



Journal of Applied Sciences

ISSN 1812-5654

science
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Breed and Seasonal Variation in the Testicular Histometric Parameters and Germ Cell Populations of the Barred Plymouth Rock and the Nigerian Indigenous Breeds of the Domestic Fowl (*Gallus domesticus*)

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Abstract: Due to the hostile climatic environment of the humid tropics characterized by high ambient temperature and humidity all year round, this study was initiated to evaluate the effect of breed and season on the testicular histometric and cellular populations of the domestic fowl. Testes obtained from forty sexually matured cocks consisting of 20 barred Plymouth Rock (bPR) and 20 non-descript Nigerian Indigenous Breeds (NIB) were processed by histological and histometric methods. Volumetric proportions of testicular germ cells remained unaffected by breed or season. Seminiferous tubules occupied 83-87% of the testicular mass in NIB and bPR, respectively. Nuclear diameter of the germ cells and their volumes remained unaffected by breed and season. The mean seminiferous tubule diameter (248.56 ± 12.56 vs. 212.93 ± 9.39 μm) and the seminiferous tubule length/paired testes (221.13 ± 12.59 vs. 133.73 ± 4.52 m) were significantly ($p < 0.01$) influenced by breed with the bPR having the higher values, the mean seminiferous tubule length/g of testicular parenchyma remained unaffected by breed or season. Sertoli Cell Index showed a linear relationship with the testes weight characterized by the equation $Y = 0.163x + 1.733$, $r = 0.9000$, $R^2 = 0.81$ ($p < 0.001$). Similar significant ($p < 0.001$) relationships occurred between seminiferous tubule length ($r = 0.8651$, $p < 0.001$), seminiferous tubule diameter ($r = 0.7658$, $p < 0.001$), volume % round spermatids ($r = 0.9904$, $p < 0.001$), testicular parenchyma volume ($r = 0.9073$, $p < 0.001$) and volume % of seminiferous tubule ($r = 0.5474$, $p < 0.001$). These parameters can be predicted from testicular weights and testicular histometry provides a reliable tool for the assessment of the reproductive state and potential sperm production capacity in the domestic fowl.

Key words: Seminiferous tubules, testicular germ cells, sertoli cells, histometry, season, domestic fowl

INTRODUCTION

In the poultry industry large numbers of infertile eggs constitute a major channel of economic losses. Among several factors that contribute to this problem (Dzoma, 2010; Dzoma and Motshegwa, 2009), is the poor knowledge of the spermatogenic potential of the cock especially as influenced by breeds and season. The importance of this knowledge becomes more evident when viewed against the hostile climatic environment encountered in the humid tropics where ambient temperature and humidity are high all year round. Light and temperature are the two environmental variables principally affecting reproductive activities in birds and other animal species. The growth of the male gonad and its later function can be strongly influenced by manipulating the light schedules during various phases of the reproductive life of the animal. The influence of

varying photoperiod on the seminiferous tubule diameter, seminiferous tubule length and volume % of seminiferous tubule has been reported in the domestic fowl (De Reviers and Brillard, 1974). The, age at sexual maturity, testes size and sperm production can also be regulated by varying the length of photoperiods in the male domestic fowl and turkey (Noirault *et al.*, 2006). However, different types of males vary in the way in which the testes grow under similar daily photoperiods.

Elevated ambient temperature impairs male reproductive performance in several animal species and some of the characteristic responses noted after prolonged exposure to whole body heating include: increases in respiratory rates and rectal temperatures as well as decline in sperm output in goats (Bitto *et al.*, 2008) boars (Wettemann and Desjardins, 1979; Egbunike *et al.*, 1976; Almeida *et al.*, 2006). Ejaculation of sperm with high proportion of morphologically abnormal spermatozoa,

lowered fertility and interference with spermatocyte/spermatid maturation has also been reported by Egbunike *et al.* (2007) and Wettemann and Desjardins, 1979). Increase in the relative humidity of the environment results in an increase in the body temperature of animals and the combined effect of high ambient temperature and relative humidity is the reduction in feed intake, productivity and general spermatogenic activity.

