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Correlation Between Testicular Biometrics and Serum Level of Reproductive Hormones of Arewa Stallions Crossbred in Ilorin, Nigeria

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ABSTRACT

The hypothalamic-pituitary-gonadal axis (HPG) controls the reproductive physiology activities of stallions. The organs involved in this axis are the hypothalamus, the pituitary, and the gonads (testes), and the endocrine secretions from these organs. This study determined the correlation between testicular measurements and reproductive hormones of cross Arewa stallion in Ilorin. Forty-three crossed Arewa breeds of stallions were sampled. Testicular measurements (left and right testicular width, length, height, total testicular volume, and total testicular weight) were taken for each sample. Serum levels of sex hormones (testosterone, luteinizing hormone, follicle-stimulating, thyroid-stimulating hormone, and estradiol) were analyzed. The data obtained were presented as Mean±SD. Thereafter, the Pearson coefficient correlation between testicular biometrics and serum level of sex hormones was determined using SPSS® 16.0. The mean of the total testicular volume, the total testicular weight, and the gonadosomatic index were 400.80cm^3, 1196.80cm^3, and 301.20kg/cm^3 respectively. There is a statistically significant correlation between testicular measurement and gonadosomatic index ($p<0.05$).These findings provide the testicular biometric data of cross Arewa stallion and can be used to determine the reproductive traits and or reproductive efficiency.

Keywords: Testicular biometrics; Reproductive hormones; