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Seminiferous Tubules Dimensions of Domestic Chicken (*Gallus Gallus Domesticus*) At Different Reproductive Stages

Musa Zakariah[1, 2 *], Agishi Geado[3], Ali Musa Wulgo[2], Esther Z. Musa[4], Josephine Joseph Dasa[4]

[1]Department of Veterinary Anatomy College of Veterinary Medicine, Federal University of Agriculture, P.M.B. 28, Zuru, Nigeria.

[2]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, P.M.B. 1069 University of Maiduguri, Maiduguri, Nigeria.

[3]Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, P.M.B. 28, Zuru, Nigeria.

[4]Department of Biology, College of Science, Federal University of Agriculture, P.M.B. 28, Zuru, Nigeria.

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Abstract: **Information on dimensions of seminiferous tubules of avian species is inadequate compared to mammalian species. The present study aimed to evaluate some parameters of seminiferous tubules of domestic chicken (*Gallus gallus domesticus*) as an avian species. A total of 30 birds were used for the study, comprising of 10 birds per each of the four reproductive cycles, namely pre-pubertal, pubertal, adult, and aged. The seminiferous tubule diameter of the aged group had the highest mean diameter (305.974 ± 26.24 ±se) even though there was no significant difference between the age groups (P>0.05). However, a significant difference (P<0.005) for the aged groups was observed for both the seminiferous tubule lumen diameter (116,273 ± 14.47 ±se) and the mean epithelium height (121.017 ± 8.89 ±se) than the pre-pubertal, pubertal, and adult age groups. The present study showed that spermatogenesis gradually declined in aged birds by presenting wider seminiferous tubule lumen which could be due to the high rate of germ cells apoptosis and fall in androgen levels, which are common indicators of aging as seen in both avian and mammalian species studied. The development of stereological research techniques yields quantitative data that is essential for creating associations between physiological and biochemical data.**

Keywords: **Domestic chicken; Reproductive stages; Seminiferous tubules.**

1. INTRODUCTION

Spermatogenesis in birds has been poorly evaluated, even though they constitute more than 50% of all the vertebrates (Aire, 2007). The efficiency of spermatogenesis generally, can be evaluated by several quantitative parameters. The measurements of seminiferous epithelium height and the tubular diameter are among the most important parameters in estimating the efficiency of spermatogenesis (Tripathi et al., 2015, Kalwar et al., 2020). Even though avian gonadal morphometry has been well investigated, but the most emphasis has been put on the endocrinological and physiological aspects of its reproductive biology. The few studies on the morphology of avian reproductive biology are mainly on the testicular structure, testicular weight, and germ cell differentiation (Artoni et al., 1999, Deviche et al., 2011, Aire et al., 2019). The seminiferous tubules of avian species are similar to mammals. Histologically, the tubule is lined with stratified epithelium comprising of different

types of germ cells that are nourished and supported by Sertoli cells (Deviche et al., 2011). The advent of stereological study methods provides quantitative information that is very crucial in establishing correlations between biochemical and physiological data (Bordbar et al., 2013). The most used parameter in evaluating seminiferous tubule is the mean tubular diameter. This is imperative because, seminiferous tubules diameter changes in testicular disorders (Wit et al., 2007). The study of testicular morphometry in animals allows the establishment of behavioral and physiological patterns that are crucial in understanding the aspects of the biology of reproduction of different species, this will make it viable to come up with protocols for assisted reproduction (Caldeira et al., 2010). In relation to the features of male reproductive biology, a strong correlation between sperm production and testicular weight has been reported (Kenagy and Trombulak, 1986). Also, the relative size of the testis can guide the biology of reproduction and the system of mating. Therefore, the size of the testis is directly proportional to reproductive behavior, since monogamous animals have a lower gonadosomatic index compared to species with polyandrous mating (Kenagy and Trombulak, 1986, Caldeira et al., 2010). The most copious portion of the testicular parenchyma is the seminiferous tubules. All quantitative parameters associated with seminiferous tubules evaluation, such as seminiferous epithelium thickness and tubular diameter have a strong correlation with spermatogenic activity (Franca and Russell, 1998). Measurement of seminiferous tubules is therefore the most reliable traditional approach employed as an indicator of spermatogenic activity in testicular function investigations (Mascarenhas, 2006). Morphometric studies on seminiferous tubules of mammalian species are well documented (Neves et al., 2002, Caldeira et al., 2010, França and Godinho, 2003, Kalwar et al., 2020). However, not much is done on the seminiferous tubules of avian species compared to mammals. The present study aimed to evaluate the seminiferous tubule diameter, seminiferous epithelium height, and seminiferous tubule lumen diameter in four reproductive cycles of the male domestic chicken.

