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Daily Sperm Production of the Domestic Fowl (*Gallus domesticus*) as Determined By Quantitative Testicular Histology and Homogenate Methods

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Abstract: The daily spermatozoa production was studied in 20 each of sexually matured barred Plymouth Rock and Nigerian indigenous breeds of domestic fowl using both the histometric and testicular homogenate methods. The exotic cocks were significantly ($p<0.01$) heavier than the locals with the respective values of 2.11±0.05 and 1.58±0.02 kg. The exotic also had larger ($p<0.01$) gross testicular weight (21.58±1.46 vs. 12.56±0.91g), paired testicular parenchymal weight (20.47±0.40 vs. 11.96±0.82 g) and paired tunica albuginea (1.11±0.18 vs. 0.60±0.11 g). The total length and the diameter of the seminiferous tubules were also significantly influenced ($p<0.01$) by breed. The volume percent occupied by seminiferous tubules though higher in the exotic birds showed no statistical significance. Daily sperm production obtained from histometric method was highly influenced by breed with $2.41\pm1.17\times10^9$ and $0.76\pm0.71\times10^9$ for exotic and local cocks, respectively. The daily sperm productions calculated on the basis of homogenization-resistant spermatids were 1.85±0.22 for exotic and 0.73±0.11 for locals. Although, the difference in DSP values based on both methods was not statistically significant there was a 23.24 and 3.90% loss in exotic and local birds respectively with the use of homogenization method. On the whole, the exotic cocks were twice as efficient in sperm production as the local birds.

Key words: Daily sperm production, domestic fowls, breeds, histology

INTRODUCTION

Quantification of the sperm production capacity in breeding animals allows for the assessment of the efficiency of spermatogenesis in males kept under different environmental conditions and enhances critical evaluation of effects of season, breed, age, bioclimatic factors, hormones, chemicals and drugs. Proper and profitable management can therefore be based on such information (Franca and Godinho, 2003; Bitto and Egbunike, 2006; Noirault *et al*., 2006; Egbunike *et al*., 2007; Gbore and Egbunike, 2008; Lupol *et al*., 2009).

Methods for quantifying the Daily Sperm Production (DSP) based on histometric data have been reviewed by Berndtson (1977) who calculated the DSP in bulls by dividing the product of the volumetric proportion of round spermatids in the testis and the volume of the testicular parenchyma by the product of the volume of a single round spermatid nucleus and the life span of round spermatids in days. With this approach, daily sperm production has been determined for boars (Swierstra, 1971; Egbunike *et al*., 1976, 2007), rabbits (Amann, 1970) and humans and rats (Johnson *et al*., 1980), goats (Bitto and Egbunike, 2006).

Daily sperm production can also be determined by physical enumeration of maturation-phase spermatid and spermatozoa in testicular homogenates. This method has been utilized in estimation of DSP in mammals (Berndtson, 1977), rabbits (Amann and Lambiase, 1969), human (Amann and Howards, 1980), Japanese quail (Clulow and Jones, 1982), boars (Egbunike *et al*., 2007), horses (Blanchard and Johnson, 1999) domestic fowl, cats (Franca and Godinho, 2003), goats (Bitto and Egbunike, 2006) turkeys (Noirault *et al*., 2006) and in bulls (Igboeli and Rakha, 1971).

Due to the paucity of information on the sperm production potentials of the domestic fowl, this research was undertaken to provide information on the Nigerian indigenous breed and to compare such information with the barred Plymouth Rock reared in most farms in the humid tropical climate of the Niger Delta of Southern Nigeria.

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MATERIALS AND METHODS

Twenty, eight-month old barred Plymouth Rock and twenty non-descript Nigerian indigenous breeds of domestic fowl previously used in natural breeding to test their fertility were used. The experiment was carried out in the Niger Delta region of Southern Nigeria between 2006-2008. This period covered the four seasons of the year-early rainy season (April-June). Late rainy season (July-Sept.), early dry season (Oct.-Dec.) and late dry season (Jan.-Mar.) (Egbunike *et al*., 1976).

Bird management: The birds were housed individually in cages and fed a standard breeder's ration containing 18% crude protein and cool clean water offered *ad libitum*.

