—Research Note—

Morphological Features of The Seminiferous Epithelium in Cat (*Felis catus*, L. 1758) Testes

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Abstract. The aim of the present study was to assess the changes in the germinal epithelium in cats of different ages. Routine histological staining was applied to perform morphological and stereological examinations. The animals were divided into five groups according to age: under 8 months (n=28), 8–12 months (n=30), 12–36 months (n=33), 3–6 years (n=14) and older than 6 years (n=13). The appearance of the gonads of the males in the first group varied the most. The seminiferous tubules of the youngest cats consisted of a monolayer of supporting cells and a few spermatogonia. No tubular lumina were present, and the diameters of the seminiferous tubules reached 132.5 μm . We noted the typical arrangement of gametogenic cells with a tubule diameter of 191.83 μm in the second group. We observed multilayer germinal epithelia with the most significant production of gametes and a seminiferous tubule diameter of 202.61 μm in the third group. The diameters of the seminiferous tubules of the fourth and fifth groups were 193.38 μm and 191.84 μm , respectively. The obtained data revealed that the most intensive morphological diversification of the seminiferous epithelium in cats occurs at about 7–8 months of age. The diameters of seminiferous tubules were highest in the third group of cats, and the activity of spermatogenesis of this group, expressed as the number of sperm per 10 mm², was also the most distinctive. The spermatogenesis process was most evident in cats between 12 and 36 months of age, which was also when the sperm concentration was highest per estimated surface.

Key words: Cat, Seminiferous epithelium, Spermatogenesis, Testis development

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The current description of postnatal development of seminiferous tubules in the cat is rather poor and mainly concerns morphological changes in the gonads of male felines younger than 12 months [1]. However, histological examination of older cats has revealed the degenerative changes in their testes, but only by taking into account the appearance of the connective tissue [2]. In human andrology, the stereological method has been used

to assess the number of different cells in the epithelium germinativum [3]. In human testes, the numbers of spermatogonia, primary and secondary spermatocytes and spermatids and spermatozoa are usually examined after hematoxylin/eosin staining [3]. Accurate assessment of testis morphology, sperm composition and the changes taking place in the male gonads is crucial in humans and other species, since this determines their future reproductive ability and enables them to be sperm donors in facilitated reproduction. There is plenty of information available concerning

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human [4–6], bull [7–9], boar [10] and stallion [11] germinal epithelium development. Gonad development takes place during the prenatal period. In the postnatal period, neurohormonal, morphological changes result in the testes assuming features that allow them to fulfill their two main activities. The most important activity of the testes is production of male germ cells. The second activity of the testes is production and release of hormones [12]. Spermatogenesis in seminiferous tubules is largely dependent on normal neurohormonal regulation between the hypothalamus, pituitary gland and testes [8, 13–15]. One or two generations of spermatogonia, spermatocytes and spermatids can normally be seen in the seminiferous epithelia of mature males. The development and differentiation of these epithelium cells has been observed to be a synchronic process during spermatogenesis. In reference to the above, no complete description of feline gonad development including spermatogenesis activity is currently available. Moreover, experiments related to the morphological changes in older cat are normally based on the features of the seminiferous tubules during histological examination [14]; however, no data is available concerning age-dependent sperm production in testes. The present study investigated and assessed the morphological development of male gonads depending on age and age-related changes in spermatogenesis activity and the diameters of the seminiferous tubules.

Materials and Methods

The materials for the examination consisted of 118 pairs of testes with epididymes from healthy European cats of different ages (between 4 months to 10 years; Table 1). The animals were external patients of the Department and Clinic of Reproduction, Ruminant Diseases and Animal Health Protection, Agricultural University of Wrocław, from an animal shelter in Łódź and a private veterinary clinic in Łapy, Poland. Testes with epididymes were collected from healthy cats after routine neutering at the owner's request.

The testes were fixed in 7 % formaldehyde neutralized CaCO_3 for 48 to 72 h. The tissue was embedded in paraffin and cut into 6- μm thick sections using a rotation microtome. The slides were stained by routine hematoxylin and eosin staining.

