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Sperm Reserves and Morphometric Analysis of Reproductive Organs in Anak 2000 Broiler Breeder Cocks

Uchechukwu, Nneka Victoria S.[1*], Odirichukwu, Ebere Obiageli [1], Rekwot, Peter I. [2], Ate Utim I. [3], Ogwu, David[4]

[1](Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria)
Corresponding Author[2]
(Animal Reproduction Research Programme, National Animal Production Research Institute, Ahmadu Bello University, Zaria, Nigeria)
[3](Department of Veterinary Surgery and Theriogenology,College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria).
[4](Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.)

Abstract: *The present study was conducted to determine the morphometry of the testes, the epididymides, and the sperm or spermatid reserves of the adult Anak 2000 broiler breeder cocks. Ten adult cocks were sacrificed following ten weeks of semen profile analysis. Their left and right testes as well as left and right epididymides were carefully excised and used for the study. The testicular and epididymal lengths and weights were taken. Testicular and epididymal sperm/spermatid reserves were also determined. The mean length and weight of the left and right testes were 5.60 ± 0.28 cm, and 5.42 ± 0.30 cm, and 21.93 ± 2.14 g and 21.27 ± 1.97 g, respectively. The mean length and weight of the left and right epididymides were 4.58 ± 0.29 cm and 4.40 ± 0.23 cm, and 1.03 ± 0.15 g and 1.19 ± 0.18 g, respectively. The mean left and right testicular, and left and right epididymal reserves were 82.18 ± 8.49 x 10^9/g testis and 76.47 ± 8.92 x 10^9/g testis, and 1.63 ± 0.28 x10^9/g epididymis and 1.99 ± 0.31 x10^9/g epididymis, respectively. No significant difference (P > 0.05) was observed between the left and right organs though the left testis appeared higher in weight and length than the right. Similarly, no significant difference (P > 0.05) existed between the mean left and right testicular reserves. Correlation analysis showed strong positive relationship between testicular weight and testicular sperm/spermatid reserves (r = 0.93, P < 0.05), testicular length and testicular sperm/spermatid reserves (r = 0.60, P < 0.05), and epididymal length and epididymal sperm/spermatid reserves (r = 0.55, P > 0.05). This study suggests that testicular size relates to sperm production.*

Keywords: *Anak 2000 broiler breeder, morphometry, sperm/spermatid reserves, testis*

I. Introduction

The knowledge of the morphometric characteristics of the mammalian testes is necessary for the assessment and estimation not only of sperm production but also of the storage potential and fertilizing ability of the breeder male [1]. Morphometric analysis of the testis is essential since gonadal development, spermatogenesis, changes in testicular components and spermatogenic functions are affected quantitatively by such factors as age, season, temperature, photoperiod, nutrition, diseases [2, 3, 4, 5, 6]. Studies have shown that testicular development and size in domestic animals are related to their short and long-term reproductive functions [5, 6]. Significant correlations have been reported between paired testes weights and body weight, sperm production and reserve potentials in boars [7]. This knowledge can be used, therefore, to determine how often and for how long a cock can be used for mating without having any depression in herd fertility [8]. The Anak 2000 broiler breeds which originated from Israel are characterized by more breast meat, early adaptation to hot weather and excellent livability [8]. A study of their reproductive organ morphometry and reserve potentials is necessary if they would be efficiently used for breeding purposes in Nigeria. Thus, the present study was designed to determine the morphometric analysis of the testes and epididymis in the Anak 2000 broiler breeder cocks, and correlating the results to the sperm/spermatid reserve in the cocks.

II. Materials And Methods

2.1 Experimental Site and Management of Birds

The study was conducted at the National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria, located in the Northern Guinea Savannah (11ºN and 12ºN, 7ºE and 8ºE) at an elevation of 650 m above sea level. The area has average annual maximum and minimum temperatures of 31.8 ±3.2ºC and 18.0 ±3.7ºC, respectively. The average monthly rainfall during the rainy season (June – November)

is 148.1± 68.4 mm (69.2 - 231.9 mm), while mean monthly relative humidity is 71.1± 9.7 %. The location has three seasons; the harmattan (November - February), the hot – dry (March - May) and the rainy (June – November) seasons [10]. The study was carried out during the rainy season.

Ten out of twenty adult Anak 2000 broiler breeder cocks whose semen characteristics were analysed for ten weeks prior to this study were randomly selected and used for this work. The birds were obtained from the Poultry Research Programme Unit of the institute, NAPRI. Each cock was tagged for easy identification and acclimatized for a period of two weeks during which they were monitored and screened appropriately. They were housed in deep litter under intensive system of management, fed restrictedly (once daily) with standard NAPRI breeders' mash containing 17 % crude protein. Water was provided ad libitum. The cocks were weighed in kilogrammes (live weights) using a mechanized weighing scale (Hana®) with sensitivity of 0.1. Thereafter, the birds were sacrificed, and their gonads carefully excised and individually marked for this study.