Histological methods: Pairs of testes were removed immediately after slaughtering, trimmed, freed of adhering fat and connective tissues and weighed. A portion of the right testis of each cock was taken for histological processing. Testicular tissues were fixed in Bouins' fixative solution for 24 h, dehydrated in a series of ethyl alcohol, cleared in chloroform and embedded in paraffin. Histological sections 7 μ thick were stained with haematoxylin and eosin and the slides observed at 800x magnification. This method was used by Egbunike *et al*. (2007) in boars.

Volumetric proportions and diameters of round spermatids and seminiferous tubules: The volume percent of round spermatids and seminiferous tubules were determined by Berndtson (1977) method using a 25-point ocular graticule. One hundred random fields were examined and all structures under each 'hit' including artifacts recorded. The mean frequencies of basement membrane, interstitial cells, Leydig cells and intertubular space was subtracted from 100% to obtain the volume % occupied by seminiferous tubules, while the volume of round spermatids was obtained according to Swierstra (1971).

Diameter of round spermatids and seminiferous tubules: The diameter of round spermatids seminiferous tubules were determined by taking the average of two perpendicular measurements of forty each of round spermatid nuclei and seminiferous tubules per animal irrespective of the stage of the cycle of seminiferous epithelium using a calibrated eyepiece micrometer. The mean values were expressed in microns (Berndtson, 1977).

The average volume of a round spermatid nucleus was calculated from the weighted mean diameter by substitution in the formula for a sphere (Swierstra, 1971).

Estimation of the daily sperm production by histometric method: The daily sperm production was estimated by histometric method using the formula:

$$DSP = \frac{CTV \times \text{Volume \% of round spermatid nuclei in testis}}{\text{Average volume per round spermatid nucleus} \times \text{Lifespan of round spermatid in days}}$$

The corrected testicular volume (CTV) was determined using the following formula modified from Swierstra (1971).

$$CTV = \frac{\text{Gross testicular weight - Tunica albuginea weight}}{\text{Testis density}} \times \text{Shrinkage correction}$$

This modification became necessary since the domestic fowl testes have no mediastinum (Swierstra, 1971).

The round spermatid nuclei were present in stages I, II, III, IV representing 55.86% of a 4 day cycle of the seminiferous epithelium. Thus, the duration of all round spermatids was calculated as 0.558×4 = 2.23 day.

DSP estimation by homogenate method: The remaining portion of the right testis was weighed again and both testes homogenized separately with a pair of sharp pointed scissors in physiological saline at 200 mg mL^{-1}, (Egbunike *et al*., 2007). The suspensions were mixed and strained through a double layer of sterile cheese cloth into graduated test tubes. All samples were covered and stored for 24 h at 4°C. A dilution of 1:25 v/v was made for counting with the Neubauer haemocytometer. The DSP for each animal was calculated by dividing the number of elongating spermatids and spermatozoa in the homogenate by the time divisor obtained by multiplying the fraction of the cycle of seminiferous epithelium occupied by these cells by the duration of a cycle. Researchers found that 48.25% of the cycle of 4 days was occupied by these cells.

Efficiency of sperm production: The efficiency of sperm production (DSP/g) by either method was calculated by dividing the DSP values by the testicular parenchymal weight according to Egbunike *et al*. (2007).

Statistical analysis: Data were subjected to analyses of variance and Student's t-test for left vs. right testes and breed effects while correlation/regression analyses according to Steel and Torrie (1996) were carried out to ascertain relationships between parameters studied.

RESULTS AND DISCUSSION

The mean body weight of the exotic birds was significantly ($p<0.01$) higher than that of the local cocks. The heavier exotic cocks also had statistically ($p<0.01$) larger mean gross testes weight, paired *Tunica albuginea* and paired parenchyma weight than the local birds (Table 1). However, there was no significant difference in the mean testis density.

Although, the volumetric proportion of the round spermatids was higher in the exotic than the local birds the difference was not statistically significant. For the round spermatids both breeds were similar and the average volume of the nucleus was 19.33±0.06 μ^3 while the time divisor was 1.93 days. However, the total length and diameter of the seminiferous tubules showed significant ($p<0.01$) breed differences with the exotic having the higher values (Table 2).

Based on histometric analysis, DSP was estimated to be $2.41\pm1.17\times10^9$ in the exotic and $0.76\pm0.71\times10^9$ in the local cocks. This difference is highly statistically significant ($p<0.01$) and shows that the exotic cocks had a three-fold superiority in daily sperm production and were twice as efficient in DSP/g testicular parenchyma when compared with locals (Table 3).