Testicular histology and histometry can provide sensitive indicators for determining the efficiency of spermatogenesis and has been used effectively in beef bulls (Berndtson *et al.*, 1987) wild boars (Costa and Silva, 2006), jaguars (Azevedo *et al.*, 2010), African lions (De Barros *et al.*, 2007), goats (Leal *et al.*, 2004; Raji *et al.*, 2008), rodents (Segatelli *et al.*, 2004) cats (Franca and Godinho, 2003). It also provides a baseline information for the assessment of induced alterations in spermatogenesis due to toxic chemicals (Berndtson and Desjardins, 1974), medicinal plant extracts (Nusier *et al.*, 2007) and Chloroquine phosphate (Asuquo *et al.*, 2007). Histological and histometric analysis of the gonads of males, together with the observations of external characteristics of gonads of males and females, may assist in identifying the reproductive condition of animals (Couto and Talamoni, 2005). Effect of season on testicular histometry and cell populations has been reported in rodents (Segatelli *et al.*, 2004, Stallions (Johnson, 1991), wild boars (Costa and Silva, 2006; Okwun *et al.*, 1996), mouse (Boiani *et al.*, 2008) and the WAD goat (Bitto *et al.*, 2008; Leal and Franca, 2006).

Despite the earlier observation that season seemed to exert no significant influence on Daily Sperm Production (DSP) or its efficiency DSP/g in the domestic fowl (Orlu and Egbunike, 2009) nor does it significantly influence the morphometry and sperm reserves (Orlu and Egbunike, 2010), there is a paucity of information on seasonal influence on the histometric parameters of the reproductive organs and the cellular dynamics in this species. Viewed against the background of the fact that high ambient temperatures impair male reproductive performance and spermatogenic activity in several animal species (Wettemann and Desjardins, 1979) this research was carried out to assess the breed and seasonal influence of tropical all year round high ambient temperature and relative humidity on the histology and histometric parameters of the reproductive organs in the domestic fowl.

MATERIALS AND METHODS

The study was carried out in the Niger Delta region of Southern Nigeria for a two year period (2006-2008) from

January to December and covered the four seasons of the year described by Egbunike *et al.* (1976) namely: Late dry season (January-March), Early rain (April-June), Late rain (July-September) and Early dry (October-December).

Birds and management: Forty adult cocks consisting of 20 barred Plymouth Rock (bPR) and 20 non-descript Nigerian Indigenous Breed (NIB) were used. They were raised in groups of 10 per season, 5 per breed and housed individually in cages. The birds were fed standard breeders' ration containing 18% crude protein *ad libitum* with cool clean water. All birds were sexually matured and had responded to abdominal massage by ejaculation.

Histological methods: Animals were killed by dislocation of the neck after which the testes were immediately removed by dissection and weighed. Samples from the mid parenchyma region of both left and right testis were fixed in 10 times their volume of Bouin's fixative solution for 24 h. The tissue samples were washed in 70% ethyl alcohol and thereafter dehydrated in increasing concentrations of ethanol and cleared in three changes of chloroform. The cleared samples were embedded in paraffin wax to form tissue blocks. Histological sections 7 μ thick were made. The processed samples were stained according to Periodic-Acid-Schiff (PAS) technique and counter-stained with haematoxylin-eosin (Egbunike *et al.*, 2007).

Total length of seminiferous tubule/paired testes: The total length of seminiferous tubules in the testes was estimated from the corrected testicular volume and volume percent of seminiferous tubules in the testes divided by the product of the squared radius and π ($R^2 \times \pi$) value in meters (m) histological sections and expressed in meters (m) (Johnson and Neaves, 1981).

Seminiferous tubule length/gram of testicular weight (m): This value was obtained by dividing the total length by the testicular weight and expressed in metres (m) according to the method described by Hochereau-de-Reviere and Lincoln (1978).

