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Correlation Between Testis Weight, Testis Volume and Total Sperm Count in the Indonesian Domestic Cats *(Felis catus)*

KORELASI ANTARA BOBOT TESTIS, VOLUME TESTIS, DAN JUMLAH SPERMA TOTAL PADA KUCING DOMESTIK INDONESIA

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ABSTRACT

Information regarding the domestic cat as a model animal in the biomedical research has not been widely carried out. This study was aimed to see the correlation between testicular weight, testicular volume and total sperm count between the left testicle, right testicle and both testes. In addition, this study was aimed to examine the effect of testicular weight and volume on quantitative histomorphometry of the testes in domestic cats in Indonesia. The results showed that there was no difference between the left and right testes. Testes weight has a positive and highly significant correlation with testes volume and total sperm count, both in the left, right, and both testes. The heavier the weight of the testes, the wider the diameter of the seminiferous tubules, the wider the lumen, and the taller the tubular epithelium. It can be concluded that the increase in testicular weight is in line with the increase in cells in the seminiferous tubules.

Keywords: correlation; domestic cat; quantitative histomorphometry; sperm; testes

ABSTRAK

Informasi mengenai kucing domestik sebagai hewan model dalam bidang biomedis belum begitu banyak dilakukan. Studi ini bertujuan melihat korelasi antara bobot testis, volume testis, jumlah sperma total antara testis kiri, testis kanan dan kedua testis. Selain itu, penelitian ini bertujuan melihat pengaruh bobot dan volume testis terhadap histomorfometri kuantitatif testis pada kucing domestik di Indonesia. Hasil penelitian menunjukkan bahwa tidak ada pebedaan antara testis kiri dan kanan. Bobot testis berkorelasi positif dan sangat signifikan dengan volume testis dan jumlah seperma total, baik pada testis kiri, kanan, maupun kedua testis. Semakin berat bobot testis, maka semakin lebar diameter tubulus seminiferus, semakin lebar lumen dan semakin tinggi epitel tubulus. Dapat disimpulkan bahwa peningkatan bobot testis sejalan dengan peningkatan sel dalam tubulus seminiferus.

Kata kunci: histomorfometri kuantitatif; korelasi; kucing domestic; sperma[testis

INTRODUCTION

The domestic cats are animals that are valued as pets, entertainers and companions by humans. However, the useful cats as an animal research model still needs to be improved because it still lags behind other domestic animals, like mice, rats, rabbits, pigs, sheep, goats and monkeys. The use of domestic cats as an animal model is valuable for study because it represents most wild animals, especially in wild Felidae, which are listed by the International Union for Conservation of Nature (IUCN) Red List (Nowell, 2002) as critically endangered, endangered, vulnerable and threatened.

The estrous cycle in the female domestic cats in the country with four seasons is seasonally polyestrous and changes in day length (the number of light hours increase), regulating seasonality (Tsutsui *et al*., 2004). The age of queens puberty is known to be at 8-10 months (Povey, 1978). In contrast, the male cats in the four seasons country are non-seasonal breeders (Spindler and Wildt, 1999), but Axnér and Forsberg (2007) and Blottner and Jewgenow (2007) show that spermatogenesis is affected by season.

The study on domestic cats spermin the country with four seasons has been reported, such as testicular weight, testicular histology, hormonal level and its correlation with sperm (Müller *et al.*, 2012; Villaverde *et al.*, 2014; Braun *et al.*, 2018). Unfortunately, the study about domestic cat sperm in Indonesia which is a tropical country with rainy and dry seasons is need to be explored more. The study about the correlation of testicular weight, testicular volume, total sperm counts and testicular quantitative histomorphometric in Indonesian domestic cats has no further investigation. In this study, we randomized utilized castrated testes which are patients at the veterinary clinic in Indonesia for several months. We investigated the correlation of testicular weight, testicular volume and total sperm counts in Indonesian male domestic cats. We examine the difference between left and right testes. We also examine the histology of the testis in different groups of weight. We calculate the tubular diameter, seminiferous epithelium height, tubular lumen diameter and ratio seminiferous epithelium height/tubular diameter. This study is necessary for developing and implementing assisted reproductive techniques, like artificial insemination, gamete preservation, *in vitro* fertilization (IVF), and many others, especially for other reseach in Indonesia.