2. MATERIALS AND METHODS

2.1. Animals used for the study

According to previously defined reproductive stages, 30 local domestic chickens were used in the study, with 10 birds in each of the three reproductive stages: pre-pubertal (2 months old), pubertal (4 months old), and adult (6 months old) (Zakariah et al., 2020, Ibrahim et al., 2022). The Animal Science Research Unit, College of Agriculture, Federal University of Agriculture, Zuru, Nigeria, is where the birds were obtained. The protocol was approved by the Federal University of Agriculture's College of Veterinary Science's Institutional Animal Ethics Committee (AEC) in Zuru, Nigeria (Approval number: FUAZ-AEC/2024-07).

2.2. Tissue samples for light microscopy

Tissue samples from both testes of each bird were collected following the opening of the thoracoabdominal cavity. The tissue samples were fixed for 5 days in 10% buffered formaldehyde and were processed conventionally for paraffin wax embedment, sectioned at 5 µm thick, and were stained with haematoxylin and eosin (H&E) for light microscopy.

2.3 Morphometrical analysis of seminiferous tubules.

The body and testicular weights of each bird in all the age groups was weighed using a digital precision balance (MII-300 digital precision weighing balance, Algen Scale Corporation^{R} Bohemia, NY). The seminiferous tubules diameter, seminiferous epithelium height, and the tubular lumen diameter were evaluated according to the previously described methods (Valença et al., 2013, Chiarini-Garcia et al., 2017). The seminiferous tubule and the tubular lumen diameters were measured across the major and minor axes, and the average taken. The 15 cross-sections of seminiferous tubules that were round or nearly round were randomly selected per bird and measured using a linear graticule micrometer, an image analyzer system (CellSens dimension software) tethered to an Olympus BX-63 microscope.

2.4 Statistical analysis

The morphometric parameters for testes, and seminiferous tubules for each age group were compared using a one-way analysis of variance (ANOVA). Age groups were used as independent variables. The body and testicular weights, the tubular diameter, seminiferous epithelium height, and the seminiferous tubule lumen diameter were used as the dependent variables. All analyses were performed using IBM SPSS version 26 software. A Tukey's Honest Significant Difference (HSD) Post hoc test was used to measure the extent of difference between groups at a 5% significant level ($p>0.005$). Data were presented as mean+ standard error (SE) and bar plots were generated using SPSS version 26.

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3. RESULTS

3.1 Seminiferous tubules parameters

The morphometric evaluation of the seminiferous tubules was performed to determine the mean difference between the age groups for the seminiferous tubule diameter, seminiferous tubule lumen diameter, and seminiferous tubule epithelium height (Table 2 and Figure 4). For the seminiferous tubule diameter, the aged group had the highest mean diameter (305.974 ± 26.24 ±se) even though there was no significant difference between the groups (P>0.05) (Table 2). However, there was a significant difference between the groups for both the seminiferous tubule lumen diameter (116,273 ± 14.47 ±se) and the mean epithelium height (121.017 ± 8.89 ±se) with the aged group being significantly higher (P<0.005) than pre-pubertal, pubertal, and adult age groups (Table 2). Following the post hoc test for multiple comparisons, there was no significant difference between all the groups compared for the seminiferous tubule diameter.