Seminiferous tubule cell populations: The volume percent of round spermatids and other spermatogenic elements was determined in sections prepared in standardized conditions, corrections made for the retraction of the testicular parenchyma during histological processing as previously described (Orlu and Egbunike, 2009). The number of germ cells and Sertoli cells in tubular cross-sections were determined in a total of 100 cross sections of the seminiferous tubule. The raw counts were transformed to nuclear/true counts by the Abercrombie (1946) formula modified by Berndtson (1977). Volume of

round spermatogenic elements nuclei were calculated from the weighted mean diameter by substitution in the formula for a sphere ($\frac{4}{3} \pi R^3$) and expressed in μm^3 as previously reported by Orlu and Egbunike (2009).

Seminiferous tubule diameter: Diameter of fixed seminiferous tubule and round spermatogenic cells were determined as earlier described by Orlu and Egbunike (2009) and expressed in μm .

Sertoli cell index (Germinal/Sertoli cell ratio): All germ cells including Spermatogonia A and B, all primary spermatocytes, secondary spermatocytes as well as round and elongated spermatids were counted and the total count divided by the total number of Sertoli cells.

Statistical analysis: Data are presented as mean \pm SEM. Breed and seasonal variation were analyzed with Students'-t test while correlation and linear regression according to Steel *et al.* (1996) were carried out to assess the relationship of the histometric parameters studied to body weight and testicular weight.

RESULTS AND DISCUSSION

The most frequently occurring spermatogenic elements were the primary spermatocytes at Pachytene stage of meiotic prophase with 6.98 and 5.92% for bPR and the NIB, respectively. The overall volume percent of the testicular cells were: spermatogonia 4.75%, primary spermatids 13.45%, round spermatids 8.57%, elongating and maturing spermatids 10.70% in the bPR. These values are not significantly ($p>0.05$) different from those in the NIB (Table 1). Season exerted no significant ($p>0.05$) influence on their relative proportions in the testes. Seminiferous tubules occupied 83.47-87.93% of the testicular mass while the inter-tubular space, interstitial

cells and the basement membrane occupied 3.77, 0.90 and 2.14%, respectively.

The mean seminiferous tubule diameter (248.56 ± 12.56 vs. $212.93\pm 9.39 \mu\text{m}$) and the seminiferous tubule length/paired testes (221.13 ± 12.59 vs. 133.73 ± 4.52 m) were significantly ($p<0.01$) influenced by breed with the bPR having the higher values. However, the mean seminiferous tubule length per gram of testicular parenchyma was not significantly ($p>0.05$) affected by breed or season (10.10 ± 1.08 vs. 12.78 ± 1.04 m) for bPR and NIB, respectively. Also significantly ($p<0.01$) affected by breed were the volume percent of round spermatids (11.92 ± 0.15 vs. 6.59 ± 0.12) and the testicular parenchyma volume (19.80 ± 3.04 vs. 9.52 ± 1.69) (Table 2). The breed

Table 1: Relative frequencies of Testicular elements in the barred Plymouth Rock and the Nigerian indigenous (NIB) of the domestic fowl

Elements	Volume % in testicular tissue*	
	bPR	NIB
Spermatogonia A	3.27	2.29
Spermatogonia B	1.48	2.15
Preleptotene 1 ^o spermatocyte	1.24	1.62
Leptotene 1 ^o spermatocyte	2.14	1.21
Zygotene 1 ^o spermatocyte	1.69	1.75
Pachytene 1 ^o spermatocyte	6.98	5.92
Diplotene 1 ^o spermatocyte	1.34	0.40
Diakinesis 1 ^o spermatocyte	0.06	0.04
Secondary spermatocyte	0.28	0.40
Round spermatids (R ₁ , R ₂)	8.57	7.81
Elongating/Elongated spermatids (e ₁ -L ₁₀)	10.70	9.55
Spermatozoa	5.52	4.98
Sertoli cell	1.01	0.98
Basement membrane	2.14	0.94
Lumen	3.55	2.42
Interstitial cells	0.90	0.67
Cytoplasm	50.60	51.52
Leydig cell	0.62	0.54
Intertubular space	3.27	4.58
Leydig cell/Sertoli cell ratio	0.61	0.68
Sertoli cell index	5.31	6.19
Seminiferous tubule	87.93	83.47

