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Breed and Seasonal Variations in the Testicular Morphometry, Gonadal and Extragonadal Sperm Reserves of the Barred Plymouth Rock and Nigerian Indigenous Breeds of the Domestic Fowl

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Abstract: Breed and seasonal influence on testicular morphometry, Gonadal Sperm Reserves (GSR), Extragonadal Sperm Reserves (ESR) was investigated in the barred Plymouth rock and the Nigerian indigenous breeds of the domestic fowl. Twenty sexually matured birds per breed were studied in the four seasons of the year. Breed significantly ($p<0.01$) influenced the body weight (2.11±0.05 vs.1.58±0.02), paired testes weight (21.92±1.46 vs. 12.43±0.91) and all other morphometric parameters with the barred Plymouth Rock showing superiority over the Nigerian indigenous breed. The GSR, ESR and their efficiencies (GSR/g, ESR/g) were highly and significantly ($p <0.01$) higher in the barred Plymouth rock. Season neither significantly ($P>0.05$) influenced the testicular morphometric parameters, GSR, ESR nor gonadal sperm reserve/gram testis (GSR/g). The higher numerical differences observed in morphometric parameters, GSR and ESR during the early and late dry seasons suggested that the dry season favors sperm production, hence sperm reserve potentials. The testicular weight was significantly ($p<0.001$) correlated not only to the body weight ($r = 0.63$) but to paired *Tunica albuginea* ($= 0.98$, $p<0.001$) and paired epididymal weight ($r = 0.69$, $p<0.001$). There was also a significant ($p<0.001$) and positive correlation between the testicular weight and GSR ($r = 0.84$, $p<0.001$). The GSR/g ($r = 0.77$; $p<0.001$) ESR ($r = 0.86$; $p<0.001$) and ESR/g ($r = 0.50$, $p<0.001$). Based on these observations it is concluded that genetic improvement of the Nigerian indigenous breed of the domestic fowl can be carried out by increasing their body weight, hence, testicular weight through crossbreeding with the heavier barred Plymouth rock to achieve improved sperm production and reserve for year round artificial insemination.

Key words: Breed, season, morphometry, sperm reserve, domestic fowl

INTRODUCTION

With increasing population in the developing countries, demand for animal protein also increases. Poultry meat and eggs present the most affordable source to mitigate the problem of protein malnutrition in Nigeria. For efficient and maximum production of chicks for meat and egg, a thorough understanding of the reproductive potential of the cock is invaluable, because, the importance of the breeder male for fertilizing eggs is rivalled only by his genetic influence on the progeny performance.

Morphometric analysis on the testis of any species or breed is necessary in assessing and estimating quantitative changes in testicular components and spermatogenic function arising from factors such as age, season, temperature and diseases (Egbunike *et al*., 1976). Nutrition also exerts some influence on testicular morphometric parameters and on gonadal sperm reserves in Corriedale rams (Bielli *et al*., 1997). For this reason, Gage and Freckleton (2003) described the mammalian testes as infallible predictors of spermatozoa production. They further asserted that knowledge of the basic morphometric characteristics of the reproductive organs is mandatory for assessment and prediction not only of sperm production but also of the storage potential and fertilizing ability of the breeder male. Direct counts of maturing spermatids in the testes have been carried out in various domestic animals and the results expressed as Gonadal Sperm Reserves (GSR). Extragonadal Sperm Reserve (ESR) represent sperm stored in the caput, corpus and cauda epididymides. Almquist and Amann (1961) developed a method for the estimation of GSR and EGR in bulls. This method with suitable modifications has been

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used to determine gonadal and extragonadal sperm reserves for various animal species: goats (Bitto and Egbunike, 2006a, b), rats (Adedapo *et al*., 2003), wild boar (Almeida *et al*., 2006), rabbits (Ogbuewu *et al*., 2009), mammals (Gage and Freckleton, 2003), partridge (Baraldi-Artoni *et al*., 2007), sheep (Bielli *et al*., 2000), donkey (Neves *et al*., 2002), domestic fowl (Obidi *et al*., 2008), Japanese quail (Clulow and Jones, 1982), bulls (Togun, 2009), rams (Gundogan, 2006), pigeons (Orsi *et al*., 2007) and elephants (Thongtip *et al*., 2008).