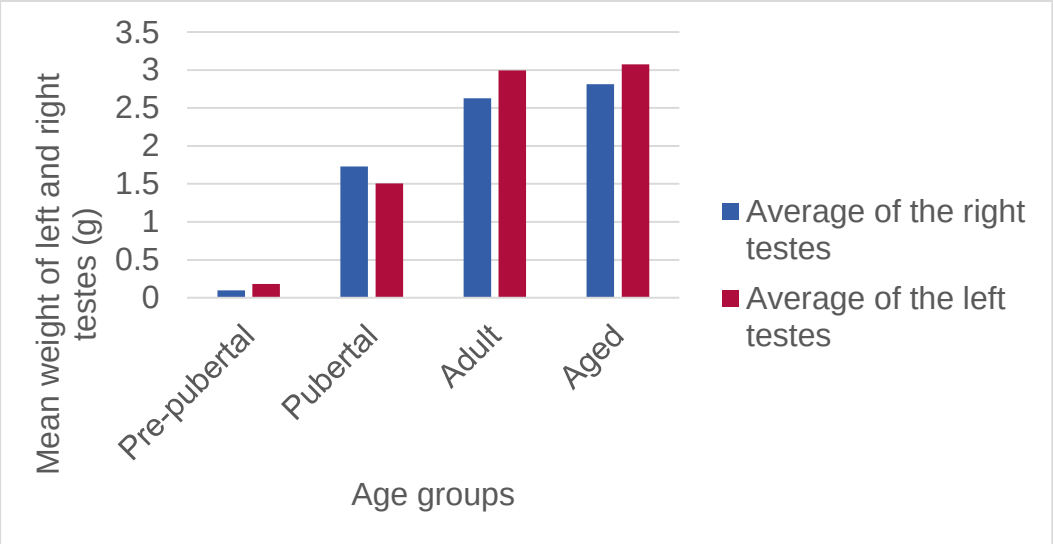

Figure 1. Bar charts showing the mean weight of the right and the left testes of all the age groups. There was a significant difference of mean testicular weight between the age groups (P<0.005), but no significant difference (P>0.005) between right and left testes.

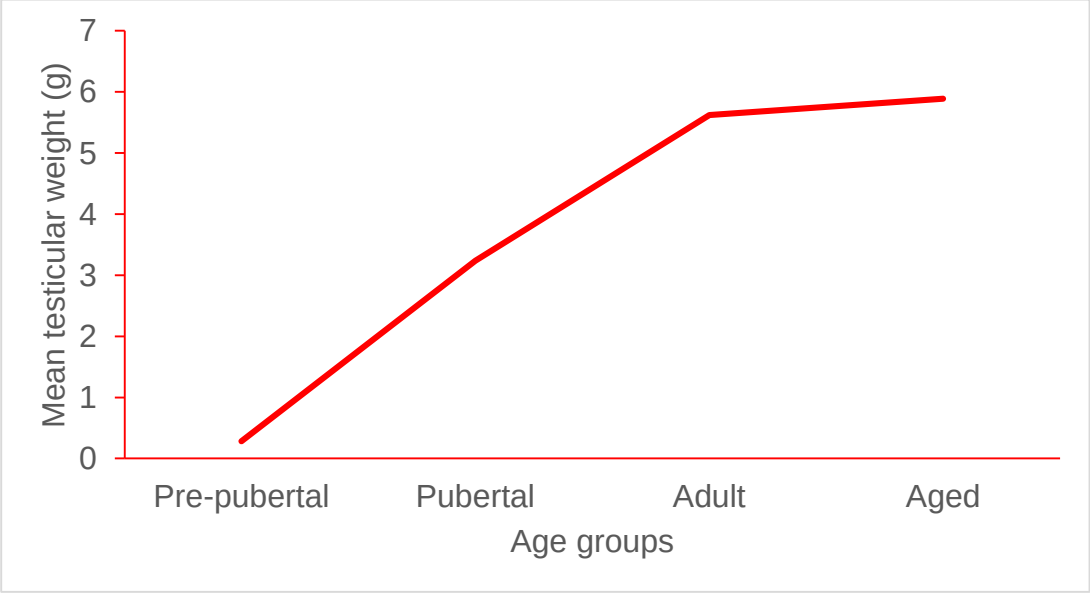

Figure 2. Graph showing the mean testicular weight of both the right and left testes of all the age groups. There was a significant difference of mean testicular weight between the age groups (P<0.005), sequentially from pre-pubertal to aged birds.

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Table 1. Showing the mean weight of right, left and both testicular weight.

Age group	weight (g) of left testes ±se	weight (g) of right testes ±se	Overall testicular weight (g) ±se	P-value
Pre-pubertal	0.182 ± 0.740	0.097 ± 0.025	0.280 ± 0.817	
Pubertal	1.507 ± 0.160	1.731 ± 0.193	3.230 ± 0.308	
Adult	2.995 ± 0.440	2.626 ± 0.193	5.621 ± 0.601	
Aged	3.075 ± 0.450	2.813 ± 0.231	5.889 ± 0.626	0.0001

Table 2. Showing the mean seminiferous tubule diameter, seminiferous tubule lumen diameter, and seminiferous epithelium height.

Age groups	Seminiferous tubule diameter (µm) ±se	P-value
Pre-pubertal	244.030 ± 28.92	
Pubertal	259.3514 ± 22.81	
Adult	210.352 ± 25.08	
Aged	305.974 ± 26.24	*0.131*
	Seminiferous tubule lumen diameter (µm) ±se	
Pre-pubertal	64.675 ± 5.60	
Pubertal	92.444 ± 12.78	
Adult	70.468 ± 9.32	
Aged	116.273 ± 14.97	*0.006**
	Seminiferous tubule epithelium height (µm) ±se	
Pre-pubertal	91.457 ± 6.01	
Pubertal	80.237 ± 7.65	
Adult	116.744 ± 13.39	
Aged	121.017 ± 8.89	*0.014**

Significant difference between the age groups for both the seminiferous tubule lumen diameter and the mean epithelium height, with the aged group being significantly higher (P<0.005) than pre-pubertal, pubertal, and adult age groups.