RESEARCH METHODS

Animals

Thirty-five sexually mature males aged between 6-48 months domestic cats were used in this study. The cats were obtained from private owners and were healthy patients at several veterinary clinics in Bogor Regency that underwent castration surgery from November 2021 until July 2022. The testicles from this castration were used as research material. All surgical procedures were performed by registered veterinarians and followed approved guidelines. Animal care was carried out at the clinic under applicable veterinary ethics. All the experiments in this research and the animal manipulation procedures were approved by the Ethics Committee of the School of Veterinary Medicine and Biomedical Science, IPB University (Ethical approval number: 006/KEH/SKE/II/2022).

Transport Media

The castrated testes were placed in physiological Natrium Chloride (NaCl) with 5 mg/mL gentamicin (Sigma, #G1397). Samples were transported at room temperature to the Laboratory of Embryology, School of Veterinary Medicine and Biomedical Science, IPB University. The preparation samples until used for the experiment were done in less than five hours. Samples were washed using phosphate buffer saline (PBS) (Merck, #P4417) with 5 mg/mL gentamicin (Merck, #G1397).

Testicular Weight, Testicular Volumeand Total Sperm Count

The washed testes are separated between the epididymis, ductus deferens and the other parts. The testes were then measured using a caliper (Facher Darex) to determine the volume of the testes. On the other hand, rhe testes were weighted by using an analytical balance (PJ Precisa Junior 60A). Measurement of testes volume was done by using the ellipsoid formula in cats, which was described previously by Howard *et al.* (1990).

Spermatozoa Collection and Total Sperm Count

This procedure was reported by Mardatillah *et al.* (2020) with some modifications. The cauda epididymis was taken from another part and put in a 35 mm dish (Biologyx) containing 1 mL of PBS with

1% of fetal bovine serum (Sigma, # F2442) and 5 mg/mL gentamicin (Sigma, #G1397). The cauda epididymis was squeezed so the spermatozoa could release from the cauda epididymis into the medium. Spermatozoa that came out were transferred in a 15 mL tube (Biologyx) to calculate the total number of spermatozoa. Spermatozoa were put in neutral buffer formalin (NBF) 10% (Medici) with a ratio 1:49 of spermatozoa:NBF. Spermatozoa were then assessed using a Neubauer chamber (Marienfeld) using a light microscope (Olympus CH20) with a magnification of 100 times.

Quantitative Histomorphometric of Testes

Based on the result of testicular morphometry, this study was divided into three groups based on the total weight of testis of each individual. The first group (A) was testis in weight 1.0-1.9 g, the second group (B) was testes in weight 2.0-2.9 g, and the last one (C) was in weight 3.0-3.9 g. The first, second and last groups consist of 14, 16 and 5 individuals in each group, respectively. The value of testis volume, testis weight and sperm count between groups was compared, such as average value, minimum value and maximum value. In addition, each group will be compared histologically.

The Histology of Testes

The testes were fixated using 4% of formaldehyde**.** After fixation, the testes were trimmed and then the tissues were processed using the paraffin embedding with a tissue embedding console (Tissue-Tek, SAKURA) and sectioned (5 µm) using a rotary microtome. The tissue sections were stained with hematoxylin-eosin (Kiernan, 2008). The slide histology was examined under a light microscope (Olympus BX 31) equipped with a CCD10 USB Camera. Morphometric parameters of 30 round or nearly round tubular profiles were chosen randomly and measured for each group of the study using ImageJ software (National Institutes of Health) at 100 times magnification (Fig. 1a, b, c). The following parameters were measured tubular diameter (µm), seminiferous epithelium height (µm), tubular lumen diameter (µm) and the ratio seminiferous epithelium height/tubular diameter were calculated.