In mammalian species significant correlations have been reported between paired testes weights and body weight, sperm production and reserve potentials in boars (Gbore and Egbunike, 2008). Changes in testicular morphometry and sperm reserves due to seasons have been observed in domestic cats (Franca and Godinho, 2003) and camel (Al-Qarawi *et al*., 2001). Due to the paucity of information on the reproductive capacity of the Nigerian indigenous breed of the domestic fowl there has been little conscious effort on its genetic improvement programs. With this in mind this research was aimed at studying the seasonal variation in the testicular morphometry, Gonadal Sperm Reserves (GSR) and Extragonadal Sperm Reserves (ESR) in the barred Plymouth Rock (bPR) reared in most poultry farms and the Nigerian indigenous breeds of domestic fowl. This knowledge with the daily sperm production (Orlu and Egbunike, 2009) can be used to determine how often and for how long a cock can be used for mating without having any depression in herd fertility. Hence, a conscious effort can be made towards the genetic improvement of the indigenous breed through crossbreeding with the superior barred Plymouth Rock.

MATERIALS AND METHODS

Twenty sexually matured barred Plymouth Rock (bPR-Exotic) and twenty non-descript Nigerian Indigenous (NI -Local) breeds of fowl were used in this research. Of these, five of each breed that responded to lumber massage by ejaculation was used in each of 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).

The experiment was conducted in the Niger Delta region of Nigeria and covered a two year period between 2006-2008.

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

Morphometry: All the birds used per season, with their identifying labels in place, were sacrificed on the same day and taken to the laboratory for dissection. The reproductive organs were removed intact and the testes and epididymides separated, freed of all adhering connective tissues for morphometric analysis.

Paired testes weight/epididymal weight: The left and right testes of both the bPR and the NI breeds trimmed and freed of all adhering connective tissues were measured separately, as were the left and right epididymides and their weights recorded to the nearest 0.01 g.

Testis and epididymal volumes: The volumes of the testes and epididymides were determined by Archimedes' principle of water displacement and results recorded in cubic milliliters.

Testis density: The testis density was calculated from the testis weight and volume and expressed in g cc^{-1}.

Tunica albuginea weight: The *Tunica albuginea* was carefully removed from the testis after cutting the testis in halves longitudinally starting from the dorsal surface. This was then weighed and the value recorded to the nearest 0.01 g.

Gonadal indices: The body weights as well as the testes weights of all forty birds used in this investigation were obtained for the estimate of gonadal indices such as:

Relative testes weights: This is testes weight relative to the body weight:

$$\frac{\text{Paired testes weight}}{\text{Body weight}} \times 100$$

Relative epididymal weight: Ratio of epididymal weight to body weight:

$$\frac{\text{Paired epididymal weight}}{\text{Body weight}} \times 100$$

Relative epididymal weight: Ratio of epididymal to testes weight:

$$\frac{\text{Paired epididymal weight}}{\text{Paired testes weight}} \times 100$$

Relative *Tunica albuginea* weight: Ratio of *Tunica albuginea* weight to testes weight:

$$\frac{\text{Paired tunica albuginea}}{\text{Paired testes weight}} \times 100$$

Sperm storage potential: Measurement of Gonadal Sperm Reserves (GSR): The left and right testes removed after slaughter and weighed were freed of *tunica albuginea*. Known weight of both testes were homogenized separately, in physiological saline at 200 mg mL^{-1} according to Igboeli and Rakha (1971). The suspensions mixed and strained through a double layer of cheese cloth into graduated tubes and a dilution of 1:25 v/v was made for enumeration in a Neubauer haemocytometer (Egbunike *et al*., 2007).

Measurement of Extragonadal Sperm Reserves (EGR): The left and right epididymal regions were separately and completely macerated in normal saline at 100 mg mL^{-1} and diluted at 1:25 v/v for counting (Egbunike *et al*., 2007). All sperm reserves were expressed in billions.

Sperm storage efficiencies: The efficiency of gonadal and Extragonadal Sperm Reserves (GSR)/g were the total gonadal and extragonadal reserves divided by the testicular parenchyma and epididymal weights, respectively.