The DSP obtained from homogenization was similar to that calculated from histometry. No statistical difference was observed with breeds based on the two methods.

The daily sperm production was highly correlated with body weight ($r = 0.61$, $p<0.01$), paired testes weight ($r = 0.98$, $p<0.01$) paired *tunica albuginea* weight ($r = 0.96$, $p<0.01$)) and DSP/g ($r = 0.97$, $p<0.01$). All morphometric characteristics except the testis density were significantly correlated with daily sperm production and its efficiency (Table 4).

Histometric parameters of the seminiferous tubule were also highly correlated with daily sperm production and its efficiency (Table 5).

A cross-section of the seminiferous epithelium of the domestic fowl is shown in Fig. 1a-h. Each plate represents a stage in the cycle of seminiferous epithelium with the associated spermatogenic elements identified on the basis of acrosomal changes, morphology of spermatid head, nuclear shape and location of spermatids.

Comparison of the morphometric characteristics indicated that the exotic barred Plymouth Rock were superior to the local breed with heavier body weight, a two-fold difference in the paired testes weight, significantly higher testicular parenchyma weight and paired *Tunica albuginea*.

The daily sperm production estimated on the basis of homogenization-resistant spermatids and spermatozoa enumerated was lower by 23.24 and 3.90% than that estimated by testicular histology in the exotic and local cocks, respectively, suggesting a higher degree of degeneration in the exotic than local cocks. It is worthy to note that with the boar no difference was found between the two methods (Egbunike *et al*., 2007).

By comparison, there was a three-fold difference in daily sperm production in favor of the exotic cocks, probably as a result of their significantly higher testicular mass. In addition, a slightly higher proportion of the testicular mass was occupied by the seminiferous tubules in the exotic cocks than in local breeds (87.93±1.04 vs. 83.48±3.07, respectively). This is an indication of the fact that the seminiferous tubular diameter and the total length which make up the percent testicular mass occupied by the seminiferous tubule are functions of the testes size.

Per gram of testicular parenchyma, the daily sperm production by histometric method was almost twice in the exotic as in the local. The results are within reasonable

Table 1: The effect of breed on body weight and some testicular parameters in the domestic fowl (Mean±SEM)

Parameters	Exotic (n = 20)	Local (n = 20)	Level of significance
Body weight (g)	2.11±0.05	1.58±0.02	p<0.01
Paired testes weight (g)	21.58±1.46	12.56±0.91	p<0.01
Paired tunica albuginea (g)	1.11±0.18	0.60±0.11	p=0.01
Paired parenchymal weight (g)	20.47±0.20	11.96±0.82	p=0.01
Mean testes density (g/cc)	1.05±0.00	1.04±0.00	ns

ns: Not significant

Table 2: Some histometric characteristics of the testis of the domestic fowl (Mean±SEM)

Parameters	Exotic (n = 20)	Local (n = 20)	Level of significance
Length of seminiferous tubule (m)	221.13±12.59	133.73±4.52	p<0.01
Diameter of seminiferous tubule (μ)	248.56±12.75	212.93±9.39	p<0.01
Volume % of seminiferous tubule	87.93±1.04	83.48±3.07	p<0.01

Table 3: Effect of breed on daily sperm production and efficiency of sperm production in the domestic fowl (Mean±SEM)

Parameters	Exotic (n = 20)	Local (n = 20)	Level of significance
Daily sperm production (DSP X 109)*	2.41±1.17	0.76±0.71	p<0.01
Daily sperm Production (DSP X 109)**	1.85±0.22	0.73±0.11	p<0.01
Daily sperm production per gram (DSP/g X 106)*	104.50±0.18	59.00±0.14	p<0.01
Daily sperm production per gram (DSP/g X 106)**	84.92±0.10	54.52±0.12	p<0.01

*Daily sperm production by histology, **Daily sperm production by homogenization method