*Breed difference not significant ($p>0.05$)

Table 2: Breed and Seasonal variation in the histometric parameters of the reproductive organ in barred Plymouth Rock (bPR) and the Nigerian indigenous (NI) breeds of the domestic fowl

Parameters	Breed	Season			
		Late dry	Early rain	Late rain	Early dry
Body weight (g)**	bPR	2.17 \pm 0.03**	2.23 \pm 0.02	2.04 \pm 0.05	2.11 \pm 0.05
	NIB	1.58 \pm 0.02	1.64 \pm 0.03	1.50 \pm 0.03	1.58 \pm 0.03
Testicular weight (g)**	bPR	21.42 \pm 2.21**	21.32 \pm 2.96	20.79 \pm 2.51	24.16 \pm 4.96
	NIB	10.54 \pm 1.71	10.76 \pm 2.12	8.91 \pm 1.52	12.48 \pm 1.91
Length of tubule/paired testes (m)**	bPR	216.10 \pm 22.62**	216.09 \pm 25.08	214.85 \pm 26.12	237.50 \pm 25.37
	NIB	142.91 \pm 10.88	140.92 \pm 11.32	117.20 \pm 10.19	133.90 \pm 11.55
Length of tubule/g of testicular weight (m)	bPR	10.09 \pm 1.04	10.14 \pm 1.12	10.33 \pm 1.28	9.83 \pm 1.20
	NIB	13.56 \pm 1.03	13.10 \pm 1.05	13.73 \pm 1.14	10.73 \pm 0.93
Seminiferous tubule Diameter (μm)*	bPR	246.12 \pm 4.16*	243.57 \pm 4.44	245.21 \pm 4.13	259.30 \pm 9.78
	NIB	206.26 \pm 3.01	211.64 \pm 4.31	209.87 \pm 3.92	223.95 \pm 3.87
Volume % of tubule	bPR	86.63 \pm 0.25	86.28 \pm 0.65	86.76 \pm 0.21	87.93 \pm 0.08
	NIB	83.16 \pm 0.11	82.98 \pm 0.53	83.22 \pm 0.86	83.47 \pm 0.05
Volume % Round** Spermatid Parenchyma	bPR	11.36 \pm 0.16**	11.38 \pm 0.20	11.04 \pm 0.12	13.97 \pm 0.15
	NIB	5.61 \pm 0.14	6.17 \pm 0.13	7.93 \pm 0.11	6.64 \pm 0.12
Volume (mm ³)**	bPR	19.36 \pm 2.08**	19.34 \pm 2.78	19.24 \pm 2.62	21.27 \pm 4.67
	NIB	9.57 \pm 1.61	9.87 \pm 1.99	7.92 \pm 1.28	11.35 \pm 1.89

Values are expressed as Mean \pm SEM; **Breed differences Significant ($p<0.01$); *Breed differences Significant ($p<0.05$); Seasonal influence ($p>0.05$)

difference observed in the volume percent of seminiferous tubule was not significant.

The mean diameter of spermatogonia A and B (6.00±0.15 and 5.50±0.04 µm), respectively, primary spermatocytes (6.00±0.34 µm) and round spermatids (3.50 µm) at stage 1 of the cycle of seminiferous epithelium were not significantly (p>0.05) affected by breed or season, nor, were the average volume of round spermatids (22.44 µm³), primary spermatocytes (113.10 µm³) and secondary spermatocytes (66.00 µm³) (Table 3).

The estimated coefficient of correlation between the testicular histometric parameters (Y) and the bodyweight (X) showed a high positive and significant (p<0.001) correlation (r = 0.64) between the testes weight and bodyweight characterized by the equation Y = 11.322x-3.3929, R² = 0.40 (p<0.001); seminiferous tubule diameter was also positively and significantly (p<0.001), correlated (r = 0.6692) to bodyweight with a regression equation Y = 33.232x+169.62, R² = 0.45(<0.001) so were the volume % of round spermatids in the testes and seminiferous tubule length with r = 0.67 and 0.49 regression equations Y = 5.5485 X + 0.4065, R² = 0.455 (p<0.001) and Y = 72,567X+57.531, R²= 0.4855 (p<0.001). Volume % occupied by seminiferous tubule showed a positive but non-significant (p>0.05) relationship with the bodyweight (Table 4).