Statistical analysis: Data obtained were subjected to Students test (Steel and Torrie, 1996) for the assessment of breed and seasonal differences. The relationship between the morphometric parameters, gonadal and extragonadal sperm reserves were analyzed with linear regression.

RESULTS

The mean body weight of the bPR was significantly ($p<0.01$) higher than that of the NI breed. The heavier bPR also had statistically ($p<0.01$) larger mean gross testes weight, paired *Tunica albuginea* and paired parenchyma weight than the NI breed (Table 1). There was no significant difference in the mean testes density.

The gonadal indices, ratios of left to right reproductive organs and derivations from testes morphometry of the bPR and the NI breeds are shown in Table 2. Generally, the left reproductive organs appeared to be superior to the right in both breeds. All morphometric parameters and their derivations showed no significant ($p>0.05$) seasonal variation, however, the early dry season (Oct-Dec) had the highest numerical values of morphometric parameters considered

Gonadal sperm reserves determined for exotic bPR ($3.56\pm1.41\times10^9$) was significantly ($p<0.01$) higher than that of the NI breed ($1.56\pm0.38\times10^9$). The efficiency of sperm reserves GSR/g was, however, similar in both breeds (Table 3). The Extragonadal Sperm Reserves (ESR) ($6.36\pm1.65\times10^9$) were significantly ($p<0.01$) higher in the

Table 1: Breed and seasonal variations of the testicular morphometry of domestic fowl (bPR –Exotic and the NI-Local) in Southern Nigeria

		Seasons				
Parameters	Breed	Late dry	Early rain	Late rain	Early dry	Mean±SEM
Body weight (kg)**	Exotic	2.17±0.03	2.23±0.02	2.01±0.03	2.04±0.05	2.11±0.05
	Local	1.58±0.02	1.64±0.03	1.59±0.03	1.50±0.03	1.58±0.02
Testes weight (g)**	Exotic	21.42±2.21	21.32±2.96	20.79±2.51	24.18±4.96	21.92±1.46
	Local	10.54±1.71	10.76±2.12	8.91±1.52	12.48±1.91	12.43±0.91
Testes parenchyma (g)**	Exotic	20.33±2.28	20.31±2.78	20.21±2.26	22.33±4.69	20.79±0.40
	Local	10.05±1.61	10.29±1.99	8.24±1.28	11.92±1.80	10.12±0.82
Mean testis density (g/cc)	Exotic	1.05	1.05	1.05	1.05	1.05±0.00
	Local	1.05	1.04	1.04	1.05	1.04±0.00
Tunica albuginea (g)**	Exotic	1.09±0.14	1.02±0.18	1.08±0.13	1.23±0.28	1.11±0.18
	local	0.50±0.10	0.68±0.13	0.53±0.09	0.56±0.12	0.56±0.11
Epididymal region (g)*	Exotic	1.34±0.14	1.28±0.11	1.13±0.08	1.63±0.10	1.93±0.11
	Local	1.11±0.12	1.05±0.24	0.86±0.16	0.91±0.005	0.99±0.14

**Breed differences significant ($p<0.01$). * Breed difference significant ($p<0.05$). No significant seasonal variation observed. Values are Mean±SEM

Table 2: Breed influence on gonadal indices and derivations from morphometric parameters of the reproductive organs in the domestic fowl (bPR-Exotic and NI breed-Local)

Parameters	Breed	Left testis (LT)	Right testis (RT)	LT:RT ratios	Total
Testes weight/body weight	Exotic	0.54±0.04	0.48±0.03	100:88.89	1.02±0.07
(%)*	Local	0.45±0.04	0.41±0.03	100:91.11	0.85±0.07
Tunica albuginea	Exotic	0.03±0.01	0.02±0.01	100:85.71	0.05±0.02
weight (g) (%)*	Local	0.02±0.01	0.02±0.01	100:90.91	0.04±0.02
	Exotic	4.99±0.07	4.82±0.14	100:95.83	9.81±0.21
(%)**	Local	5.94±0.03	5.38±0.03	100:90.57	11.32±0.09
Epididymal weight	Exotic	0.04±0.001	0.03±0.001	100:80	0.07±0.002
(g) (%)*	Local	0.04±0.001	0.03±0.001	100:80	0.07±0.002
	Exotic	6.69±0.35	6.14±0.41	100:91.78	12.83±0.76
(%)**	Local	9.21±0.67	9.10±0.78	100:98.81	18.31±1.45

