A stereological approach. Exp. toxicol. pathol., 63: 627-633.

Nyengaard, J.R., 1999. Stereologic methods and their application in kidney research. J. Am. Soc. Nephrol., 10: 1100-1123.

Rocha, D.C., L. Debeljuk and L.R. Franca, 1999. Exposure to constant light during testis development increases daily sperm production in adult Wistar rats. Tissue Cell, 31: 372-379.

Roosen-Runge, E.C., 1977. The process of spermatogenesis in animals. Cambridge University Press, New York, ISBN-13: 9780521212335, pp: 214.

Russell, L.D., 1996. Mammalian Leydig Cell Structure. In: The Leydig Cell, Payne, A.H., M.P. Hardy and L.D. Russell (Eds.). Cache River Press, Vienna, Austria, ISBN: 9780962742279, pp: 43-96.

Russell, L.D., H.P. Ren, I.S. Hikim, W. Schulze and A.P.S. Hikim, 1990a. A comparative study in twelve mammalian species of volume densities, volumes and numerical densities of selected testis components, emphasizing those related to the Sertoli cell. Am. J. Anat., 188: 21-30.

Russell, L.D., R.A. Ettlin, A.P. Sinha Hikim and E.D. Clegg, 1990b. Histological and Histopathological Evaluation of the Testis. Cache River Press, Clearwater, Pages: 286.

Sakamoto, H., K. Saito, M. Oohta, K. Inoue, Y. Ogawa and H. Yoshida, 2007. Testicular volume measurement: Comparison of ultrasonography, orchidometry and water displacement. Urology, 69: 152-157.

Schiff, J.D., P.S. Li and M. Goldstein, 2004. Correlation of ultrasonographic and orchidometer measurements of testis volume in adults. BJU Int., 93: 1015-1017.

Segatelli, T.M., L.R. Franca, P.F.F. Pinheiro, C.C.D. Alemida and M. Martinez *et al*., 2004. Spermatogenic cycle length and spermatogenic efficiency in the gerbil (*Meriones unguiculatus*). J. Andrology, 25: 872-880.

Setchell, B.P., S. Maddocks and D.E. Brooks, 1994. Anatomy, Vasculature, Innervations and Fluids of the Male Reproductive Tract. In: The Physiology of Reproduction, Knobil, E. and J.D. Neill (Eds.). Raven Press Ltd., New York, pp: 1063-1175.

Silva, M.A.J. and J. Merzel, 2001. Stereological determination of the volume of the rat hemimandible tissues. Anat. Rec., 263: 255-259.

Wing, T.Y. and A.K. Christensen, 1982. Morphometric studies on rat seminiferous tubules. Am. J. Anat., 165: 13-25.