Data Analysis

The data obtained from testicular weight, testicular volume and total sperm count were differentiated between the left testes and right testes using the t-student test. The data reported as p values <0.05 were considered significant. The correlation between testicular weight, testicular volume and total sperm count was tested by Pearson analysis. The data of quantitative histomorphometric testes from all groups were differentiated using one-way analysis of variance (ANOVA) test and Tukey's post-hoc tests ($p<0.05$). All the tests were carried out by using the Minitab 18 application.

RESULTS AND DISCUSSION

Testicular Weight, Testicular Volume and Total Sperm Count

The differences between testes weight, testes volume and total sperm count between left and right testes are shown in Table 1. The differences in the left testes weight (1.112 ± 0.441 g) and the right testes weight (1.125 ± 0.454 g) were not significantly different ($P<0.05$). There were no significantly different between the left and right testes volume (1024 ± 385 mm^3, 1034 ± 423 mm^3, respectively, $P<0.05$) and total sperm count between the left (19.8 ± 38.85 x 10^6) and the right (21.5 ± 49.68 x 10^6) testes ($P<0.05$).
The Pearson correlations between testes weight and testes volume and total sperm count in the right, left and both testes are shown in Tables 2, 3 and 4. Testes weight in the right, left and both testes were correlated positively with testes volume ($r=0.892$, $r=0.837$, $r=0.912$, respectively, $P<0.05$) and total sperm count ($r=0.355$, $r=0.441$, $r=0.478$, $P<0.05$). In addition, the testes volume and total sperm count in the left testes were significantly different ($r=0.375$, $r=0.478$, $r=0.492$, $P<0.05$).

Quantitative Histomorphometric of Testes

The mean of testis weight in the A, B, and C groups were 1.399±0.27 g, 2.66±0.39 g, and 3.61±0.13 g, respectively. There were significantly different between testes volume between group A (1442±419 mm^3), group B (2355±422 mm^3) and group C (3334±497 mm^3) but total sperm count in all groups were not significantly different (Table 5). Seminiferous epithelium height (µm), tubular lumen diameter (µm) and the ratio seminiferous epithelium height per tubular diameter (%) were shown in Table 5 and Figure 1.

The tubular diameter and seminiferous epithelium height of group A were lower and significantly different from groups B and C, but groups B and C were not different. Significantly

differences in lumen diameters were found between groups A and C, but group B was not different from the other groups. The last was the ratio of seminiferous epithelium height per tubular diameter. Group A had the lowest percentage of it and was significantly different between groups B and C.

Table 1. Testes weight, testes volume, and total sperm count in the domestic cats.

	Left Testes	Right Testes
Testes Weight (g)	1.112 ± 0.441[a]	1.125 ± 0.454[a]
Testes Volume (mm^3)	1024 ± 385[a]	1034 ± 423[a]
Total Sperm Count (10^6)	19.8 ± 38.85[a]	21.5 ± 49.68[a]

*Significant differences are indicated by different letters in the same row ($p<0.05$)

Table 2. Correlation testes weight, testes volume, and total sperm count of right testes in the domestic cats (*Pearson correlation coefficient* (r): *p*-value)

	Testes Weight	Testes Volume
Testes Volume	0.892 ; 0.000	
Total Sperm Count	0.355 ; 0.036	0.375 ; 0.026

Table 3. Correlation weight, volume, and total sperm count of left testes in the domestic cats (*Pearson correlation coefficient* (r): *p*-value)

	Testes Weight	Testes Volume
Testes Volume	0.837 ; 0.000	
Total Sperm Count	0.441 ; 0.008	0.478 ; 0.004

Table 4. Correlation testes weight, testes volume, and total sperm count of both testes in the domestic Cats (*Pearson correlation coefficient* (r): *p*-value)

	Testes Weight	Testes Volume
Testes Volume	0.912;0.000	
Total Sperm Count	0.478;0.004	0.492;0.003