*Organ weight relative to body weight. **Organ weight relative to testes weight. Values are Mean±SEM

Table 3: Breed and seasonal variation in the Gonadal Sperm Reserve (GSR) and Extragonadal Sperm Reserves (ESR) of the domestic fowl (bPR -Exotic and the NI- Local breed)

		Seasons				
Parameters	Breed	Late dry	Early rain	Late rain	Early dry	Mean±SEM
GSR (X109)**	Exotic	3.38±0.87	2.92±0.29	3.11±0.42	4.83±1.22	3.56±0.70
	Local	1.38 ±0.28	1.32±0.17	1.68±0.19	1.62±0.44	1.50±0.27
GSR/g (X109)**	Exotic	156.51±22.75	150.65±19.46	154.74±6.25	186.42±26.29	164.50±18.69
	Local	137.94±11.05	124.65±15.19	118.07±4.40	125.03±18.42	128.00±12.27
ESR (X109)**	Exotic	5.93±1.11	5.71±0.54	5.93±0.72	7.88±1.73	6.36±1.65
	Local	2.80±0.57	2.62±0.34	3.26±0.38	2.81±0.69	2.87±1.10
ESR/g (X109)**	Exotic	4.50±0.80	4.68±0.60	4.46±0.64	4.27±0.39	4.48±1.18
	Local	2.57±0.53	2.88±0.37	3.01±0.47	3.07±0.80	2.88±0.54

**Values are significant ($p<0.01$). Values are Mean±SEM

Table 4: Correlation coefficients of morphometric parameters of the reproductive organs with gonadal and extragonadal sperm reserves in the barred Plymouth rock and Nigerian indigenous breed of domestic

	Dependent variables								
Independent variables	9	8	7	6	5	4	3	2	1
Body weight	0.53^{3}	0.39^{2}	0.60^{3}	0.54^{3}	0.35^{1}	0.65^{3}	0.07	0.64^{3}	-
Paired testes weight	0.50^{3}	0.84^{3}	0.86^{3}	0.84^{3}	0.69^{3}	0.98^{3}	0.03	-	
Testes density	-0.03	-0.03	-0.03	-0.03	-0.06	-0.06	-		
Paired *Tunica albuginea*	0.49^{2}	0.48^{2}	0.85^{3}	0.84^{3}	0.68^{3}	-			
Paired epididymal weight	0.68^{3}	0.40^{2}	0.68^{3}	0.67^{3}	-				
Gonadal sperm reserve	0.57^{3}	0.77^{3}	0.97^{3}	-					
Extragonadal reserve	0.64^{3}	0.74^{3}	-						
Gonadalsperm reserve/g	0.53^{3}	-							
Extragonadal reserve/g	-								

Only superscripted values are significant. [1]: Significant ($p<0.05$), [2]: Significant ($p<0.01$), [3]: Significant ($p<0.001$)

bPR than in the NI breed ($2.87\pm1.10\times10^{9}$). No breed differences were observed in the efficiency of Extragonadal Sperm Reserves (ESR/g). The values represent the total mean ESR as the epididymides were not differentiated into corpus, caput and caudal regions.

All morphometric parameters were highly correlated ($p<0.001$) with the body weight and with each other (Table 4) except the testis density. Gonadal Sperm Reserve (GSR) was significantly ($p<0.05$) correlated with body weight and highly correlated ($p<0.001$) with all morphometric characteristics except testis density ($r = -0.03$). Extragonadal reserves (ESR) were highly significantly ($p<0.001$) correlated with paired testes weight ($r = 0.86$), paired t*unica albuginea* ($r = 0.85$) and ESR/g ($r = 0.64$) (Table 4).