Most of the histometric parameters analysed showed very high significant (p<0.001) correlation with the testicular weight. The relationship between paired testes weight (X, in grams) and the histometric parameters (Y) are shown in Fig. 1a-f. Sertoli Cell Index showed a linear relationship with the testes weight that was characterized by the equation Y = 0.163x+1.733, r = 0.9000, R² = 0.81 (p<0.001) (Fig. 1a). Similar significant correlative relationship with the paired testes weight (X) existed between volume occupied by round spermatids, Y = 0.5276x+0.0021, r = 0.98, R² = 0.97 (p<0.001) (Fig. 1b); Seminiferous tubule length, Y = 2.644x-427.61, r = 0.87, R² = 0.75 (p<0.001) (Fig. 1c); Seminiferous tubule diameter, Y = 2.3723x + 194.64, r = 0.98, R² = 0.97 (p<0.001) (Fig. 1d); Volume percent occupied by seminiferous tubule Y = 0.3379x + 79.457, r = 0.55, R² = 0.30 (p<0.001) (Fig. 1e) and Paired parenchyma volume, Y = 0.717x +4.2206 r = 0.9073, R² = 0.8232 (p<0.001) (Fig. 1f).

The relative contribution of the testicular histometric parameters to the bodyweight and testicular weight were evaluated in histological sections of the testes. The volume percent occupied by the various testicular elements did not differ significantly between the two breeds nor were they affected by season. Differences observed in the populations of Spermatogonia A and B, primary and secondary spermatocytes, spermatids and other spermatogenic elements were numerical without any statistical significance with reference to breed and season. This is expected since testicular germ cell populations are species specific and can only be influenced by age in most animals and perhaps seasons in seasonal breeders. The volume percent occupied by the seminiferous tubules ranged between 83-87% in the NIB and bPR respectively. The 87.93% for the bPR compares favourably with 87-90% reported for M15 crossbreeds of the domestic fowl (De Reviere and Brillard, 1974) and turkeys (Noirault *et al.*, 2006). Of interest is the observation that 83-87% is quite comparable to those of some mammalian species ranging between 70-90% (França and Russell, 1998). The seminiferous tubular diameter remained stable throughout the four seasons of the year with variations that were statistically non-significant (Table 2). Breed, however, exerted significant (p<0.01) influence on the tubular diameter with 212.93±3.78 µm for NIB and 248.56±5.63 for bPR. The 248.56±5.63 µm observed in this study agrees with those reported for domestic fowls raised under varying photoperiods (De Reviere and Brillard, 1974) and higher than those reported for turkeys (Noirault *et al.*, 2006). Seminiferous tubular diameter has been described as an indicator for the assessment of active

Table 3: Variation in germ cell nuclear diameter (µm) and volume (µm³) in the domestic fowl

Cellular nomenclature	Diameter (µm)*	Volume (µm ³)
Spermatogonia A	6.00±0.15	113.10
Spermatogonia B	5.50±0.04	87.11
Preleptotene 1 ^o spermatocyte	4.50±0.02	47.71
Leptotene 1 ^o spermatocyte	6.20±0.11	124.79
Zygotene 1 ^o spermatocyte	6.25±0.10	127.83
Pachytene 1 ^o spermatocyte	6.50±0.12	143.79
Diplojene 1 ^o spermatocyte	6.55±0.02	147.14
Secondary spermatocyte	5.00±0.01	65.45
Round spermatid		
R ₁	3.50±0.01	22.44
R ₂	3.50±0.01	22.44
R ₃	3.00±0.01	14.14
Mean of all round spermatids	3.33±0.04	19.33