Discussion

Spermatogenesis is process to differentiate spermatogonia into mature spermatozoa. This process occurs in the tubules seminiferous testes of male mammals. In subtropical countries, reproduction in the mammal is commonly seasonal variation as an adaptation to annual changes in the habitat. The regulation of pituitary and gonadal function is mainly influenced by photoperiodic signals (Lincoln, 1989). Seasonality in testis function ranges from cycles of highly active and ultimately arrested spermatogenesis to moderate fluctuation in sperm production throughout the year (Blottner and Jewgenow, 2007). Nevertheless, Indonesia has no four seasons like summer, fall/autumn, winter or spring. Indonesia gets sunlight over the year and the mammal reproduction cycle occurs at any time. In this study, the left testes and the right testes in the same individual have no difference in the testes weight, testes volume and total sperm count (Table 1). This study is the same as stated by Scott and Scott (1957) that there are no significant differences between the left and right testes. It indicates that both testes have good physiological and endocrine conditions to produce spermatozoa. It is frequently found in domestic cats that both testes could not reproduce spermatozoa due to cryptorchidism, which is a disorder of sexual development because the condition results from defective transmigration of one or both testes into the scrotum (Centerwall and Benirschke, 1975; Millis *et al.,* 1992; Yates *et al.*, 2003). Normally, testicular migration through the inguinal canal into the scrotum occurs before birth (Romagnoli and Schlafer, 2006). There is no side predilection (left or right) for the development of feline cryptorchidism (Richardson and Mullen, 1993). The prevalence of this evidence has been reported as 1-8.6% (Yates *et al.,* 2003).

In this study, the testicular weight, testicular volume, and total sperm count are correlated positively (Tables 2, 3 and 4). This research is in line with Bengal bucks (Sanjoy *et al.,* 2011), Murrah buffalo bulls (Pant *et al.,* 2003), and Brahman Bulls (Vásquez *et al.,* 2003). In common, the advantage of measuring testicular weight, testicular volume and total sperm count in mammals is to predict the fertility of the animals. This study in the domestic cats could be used as a model in breeding soundness assessment to select fertile wild felines in their natural habitat.

Table 5. Testis weight, testis volume, total sperm count, tubular diameter (µm), seminiferous epithelium height (µm), tubular lumen diameter (µm), and the ratio seminiferous epithelium height/tubular diameter (%) in the domestic cats.

	A	B	C
Testes Weight (g)	1.399±0.27[a]	2.66±0.39[b]	3.61±0.13[c]
Testes Volume (mm^3)	1442±419[a]	2355±497[b]	3334±365[c]
Total Sperm Count (10^6)	3.6±3.3[a]	65.4±98.1[a]	84.4±71.6[a]
Tubular Diameter (µm),	247.8±60.8[b]	289.3±72.1[a]	292.91±32.6[a]
Seminiferous Epithelium Height (µm)	68.15±20.5[b]	95.57±31.5[a]	106±22.1[a]
Tubular Lumen Diameter (µm)	78.32±21.93[b]	104.71±36.97[ab]	135.9±109.7[a]
Ratio Seminiferous Epithelium Height/Tubular Diameter (%)	27.95±7.1[b]	33.80±12.8[a]	36.45±5.6[a]

*Significant differences are indicated by different letters in the same row ($p<0.05$). A) First group (testis weight 1.0-1.9 gr); B) second group (testis weight 2.0-2.9 gr); C) Third group (testis weight 3.0-3.9 gr).