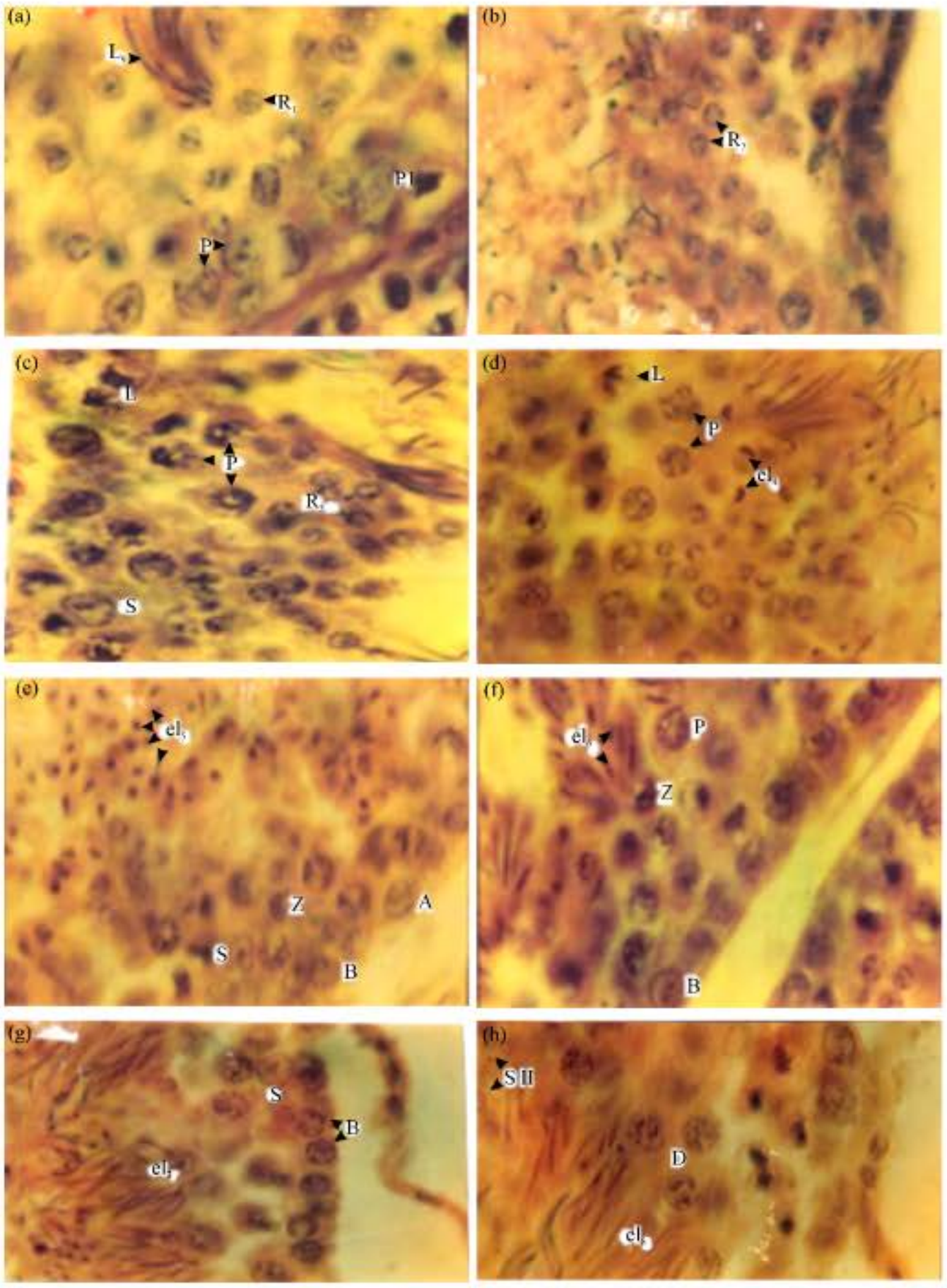

Fig. 1: Cross-section of the testis of the domestic fowl showing the seminiferous epithelium and the spermatogenic elements at various stages of differentiation and maturation: (a) Stage I: Pl, P, R_1, L_9, (b) Stage II: Pl, P, R_2, (c) Stage III: L, P, S, R_3, L_{10}, (d) Stage IV: L, P, el_4, L_{10}, (e) Stage V A, B, Z, S, el_5, (f) Stage VI B, Z, P, el_6, (g) Stage VII B, S, el_7, (h) Stage VIII A, D, SII el_8. Spermatogenic elements observed include: A, Spermatogonia type, A, B: Spermatogonia type B, Pl: Preleptotene primary spermatocytes, L: Leptotene primary spermatocytes, Z: Zygotene primary spermatocytes, P: Pachytene primary spermatocytes, D: Diplotene primary spermatocytes, S: Sertoli cells, R_1, R_2, R_3: Round spermatids at steps 1, 2 and 3. el_4, el_5, el_6, el_7, el_8: Elongating spermatids at steps 4, 5, 6, 7 and 8, L_9, L_{10}: Mature spermatozoa at spermiation

range when compared with the total number of round spermatids per gram of testicular parenchyma per day reported by De Reviers (1971). However, there has been no previous work on the Nigerian indigenous breed with which to compare the $59.00\pm0.14\times10^6$ obtained in this study.