*(Means±Sem) Sem = Standard error of mean

Table 4: Linear regression of histometric parameters of the reproductive organs on the Body weight of the domestic fowl

Dependent variables (Y)	Regression equation X = Body weight	R	R ²
Paired Testes weight	Y= 11.322x -3.3929	0.6356***	0.4041**
Seminiferous tubule diameter	Y= 33.232x +169.62	0.6692***	0.4478**
Seminiferous tubule length	Y= 72.567x +57.531	0.4855**	0.2357
Volume % seminiferous tubule	Y= 2.873x + 80.073	0.2612	0.06
Volume % round spermatids	Y = 5.5485x -0.4065	0.6745***	0.455**

*=(p<0.05); **=(p<0.01); ***=(p<0.001)

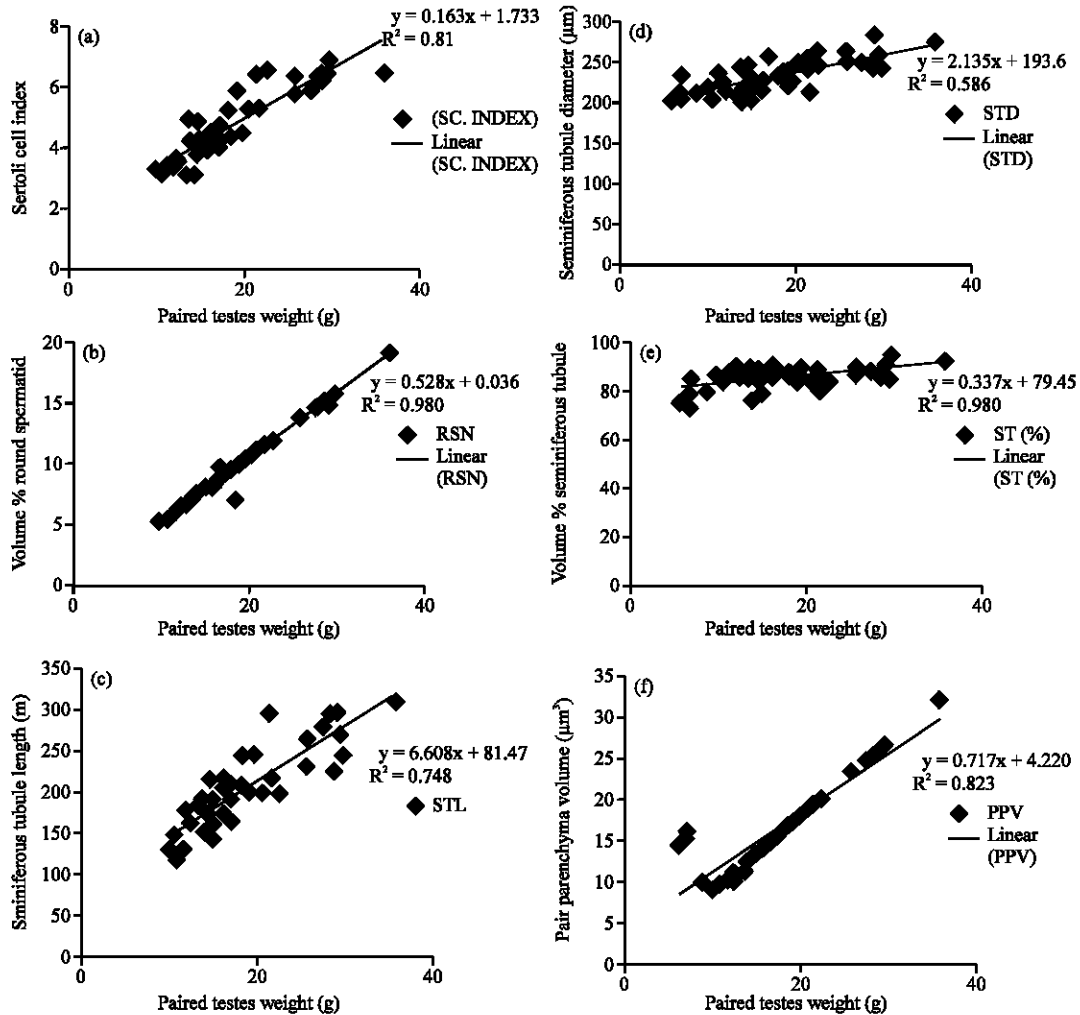


Fig. 1: (a-f) The relationship between Paired testes weight (X) and the Testicular histometric parameters (Y)

spermatogenesis hence the non-seasonal animals or birds would not exhibit significant variation in tubular diameter with season after sexual maturity (França and Russell, 1998).