Fifty cross-sections of seminiferous tubules that were round or nearly round were chosen randomly for each testis, and their diameters were measured using a Nikon Microphot-FXA (Nikon Corporation, Tokyo, Japan) equipped with the Olympus Micro Image software. Furthermore, 50 randomly selected cross-section were histologically evaluated using a light microscope with an EFM-16X Filar Micrometer Eyepiece (LOMO, St. Petersburg, Russia). A quantitative investigation of spermatogenesis was then conducted. For this purpose, the total number of round to elongated spermatids and spermatozoa in a area of 10 mm² was evaluated.

All analyses were performed using repeated measures ANOVA tests followed by Newman-Keuls Multiple Comparison Tests (GraphPAD PRISM version 4; GraphPad Software, San Diego, CA, USA; $P < 0.05$ was considered significant). Differences between groups were analyzed using T-Student test (GraphPAD PRISM version 4). The obtained data is presented in a table as means $\pm$ standard deviation. Values of $P < 0.05$ were considered significant.

Results

The morphometric (the diameter of the seminiferous tubule depending on age) and stereologic (number of sperm and spermatides per 10 mm²) data are presented in Table 1.

The germinal epithelia of the testes of the group I animals (<8 months) were composed of one layer of cells, and the Sertoli cells (supporting cells) were very visible. A few spermatogonia were observed on the basement membrane. In the cats neutered at four months of age, the lumen of the seminiferous tubule was not present (Fig. 1a). Changes preceding maturation, such as the presence of mucus in the tubule lumen, were observed (Fig. 1b). The regular lumen and stratified epithelium were observed in cross-sections of seminiferous tubules at seventh-eighth months of age. Spermatogenesis was only observed in 12 animals in group I. Group I consisted of 28 animals: 2 cats aged 4 months (spermatogenesis not observed), 3 cats aged 5 months (spermatogenesis not observed), 4 cats aged 6 months (spermatogenesis not observed), 10 cats aged 7 months (spermatogenesis was observed in 3 cases) and 9 cats aged 8 months (spermatogenesis was observed).

Table 1. Data obtained from histological examination of cat testes (diameters of seminiferous tubules and total numbers of spermatids and spermatozoa in areas of 10 mm²)

n=118	Diameter of seminiferous tubules (μm)		Means for both testes	Total number of spermatids and spermatozoa in a area of 10 mm ²		Means for both testes
	Left testis	Right testis		Left testis	Right testis	
I (n=28) < 8 months of age	128.58 ^A ± 19.17	135.53 ^A ± 22.88	132.05	415.20 ^A ± 6.46	750.80 ^{A***} ± 5.65	583.00
II (n=30) 8–12 months	189.47 ^B ± 15.07	194.20 ^{BC} ± 13.17	191.83	852.08 ^B ± 59.6 ^a	890.00 ^{B*} ± 56.87	871.00
III (n=33) 1–3 years old	207.39 ^C ± 5.78	197.83 ^{B***} ± 11.69	202.61	947.96 ^C ± 64.50	880.96 ^{B***} ± 50.46	914.44
IV (n=14) 3–6 years old	199.51 ^D ± 15.93	187.26 ^C ± 17.59	193.38	723.44 ^D ± 16.43	719.76 ^C ± 25.19	721.60
V (n=13) > 6 years	192.34 ^B ± 5.37	191.34 ^{BC} ± 5.37	191.84	745.60 ^D ± 46.39	733.84 ^A ± 25.19	739.70

Different letters indicate significance differences between groups (in column); $P < 0.05$ was considered significant. Asterisks indicate differences between the left and right testis (in row); * $P < 0.05$ and *** $P < 0.001$ were considered significant.