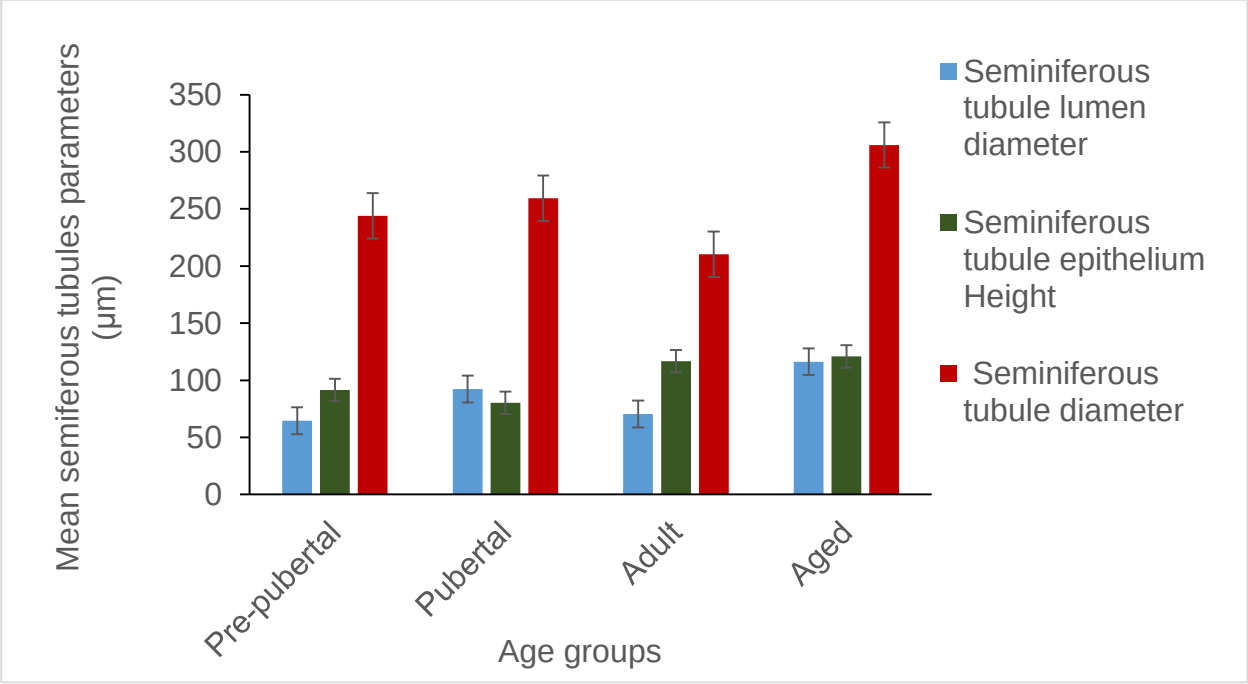

Figure 3. Bar charts showing mean seminiferous tubules parameters of all the age groups. There was a significant difference (P<0.005) between the groups for both the seminiferous tubule lumen diameter and epithelium height but no significant difference (P>0.005) for seminiferous tubule diameter among the age groups.