The mean seminiferous tubule length showed significant ($p < 0.01$) breed difference 221.14 ± 24.79 m per paired testes in the bPR and 133.73 ± 10.98 m in the NIB. This report is in agreement with the 229 m reported by De Reviers and Brillard (1974) but significantly lower than the 529 m reported for turkeys (Noirault *et al.*, 2006). There is little or no parallel information on the histometric parameters of the non-descript Nigerian indigenous breed for comparative purposes. However, a critical look at the data obtained indicate that the mean seminiferous tubule length of the barred Plymouth Rock throughout the four seasons of the year remained approximately twice that of the NIB. There was also a two-fold increase in the volume

percent of the round spermatid nucleus in the bPR, and, since the diameter of the seminiferous tubule is an indicator of active spermatogenesis, then the spermatogenic activity of the NIB is 80% of the capacity of the bPR. This result confirms earlier report (Orlu and Egbunike, 2009) that the bPR has a three-fold superiority in sperm production and a two-fold sperm production efficiency over the NIB. Since the seminiferous tubule length is estimated from corrected testicular volume and volume percent of seminiferous tubules in the testes, animals that have larger testicular weight and volume percent of seminiferous tubule would definitely have longer seminiferous tubules. This deduction agrees with an earlier report that birds with heavier testes had higher daily sperm production (Orlu and Egbunike, 2009) as well as higher sperm reserves (Orlu and Egbunike, 2010). There was, however, no significant breed variation in the mean

seminiferous tubule length per gram of testicular weight (11.65±0.12 m) in the bPR and (12.48±1.04 m) in the NIB. Most mammalian species have about 10-20 m of seminiferous tubules per gram of testicular weight (Costa and Silva, 2006; Segatelli *et al.*, 2004; Leal *et al.*, 2004; Johnson, 1991; França and Russell, 1998).

The volume of round spermatogenic cells varied with the type and maturation phase of the cell. Spermatogonia A with a diameter of 6.00±0.15 µm and volume of 113.19 µm³ produces spermatogonia B (diameter 5.50±0.04 and volume 87.11 µm³). Once spermatogonia B becomes committed to meiosis at Preleptotene stage, the nuclear volume increases steadily from 47.14 µm³ through Leptotene (124.79 µm³), Zygotene (127.83 µm³), Pachytene (143.79 µm³) to Diplotene (147.14 µm³) for the first maturation division. A very transient diakinesis was observed at stage VIII (Orlu and Egbunike, 2009) to produce secondary spermatocytes for the second maturation division and formation of round spermatids. Thereafter, the nuclei volume reduced from 22.44 µm³ to 19.30 µm³ before elongation and maturation (Table 3). The volume of round spermatogenic cells was not affected by breed or season and the values observed are within the range reported for domestic fowl (De Reviere and Brillard, 1974). However, the largest spermatogenic cell observed in this study was the primary spermatocyte at Diplotene which is at variance with the Preleptotene reported for turkey (Noirault *et al.*, 2006).