The typical arrangement of the gametogenic cells was present in the group II animals (8–12 months), with the spermatogonia present in the nearest basement membrane. The tubular lumens of the primary and secondary spermatocytes were visible. Spermatozoa were observed, and elongated and round spermatids were present below them. All cells belonging to the spermatogenic epithelium were connected to supporting cells. The number of spermatids and sperm was 871.00 per 10 mm², and the diameter of the tubule was 191.83 μm .

The germinal epithelia of the group III animals (12–36 months) were multilayered and possessed the most significant production of gametes (Fig. 1c). We observed 914.44 sperm and spermatids per 10 mm², in and the diameter of the seminiferous tubule reached 202.61 μm .

The diameters of the seminiferous tubules in groups IV and V (3–6 years and >6 years old, respectively) were 193.38 and 191.84 μm , respectively, and the total numbers of spermatids and spermatozoa in a area of 10 mm² were 721.60 and 739.70 per 10 mm², respectively.

Comparison of the diameters of the seminiferous tubules in both the left and right testes for all the groups revealed that the most important statistical differences ($P < 0.05$) were observed between the first group and all the other groups. There was no statistical difference in diameter of the left testis between groups II and V ($P < 0.05$). The diameters of

the left testes of group II, III and IV were statistically different ($P < 0.05$; Table 1). The diameters of the right testes in group I were statistically different from those of groups II, III, IV and V ($P < 0.05$). The diameters of the right testes in groups II and V were not statistically different ($P > 0.05$). The diameters of the right testes in groups III and IV were significantly different ($P < 0.05$). No differences were observed between the diameters of the left and right testes in groups I, II, IV and V ($P > 0.05$). The diameters of the left and right testes of group III differed significantly ($P < 0.001$).

The total number of spermatids and spermatozoa in a area of 10 mm² in the left testis did not differ statistically ($P < 0.05$) between groups IV and V. However, the total number of spermatids and spermatozoa in a area of 10 mm² in the left testis of group I was statistically different ($P < 0.05$) compared with the other groups. There was a statistically significant difference ($P < 0.05$) in the total number of spermatids and spermatozoa in a area of 10 mm² in the left testis between groups II and III, II and IV, II and V, III and IV and III and V. No differences in the total number of spermatids and spermatozoa in a area of 10 mm² in the right testis were observed ($P > 0.05$) between groups I and V and II and III. However, there was a statistically significant difference ($P < 0.05$) for group I compared with groups II, III, IV ($P < 0.05$) and for group II compared with groups IV, II and V ($P < 0.05$).

Fig. 1. The cross-sections of seminiferous tubules from cats at different ages. The lumens of seminiferous tubules were not observed in 4–5-month-old cats (a). Lumens were observed in the testes of 6–7-month-old cats (b). The seminiferous tubules of cats aged 1–3 years old were characterized by the facts that they possessed the most regular lumens, the largest diameters and multilayer epithelia.

increase in fluid in the central compartment of the tubule produced by supporting cells as a result of hormonal stimulation [17, 18]. We demonstrated that the diameter of the right testis did not differ statistically ($P>0.05$) among groups II, III and V. This suggests that cats mature between 8–12 months. However, there was a significant difference in diameter of the left testis between group II and groups I, III and IV ($P<0.05$); there was no significant difference between groups II and V ($P>0.05$). The result indicates that the tubule diameter may differ during lifetime. One of the reason may for this be different fluid production by Sertoli cells [17, 18].

Kirkpatrick *et al.* [18] demonstrated that the germinal epithelium begins to produce sperm at 8 months of age and that spermatides can be observed in the lumen of seminiferous tubules in animals above 8 months of age, irrespective of the season. However, our study pointed to the period between 8 to 12 months of age being a rather temporary stage in preceding adolescence. This is in agreement with Elcock and Schoning's [2] study, which is based on the degenerative changes appearing in cat testes at different ages.