De Reviers (1971) also reported a value of 27.00×10^6 for the number of primary spermatocytes per gram of testis per day for Rhode x Wyandotte Stock M14 cocks. This is useful in predicting the daily sperm production. However, there is no significant difference between De Reviers (1971) result and the report presented

Table 4: Correlation coefficients of testicular parameters, body weight and daily sperm production of the domestic fowl

	Dependent variables					
Independent variables	1	2	3	4	5	6
Body weight	-	0.61[a]	0.63[a]	0.64[a]	0.07	0.65[a]
Daily sperm production	-	0.97[a]	0.98[a]	-0.05	0.96[a]	
Daily sperm production/gram	-	0.99[a]	-0.04	0.98[a]		
Paired testes weight	-	-0.03	0.98[a]			
Testis density	-	-0.06				
Paired tunica albuginea	-					

n = 40, a: Values superscripted are significant (p<0.01)

Table 5: Relationship between histometric characteristics and sperm production the domestic fowl

	Dependent variables				
Independent variables	1	2	3	4	5
Seminiferous tubule diameter	-	0.69[a]	0.57[a]	0.75[a]	0.75[a]
Seminiferous tubule length	-	0.72[a]	0.80[a]	0.83[a]	
Percent seminiferous tubule in the testis	-	0.50[a]	0.55[a]		
Daily sperm production	-	0.97[a]			
Daily sperm production/g	-				

in this study. This is because her result is based on primary spermatocytes while this work is based on round spermatids (Swierstra, 1971). The four primary spermatocytes produced by each spermatogonium type A undergo two maturation divisions to produce four spermatids each. Therefore, the sperm production efficiency (DSP/g) presented by De Reviers is 4 $(27.00\times106) = 108.00\times10^6$ by the round spermatids method and compares favorably with $104.50\pm0.18\times10^6$ observed in this study. Thus the Barred Plymouth Rock can be used for the production of semen for commercial artificial insemination breeding program in the Niger Delta region of Southern Nigeria.

Theoretically, sperm production can be estimated by histometric method for any meiosis-committed germinal cells, but such calculations are subject to overestimation due to the possible degeneration as spermatogenesis advance. To minimize this bias, round spermatids were selected for DSP calculation in the histometric method. This method gives a more accurate result because there would be less germ cell degeneration during the elongation and maturation processes in spermatids.

Johnson *et al*. (1980) had suggested that the discrepancy between values obtained from homogenization and histometric methods in the estimation of DSP may arise from attrition between round spermatids and maturation phases of spermatids development. Thus, the difference in the DSP and DSP/g calculated by testicular histology and homogenate methods may be due to a number of variables. The time divisor obtained by the calculation of the relative percentage of occurrence of stages of the cycle of seminiferous epithelium which contain both round spermatids and maturation-phase spermatids could be partly responsible. It is possible that stage I contains some early maturation-phase spermatids which are not homogenization-resistant or that spermiation actually occurs at stage III, such that inclusion of stage IV results in an over-estimation of the time divisor. Also, the difference between the two methods suggests some degeneration of spermatids between the round type (used for the histology) and the elongated type which forms part of the gonadal reserves used in the homogenate method. In spite of such uncertainties, results obtained by these methods remain valid since only absolute and not relative values of both breeds are affected. However, if in future a more appropriate time divisor for homogenization studies in the domestic fowl is established, previous results can readily be corrected.

It is worthy of note that the relative inefficiency of the local cock is the cumulative expression of several interactive factors such as low body weight and testes weight and relatively lower seminiferous tubule length and diameter which are also functions of the testes weight. Since, sperm production is highly positively correlated ($r = 0.61$, $p<0.01$) with body-weight and testis weight ($r = 0.98$, $p<0.01$), improved sperm production in the local cocks requires a concerted effort towards increased body weight and testicular weight by crossbreeding the local breeds with the larger and heavier exotic breeds.

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