2.2 Testicular and Epididymal Measurements

The procedure for the study was carried out as described and modified by [11]. Each of the testes (right and left) was excised from each cock, and the epididymis was carefully separated from the testes using a scapel blade. Each testis was marked and weight determined in grammes using an electronic chemical balance (Citizen®) to the nearest 0.001. The total weight of the paired testes obtained for each cock was further used in calculating its percentage weight per live weight of the cock as described by [8] in (1):

$$\frac{\text{Weight of the paired testes}}{\text{Live weight of the cock}} \times 100\ \%$$

The length of each testis was also determined along the longitudinal axis using a measuring tape in centimeters. Similarly, the length of each epididymis was taken using a measuring tape in centimeters and the weight obtained in grammes using the electronic chemical balance.

2.3 Testicular and Epididymal Sperm/Spermatid Reserve Study

The Tunica albuginea from each marked testis was removed using a scapel blade. Each testis was homogenized in 20 ml of normal saline solution containing antibiotics using a high speed blender at full speed for about 2 minutes. The homogenate was transferred into a measuring cylinder. The blender container was rinsed with 50 ml of saline solution and added to the homogenate and the new volume was measured. Thereafter, 5 ml of the homogenate was transferred to a conical flask and further diluted with 30 ml of the saline solution. The mixture was stored overnight at 5 °C to let out sperm cells. The next day, the mixture was filtered through gauze and 5 ml of the filtrate used for testicular sperm/spermatid reserve determination. The sperm/spermatid concentration was determined using the erythrocyte counting chamber of the haemocytometer, according the method of [12]. Sperm cells and spermatids were counted diagonally from top left to bottom right in five large squares, and the reserves determined according to the standard method of [13] and [11].

The epididymides were each minced separately in 10 ml of normal saline solution containing antibiotics with sharp scissors and stored overnight to let out the sperm cells. The mixture was then filtered through guaze and the volume of the filtrate taken. Thereafter, 0.5 ml of the epididymal filtrate was diluted with 1 ml of the saline solution and used for epididymal sperm/spermatid reserve determination. Using the erythrocyte counting chamber of the haemocytometer, as described by [12], the sperm/spermatid concentration was determined.

Sperm cells and spermatids were counted diagonally from top left to bottom right in five large squares according to the method of [11]. The determination of sperm and spermatid reserves was done according to the standard method of [13] and [11].

III. Data And Statistical Analyses

The measurements for the right and left testes, and the right and left epididymides, respectively were presented as mean ± S.E.M (Standard Error of Mean) and compared using the paired t – test. The weight of the paired testes in relation to the live weight of the cocks was presented as range in percentage and as mean ± S.E.M. The testicular and epididymal sperm/spermatid reserves from the right and left testes and from the right and left epididymides, were presented as mean ± S.E.M and appropriately compared using the paired t– test. Pearson correlation analysis was also carried out to determine the relationship between the sperm/spermatid reserves and their corresponding organ measurements. All the results were analyzed using The Analyse –it version 2.21 Excel 12+ (2008). Values at $P < 0.05$ were considered to be significant.

IV. Results And Discussion

4.1 Testicular and Epididymal Parameters of Anak 2000 Broiler Breeder Cocks

Percentage weight of the paired testes per live weight of the cock using (1) gave a range of 0.41 – 1.11 % and a mean ± S.E.M value of 0.8 ± 0.07 % in this study. Mean ± S.E.M weight of the cocks was 5.64 ± 0.10

kg. Percentage weight of the paired testes per live weight of the cock obtained from the present study agreed with the findings of [14]. It also compared favourably with an earlier report by [15] who stated that in sexually active cocks, the testis made up to 1 % of the body weigh

The mean values of the testicular and epididymal parameters are shown in Table 1. Comparisons of the left and right organ parameters (weight and length) showed that the left organs appeared higher in the parameters taken that the right except for epididymal weight, though with no statistical significance ($P > 0.05$). This observation agrees with the findings of [14] from their work on Sahel strains of the local cocks in Nigeria, and that of [16] on Ross broiler breeder cocks.

The mean testicular length measurement obtained in this study is in agreement with earlier reported range of 3.2 – 5.60 cm [17] but higher than that reported by [14] which can be attributed to breed variation.