4. DISCUSSION

The present study showed a significant change in the body weight, testicular weight, seminiferous tubule epithelium height, and the seminiferous tubule lumen diameter between the age groups. However, seminiferous tubule diameter was not significantly different among the age groups. This is in accordance with the reports of the previous study (Müller and Skakkebæk, 1983, Gaytan et al., 1986, Artoni et al., 1999). Muller and Skakkebaek, (1983) observed that testicular weight increase with age which was highly influenced by the size of the germ cell proliferation. They also found no significant difference in the mean tubular diameter during the 0 – 10year period of pre-pubertal boys. Most of the morphometric studies on mammalian and avian testes have measured the testicular parameters under experimental conditions. To our knowledge, this is the first study that reports morphometric changes in body weight, testicular weight, seminiferous tubule diameter, seminiferous epithelium height, and tubular diameter in four reproductive cycles of Japanese quails. Shil et al., (2015) evaluated the morphometry of testes of one reproductive cycle of Japanese quails and reported positive correlations between mean body and testicular weights, which is similar to the observations in one of the reproductive cycles (adult age group) of the present study. In birds, testes are located in the abdominal cavity deeply, they are only visible after removal of other organs such as the intestine. They are surrounded by a fibrous tissue capsule comprising of contractile fibers and connective tissue (Aire and Ozegbe, 2007). The main component of the testes is seminiferous tubules, where spermatogenesis takes place. The left and the right testes in the present study are symmetrical, as there was no significant difference observed. This is in agreement with the reports of (Kempenaers et al., 2002) in tree swallow (*Tachycineta bicolar*). The cross-sections of the seminiferous tubules observed in the present study are similar to the previously described testicular structure of avian species (Deviche et al., 2011). They reported that seminiferous tubules are the major component of testicular tissue and it is surrounded and anastomosed by a basal lamina consisting of myoepithelial cells, fibroblasts, and connective tissue. They also described its epithelium where germ cells proliferate and differentiate as they move from the basement membrane to the tubular lumen. The changes in shapes and sizes of the seminiferous tubules in avian species as observed in the present study were originally attributed to changes in androgen levels. Older animals secretes more androgen than younger ones, which stimulates testicular activity and maintenance of the seminiferous tubule integrity (Thurston and Korn, 2000). The significant increase in the seminiferous tubules lumen diameter observed in the aged birds in the present study could be due to high rates of germ cell apoptosis (Zakariah et al., 2020) and a fall in androgen levels (Zirkin and Chen, 2000). Germ cell deaths create intervening spaces in the seminiferous tubules thereby leady to the larger tubular lumen.

Even though, evaluation of spermatogenesis can be highly inconsistent (Briskie and Montgomerie, 2007), testicular size is often used to measure sperm production, as greater components of testicular tissue are dedicated to spermatogenesis. Generally, sperm production is proportional to testicular size as demonstrated in the zebra finch (Birkhead et al., 1993) and the house sparrow (Birkhead et al., 1994). However, not much information is available concerning sperm production in all avian species, especially wild birds. Therefore, the correlation between testicular size and sperm production cannot be generalized since several factors can alter the process of spermatogenesis (Briskie and Montgomerie, 2007). Age-related studies in relation to testicular size, having larger testis in older animals than younger ones as observed in domestic chicken in the present study, have also been documented in other avian species (Deviche et al., 2000, Graves, 2004, Laskemoen et al., 2008). The differences in testicular size in relation to age may be due to younger birds secreting less gonadotropic hormones (GTHs) than older birds (Silverin et al., 1997). Also, the differences in the testicular size in relation to age could be due to a lower level of testosterone in younger adults compared to older ones (Deviche and Sharp, 2001). Inter- and intraspecies comparisons indicate an allometric relationship between testis and body sizes, but the strength of this relationship also differs (Pitcher et al., 2005, Gunn et al., 2008). It is common knowledge that climate change can affect factors that are responsible for the reproductive development of birds (Deviche et al., 2011). This is evident as the breeding season of some avian species has been shifted from the historic breeding season as observed through a long-term study (Pearce-Higgins et al., 2005, Visser et al., 2006). However, in Japanese quails, seasonal variations do not have significant effects on live body weight (Shil et al., 2015). As previously observed, the mean diameter of the seminiferous tubule and the seminiferous tubule epithelial height in mammals is directly proportional to the spermatogenic activity (Franca and Russell, 1998, Valença et al., 2013). This is because the seminiferous tubule lumen diameter and seminiferous epithelium height are determined by the number of germ cells present in the seminiferous tubules. Even though these values can vary, as germ cell apoptosis is not sequential, it can be affected by several factors because of its complexity (Billig et al., 1995). Therefore, the degree of variations in the morphometric data of the present study is not sufficient enough to an inference for the reproductive cycles of domestic chicken, but it can be used as baseline data for future study.

5. CONCLUSION

The mean tubular diameter is the most commonly used metric for assessing seminiferous tubules. This is necessary because testicular disorders cause changes in the diameter of the seminiferous tubules. Animal testicular morphometry research enables the identification of physiological and behavioral patterns that are essential for comprehending various facets of the biology of reproduction in various species, which will enable the development of assisted reproduction protocols. By displaying a wider seminiferous tubule lumen, the current study demonstrated a gradual decline in spermatogenesis in older birds. This could be because of the high rate of germ cell apoptosis and the decline in androgen levels, which are common markers of aging observed in both the mammalian and avian species under study. The development of stereological research techniques yields quantitative data that is essential for creating associations between physiological and biochemical data.

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