DISCUSSION

The mean body weight of the Nigerian Indigenous (NI) breed used compares favourably with those reported by Abu *et al.* (2006). Comparison of the morphometric parameters in the two breeds (bPR and the NI breeds) showed significant ($p<0.01$) differences in the paired testes weight, paired parenchyma and paired *Tunica albuginea* weights. No breed difference was observed in the mean testis density. Season also appeared to exert no significant influence on any of the morphometric parameters, the left to right ratios as well as their derivations. Bitto and Egbunike (2006a, b) reported similar observation in the West African dwarf bucks. Gonadal Sperm Reserves (GSR) and extragonadal Sperm Reserves (ESR) appeared to be breed dependent with the bPR having significantly ($p<0.01$) higher values than the NI breed. Obidi *et al.* (2008) obtained slightly higher values for the GSR/g in the Shikabrown red breed of the domestic fowl.

A significant ($p<0.01$) and positive correlation exists between the body weight and testicular weight and the testicular weight is highly significantly ($p<0.001$) correlated with other morphometric parameters as well as the left and right organ weights. This observation obtains in most breeding animals; goats (Bitto and Egbunike, 2006a, b); boars (Egbunike *et al.*, 2007; Gbore and Egbunike, 2008); domestic fowl (Orlu and Egbunike, 2009), wild boars (Almeida *et al.*, 2006), donkeys (Neves *et al.*, 2002) rats (Adedapo *et al.*, 2003) Corriedale rams (Bielli *et al.*, 1997, 2000) buck rabbits (Ogbuewo *et al.*, 2009).

The higher values of GSR and ESR observed in the barred Plymouth rock is associated with its significantly ($p<0.01$) higher body weight and testicular weight. This deduction is validated by the highly significant ($p<0.001$) correlation between the GSR and paired testes weight as well as the lack of significant difference ($p>0.05$) between the breeds in the efficiency of Gonadal Sperm Reserves (GSR)/g. In addition, the linear regression for paired testes

weight (Y) on the body weight (X) was Y = 11.48x-3.80, R^2 = 0.404, with a correlation value r = 0.64 ($p<0.01$) while a regression of GSR (Y) on Paired Testes Weight (PTW) X was Y = 0.93X+0.51, R^2 = 0.70566 (r = 0.84, $p<0.001$). The gonadal indices presented in Table 2 indicate that breed and season had no significant ($p>0.05$) influence on any gonadal index calculated. Nevertheless, it was interesting to note that in the forty cocks studied. The paired testicular weight was approximately 1% of the body weight; paired tunica albuginea about 10% of the paired testes weight and the epididymal weight approximately 15% of the paired testes weight.

The mean GSR 3.56±1.41 vs. 1.50±0.83 (Table 3) for the bPR and NI breeds, respectively, represent approximately 2 days sperm production (Orlu and Egbunike, 2009). The low correlative value of the GSR on the body weight (r = 0.39, $p<0.05$) does not permit accurate prediction of the GSR from the body weight. The Extragonadal Sperm Reserves (ESR) is a function of the Gonadal Sperm Reserves (GSR) which is, in turn, a function of the Daily Sperm Production (DSP) (Orlu and Egbunike, 2009). This relationship is reflected in the highly significant ($p<0.001$) and positive correlation between GSR and ESR (Table 4). The ESR in both the bPR (6.36±1.65) and the NI breeds (2.87±1.10) represent 3 days sperm production in both breeds. Season had no significant effect on the GSR, ESR or their efficiencies (GSR)/g and (ESR)/g. However, the trend observed in GSR and ESR indicate that the Early Dry Season (EDS) favours increased spermatogenic activities in domestic fowl resulting in higher GSR and ESR. The apparent lack of significant seasonal influence on testicular morphometry, GSR and ESR is shows that the domestic fowl is not a seasonal breeder.

Since, the testicular weight is highly significantly ($p<0.001$) related to gonadal and extragonadal sperm reserves the possibility exists that daily sperm production, GSR and ESR could be accurately estimated from testicular weight. Hence, genetic improvement of the Nigerian indigenous breed should involve a deliberate effort at increasing the body weight, hence, the testis weight by crossbreeding it with the heavier superior barred Plymouth rock for increased sperm production and reserves.

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