In group III (12–36 months), the increase in the diameter of the seminiferous tubule reached 202.61 μm , and the differences between the diameters of the left testes in all groups were statistically significant ($P<0.05$). The diameters of the right testes of the group III cats (12–36 months) were statistically different ($P<0.05$) compared with groups I and IV. A statistically significant difference ($P<0.001$) was observed between the diameters of the left and right testes in group III. The highest number of spermatides and sperm were observed in this group compared with the other groups. We attempted to investigate whether season changes in the gametogenic epithelium of domestic cats, such as epithelium involution, could be observed. Changes in testicular size and weight and appearance of the seminiferous epithelium have been observed in some animals with seasonal breeding activity, such as raccoon dogs [19], bank voles [20] and silver foxes [21]. In these species, the diameters of the seminiferous tubules are lower during the non-breeding season and spermatogenesis changes seasonally. All types of spermatogenic cells may be found during the breeding season [19]. We did not notice any significant changes in the appearance of spermatogenic cells in our examinations of domes-

tic cat testes. The photoperiod does not affect animals kept at home in unchanging light conditions. A small amount of interstitial connective tissue was present.

The data concerning the diameters of the seminiferous tubules in groups IV (3–6 years) and V (>6 years) demonstrate that there was a statistically significant difference between group IV compared with the others groups in relation to the left testis ($P < 0.05$) and between group IV compared with group I and III in relation to the right testis. Moreover, the activity of spermatogenesis diminished in groups IV and V and reached 721.60 and 739.70 per 10 mm^2 , respectively; these were statistically different ($P < 0.05$) compared with the groups II and III left and right testes. The differences between the group IV and V left and right testes were not statistically important ($P > 0.05$). However, the number of sperm/spermatids per area is a rather indirect index of sperm producing activity, and the senile changes in the testes may be the results of the thickness of adventitia and the number of interstitial cells increasing with age [2]. The decrease in mature germ cells could be the result of a diminished number of spermatogonia in the older cats; however, despite an increase in interstitial cell number, Elcock and Schoning did not observe a decrease in tubule diameter [2].

No differences in the tubule diameters between left and right testes or in total numbers of spermatids and spermatozoa in a area of 10 mm^2 were observed ($P > 0.05$) in groups I, IV and V. However, a significant difference ($P < 0.001$) was observed between the diameters of the left and right testes of group III. Statistically important differences ($P < 0.001$) were observed for germ cell number in the left and right testes of the youngest cats (<8 months) and in group III (12–36 months). We do not know the reason for these results, especially taking into account such a great difference in group I (the right testis contained almost two times the total number of spermatids and spermatozoa in a area of 10 mm^2 as the left testis). Taking this data

into account, it seems reasonable to collect cells for assisted reproductive techniques or cell culture from cats older than eight months of age because of the functionally and morphologically immature testes of younger animals. This study provides a baseline for future study concerning local immune and endocrine regulation in cat gonads. The potential development of different cells isolated from feline testes for cell culture plays an important role in *in vitro* study. The number of sperm/spermatids per area gives indirect information about spermatogonia activity at different ages and helps with selection of the best group of animals for *in vitro* experiments.

In conclusion, development of the male gonads of pet cats occurs before 8 months of age, and this prepares the testes to fulfill their endocrine and external secretion functions. Regular spermatogenic activity, which occurs when the cat reaches the puberty, begins after 8 to 12 months of age. The most significant epithelial divisions occur in males between 1 to 3 years of age.

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References

1. **Sánchez B, Pizarro M, Garcia P, Flores JM.** Postnatal development of seminiferous tubules in the cat. *J Reprod Fertil* 1993; 47 (Suppl): 343–348.
2. **Elcock LH, Schoning P.** Age-related changes in the cat testis and epididymis. *Am J Vet Res* 1984; 45: 2380–2384.
3. **Raleigh D, O'Donnell L, Southwick GJ, Kretser DM, McLachlan RI.** Stereological analysis of the