The histometric parameters and the paired testes weight positively and significantly (p<0.001) correlated with the bodyweight. A regression analysis between the bodyweight (X) and paired testes weight (Y) showed that this relationship was linear and characterized by the equation $Y = 11.322X - 3.3929$, $r = 0.6356$ (p<0.001) $R^2 = 0.4041$ (p<0.01). Although this relationship is highly significant, differences in the paired testes weight could only account for 40.41% of the variation in the bodyweight of domestic fowl. This result agrees with an earlier report (Orlu and Egbunike, 2009; Raji *et al.*, 2008; Bitto *et al.*, 2008). The seminiferous tubule diameter and length were also positively correlated with the bodyweight and characterised by the following equations $Y = 33.232X + 169.62$, $r = 0.6692$, $R^2 = 0.4478$ (p<0.001) for the diameter and $Y = 72.567X + 57.531$, $r = 0.4855$, $R^2 = 0.2357$ for the length, respectively. Also, significant (p<0.001) and positively correlated (r = 0.6745) with the bodyweight was the volume % of round spermatids in the testes with a regression equation $Y = 5.5485X - 0.4065$, $R^2 = 0.455$, (p<0.001). The significant correlative relationship and the regression model indicate that these histometric parameters can be predicted from

the body weight of the birds. Bitto *et al.* (2008) reported similar results while working with the WAD goats. However, based on the coefficient of determination, only 23.57% of the variation in the seminiferous tubule length can be attributed to the differences in the bodyweight. The volume percent of seminiferous tubules has no relationship with the bodyweight as shown in the lack of correlation (r = 0.2612, p>0.05) and low coefficient of determination ($Y = 2.873X + 80.073$, $R^2 = 0.06$, p>0.05). This is in agreement with the observation that the volume percent of seminiferous tubule is species specific and cannot be predicted from bodyweight (Segatelli *et al.*, 2004).

A positive and highly significant (p<0.001) correlative (r = 0.90) relationship was observed between the Sertoli cell index and the paired testes weight. This relationship was linear and typified by the equation $Y = 0.1631X + 1.733$, $R^2 = 0.81$ (p<0.001) where X is the testes weight in grams, Y is the Sertoli cell index (Germinal cell/Sertoli cell ratio) (Fig. 1a). The Sertoli cell index reflects the functional capacity of the Sertoli cell to support germ cells in the seminiferous epithelium and is reported to be species specific. This result agrees with earlier reports (Berndtson *et al.*, 1987; França and Russell, 1998; Leal *et al.*, 2004; Costa and Silva, 2006; Segatelli *et al.*, 2004). Thus species in which Sertoli cell supports larger number of germinal cells tend to have heavier testes, hence, higher daily sperm production capacity (Berndtson *et al.*, 1987; Orlu and Egbunike, 2009). Similar linear relationships occurred between testis weight and volume percent occupied by round spermatids characterized by the equation $Y = 0.528X + 0.0364$, $r = 0.9904$ (p<0.001), $R^2 = 0.9809$ (p<0.001) (Fig. 1b). This result is quite similar to the value reported by De Reviere and Brillard (1974) for 32 weeks old cockerels raised under 16 hours photoperiod. Significantly high correlation coefficients and linear relationships between seminiferous tubule length, (r = 0.8651, p<0.001), seminiferous tubule diameter (r = 0.7658, p<0.001), Volume % seminiferous tubule (r = 0.5474, p<0.001) and paired parenchyma volume (r = 0.9073, p<0.001) were also observed in this study. The linear nature of the regression equations (Fig. 1c-f) indicate that larger and heavier testes would obviously be occupied by higher percentage of seminiferous tubules, possess greater tubular length as well as diameter, higher Sertoli cell supporting capacity and perhaps, higher daily production capacity. More so, since sperm production indicator histometric parameters such as Sertoli cell index, seminiferous tubule diameter, seminiferous tubule length/g of testicular weight and volume % of round spermatids can be predicted from both the body weights

and from known testicular weights valuable breeder cocks can be selected for breeding in commercial and research farms. Despite the hostile climatic environment of the humid tropics with year round high ambient temperature and relative humidity, season exerted no significant influence on the testicular histometric parameters, the volumetric proportions of testicular germ cells or their sizes. Thus, testicular histometry provides a reliable tool for the assessment of the reproductive state and potential sperm production capacity of the domestic fowl. Breeder cocks raised in the humid tropics with proper management can be used for year round breeding purposes without adverse effect on fertility.

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