Table 1: Testicular and Epididymal Measurements of Anak 2000 Broiler Breeder Cocks (n=10)

Parameters	Mean (± S.E.M) Value		
	Left	Right	Mean Difference
Testicular weight (g)	21.93 ± 2.14^a	21.27 ± 1.97^a	0.65 ± 0.17
Testicular length (cm)	5.60 ± 0.28^a	5.42 ± 0.30^a	0.18 ± 0.06
Epididymal weight (g)	1.03 ± 0.15^a	1.19 ± 0.18^a	-0.16 ± 0.04
Epididymal length (cm)	4.58 ± 0.29^a	4.40 ± 0.23^a	0.18 ± 0.05

Means within rows with different superscripts are significantly different ($P < 0.05$).
S.E.M. = Standard error of mean

4.2 Testicular and epididymal sperm/spermatid reserves of Anak 2000 Broiler Breeder Cocks

The mean values of the testicular and epididymal sperm/spermatid reserves of Anak 2000 broiler breeder cocks are shown in Table 2 below. There was no significant difference ($P > 0.05$) between the left and right sperm reserves for each of the organs.

Table 2: The Mean Values of Left and Right Testicular and Epididymal Sperm/Spermatid Reserves of Anak 2000 Broiler Breeder Cocks (n = 10)

Parameter	Mean (± S.E.M) Value		
	Left	Right	Mean difference
Testicular sperm/spermatid reserves ($x10^9$/g testis)	82.18 ± 8.49^a	76.47 ± 8.92^a	5.71 ± 8.91
Epididymal sperm/spermatid reserves ($x10^9$/g epididymis)	1.63 ± 0.28^a	1.99 ± 0.31^a	0.37 ± 0.45

Within rows, means with different superscripts are significantly different ($P \leq 0.05$).
S.E.M. = Standard error of mean

The mean values for the testicular sperm/spermatid reserves obtained in this study did not compare favourably with the findings of [8] in their study on barred Plymouth Rock broiler breeders. However, the values obtained for the left testicular sperm/spermatid reserves in the present study showed some similarities to that obtained by [18] in the left testis of Shika Brown Red cocks. Lack of significant difference in the sperm/spermatid reserves of the left and the right testes and between the left and right epididymides in this study was contrary to the findings of [18] who reported that the left organ reserves significantly differed from the right in Shika brown Red and Shikabrown White layer strains. The disparity in these results may be due to breed variation.

4.3 Correlations between Organ Parameters and the Sperm/Spermatid Reserves of Anak 2000 Broiler Breeder Cocks

Correlation analysis of the organ parameters (testicular length and weight; epididymal length and weight) and the sperm/spermatid reserves (testicular and epididymal) is presented in Table 3. The analysis showed positive correlations between testicular sperm/spermatid reserves and testicular weight ($P < 0.05$); testicular sperm/spermatid reserves and testicular length ($P<0.05$); epididymal sperm/spermatid reserves and epididymal length ($P > 0.05$).

The positive relationship observed between the testicular parameters (weight and length) and testicular sperm/spermatid reserves in this study agreed with the reports of other authors who further stated that

testes size positively relate to sperm production [19, 20, 21]. The positive relationship observed between the epididymal length and epididymal sperm/spermatid reserves was expected since the epididymis in the avian species serves as a transport organ for spermatozoa.

Table 3: Correlation Coefficients of Testicular and Epididymal Parameters and the Sperm/Spermatid Reserves of Anak 2000 Broiler Breeder Cocks (n = 10)

Parameter	Testicular weight (g)	Testicular length (cm)	Epididymal weight (g)	Epididymal length (cm)	Testicular sperm/ spermatid reserves ($x10^9$/g testis)	Epdidymal sperm/ spermatid reserves ($x10^9$/g epididymis)
Testicular weight (g)	-					
Testicular length(cm)	0.60	-				
Epididymal weight (g)	0.12	0.24	-			
Epididymal length (cm)	-0.19	-0.30	0.71[a]	-		
Testicular sperm/ spermatid reserves ($x10^9$/g testis)	0.93[a]	0.60	0.21	0.18	-	
Epdidymal sperm/ spermatid reserves ($x10^9$/g epididymis)	0.15	-0.27	0.35	0.55	0.02	-

[a] Values within rows with superscript indicate statistical significance ($P < 0.05$)

V. Conclusion

There was no statistical significant difference between the left and right testicular parameters though the left testis appeared higher in weight and length. No significant difference was obtained in the sperm/spermatid reserves of the left and the right testes as well as in the sperm/spermatid reserves of the left and right epididymides. Positive and significant relationship was observed between the testicular parameters (weight and length) and testicular sperm/spermatid reserves in the present study. This points out the fact that reproductive organ morphometry especially when done uninvasively could be used to assess the breeding potential of any breeder cock.

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