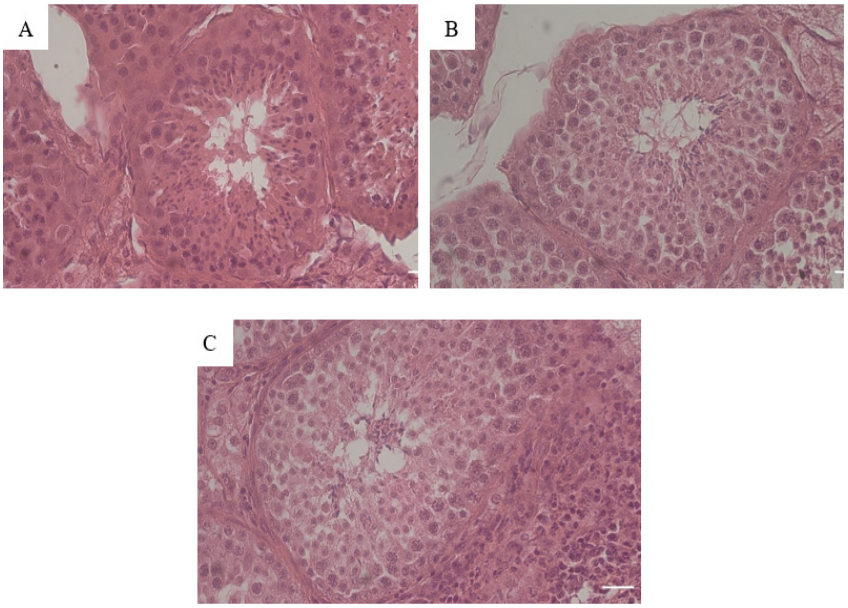

Figure 1. The seminiferous tubular histology of Indonesian domestic cats in 400 magnifcation. A) First group (testis weight 1.0-1.9 gr); B) second group (testis weight 2.0-2.9 gr); C) Third group (testis weight 3.0-3.9 gr). Scale bar: 25 µm.

In the quantitative histomorphometric experiment of testes, as expected, group C, which has the most enormous testicular weight, has the most considerable volume and is significantly different from all groups. Although total sperm count is not different from all groups, the bigger the testicular weight, the higher the seminiferous epithelium, as well as tubular diameter, seminiferous epithelium height, tubular lumen diameter and ratio seminiferous epithelium height/tubular diameter.

Based on the data of this study, the increase in the weight and volume of the testis is correlated with the tubular diameter and tubular seminiferous epithelium height, which is correlated with sexual maturity (Kutzler 2022). At birth, the seminiferous tubules consist of a monolayer of Sertoli cells with few spermatogonia and no tubular lumen present (Sánchez *et al.*, 1993; Siemieniuch and Wocławek-Potocka, 2007). At the age of 2-2.5 months, the tubular diameter starts to increase

steadily and the mean tubular diameter and the mean tubular seminiferous epithelium height in adult male cats are 223 μm and 81 μm, respectively (Franca and Godinho 2003).

In this study, the tubular diameter, seminiferous epithelium height, tubular lumen diameter (μm) and ratio seminiferous epithelium height/tubular diameter are correlated with total sperm count. Sperm production is significantly correlated with the age of the male cats. Based on the report pf Bohrer *et al.* (2015), the percentage of cats with sperm present in the testicular seminiferous tubule lumen increases over time from 0% at the age of 2–2,5 months old) and to100% at the age of 12–24 months old. The total number of germ cells and the number of round spermatids per seminiferous tubule cross-section at stage 1 of the cycle (Franca and Godinho, 2003)

In the seminiferous tubule, there are Sertoli cells that function in the spermatogenesis process to produce spermatozoa. The Sertoli cells support the development of the germ cells from spermatogonia to spermatocytes (Kutzler 2022). Moreover, clusters of Leydig cells in the interstitial tissue are the primary source of testosterone or androgens in males that play a crucial role in many vital physiological processes in males, i.e. spermatogenesis (Preston *et al.* 2012). Based on that evidence, the higher the tubular diameter the higher spermatogenic cells and Leydig cells that correlated with spermatogenesis to produce spermatozoa.

CONCLUSION

There are no significantly different in testes weight, testes volume, and total sperm count between the left and right testes. The testes weight is correlated positively with testes volume and total sperm count in the left, right, and both testes. The last, the heavier the testes weight, the bigger the testes volume, tubular diameter, tubular lumen diameter, seminiferous epithelium height, and the ratio of seminiferous epithelium height per tubular diameter.

SUGGESTION

The number of male domestic cats could be added and the sampling period can be done longer so that it can add data and represent how the season in Indonesia could influence Indonesian domestic cat spermatogenesis.

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