- human testis after vasectomy indicates impairment of spermatogenic efficiency with increasing obstructive interval. *Fertil Steril* 2004; 81: 1595–1603.
4. **Lamont MA, Faed MJ, Baxby K.** Comparative studies of spermatogenesis in fertile and subfertile men. *J Clin Pathol* 1981; 34: 145–150.
 5. **Syed V, Hecht NB.** Disruption of germ cell-Sertoli cell interactions leads to spermatogenic defects. *Mol Cell Endocrin* 2002; 186: 155–157.
 6. **Schulze W.** Evidence of a wave of spermatogenesis in human testis. *Andrologia* 1982; 14: 200–207.
 7. **Amman RP.** Endocrine changes associated with onset of spermatogenesis in Holstein bulls. *J Dairy Sci* 1983; 66: 2606–2622.
 8. **Moura AA, Erickson BH.** Testicular development, histology, and hormone profiles in three yearling angus bulls with spermatogenic arrest. *Theriogenology* 2001; 55: 1469–1488.
 9. **Parks JE, Lee DR, Huang S, Kaproth MT.** Prospects for spermatogenesis *in vitro*. *Theriogenology* 2003; 59: 73–86.
 10. **Garcia-Gill N, Pinart E, Sancho S, Badia E, Bassols J, Kádár E, Briz M, Bonet S.** The cycle of the seminiferous epithelium in Landrace boars. *Anim Reprod Sci* 2002; 73: 211–225.
 11. **Johnson L, Blanchard TL, Varner DD, Scrutchfield WL.** Factors affecting spermatogenesis in the stallion. *Theriogenology* 1997; 48: 1199–1216.
 12. **Leme DP, Papa FO, Trinca LA.** Assessment of seasonal influence of equine spermatogenic cells and Sertoli cells by testicular fine needle aspiration cytology. *Theriogenology* 2002; 58: 269–272.
 13. **Johnson L, Blanchard TL, Varner DD, Scrutchfield WL.** Factors affecting spermatogenesis in the stallion. *Theriogenology*, 1997; 48, 1199–1216.
 14. **McLachlan RI, Wreford NG, O'Donnell L, Kretser DM, Robertson DE.** The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH. *J Endocrinol* 1996; 148: 1–9.
 15. **Moura AA, Erickson BH.** Testicular development, histology, and hormone profiles in three yearling angus bulls with spermatogenic arrest. *Theriogenology* 2001; 55: 1469–1488.
 16. **Parks JE, Lee DR, Huang S, Kaproth MT.** Prospects for spermatogenesis *in vitro*. *Theriogenology* 2003; 59: 73–86.
 17. **França LR, Godinho CL.** Testis morphometry, seminiferous epithelium cycle length and daily sperm production in domestic cats (*Felis catus*). *Biol Reprod* 2003; 68: 1554–1561.
 18. **Russel LD, Bartke A, Goh JC.** Postnatal development of the Sertoli cell barrier, tubular lumen and cytoskeleton of Sertoli and myoid cells in the rat and their relationship to tubular fluid secretion and flow. *Am J Anat* 1989; 14: 179–189.
 19. **Kirkpatrick JF.** Seasonal testosterone levels, testosterone clearance and testicular weights in male domestic cats. *Can J Zool* 1985; 63: 1285–1287.
 20. **Weng Q, Medan MS, Xu M, Tsubota T, Watanabe G, Taya K.** Seasonal changes in immunolocalisation of inhibin/activin subunits and testicular activity in wild male raccoon dogs (*Nyctereutes procynoides*). *J Reprod Dev* 2006; 52: 503–510.
 21. **Takha KM, Kaipia A, Toppari J, Takha S, Tuuri T, Tuohimaa P.** Hormonal and photoperiodic modulation of testicular mRNAs coding for inhibin/activin subunits and follistatin in *Clethrionomys glareolus*, Schreber. *J Exp Zool* 1998; 281: 336–345.
 22. **Andresen Berg K, Wiger R, Dahl E, Torp T, Farstad W, Krogenaes A, McNeilly AS, Paulenz H, Ropstad E.** Seasonal changes in spermatogenic activity and in plasma levels of FSH, LH and testosterone, and the effect of immunization against inhibin in the male silver fox (*Vulpes vulpes*). *J Androl* 2001; 24: 284–294.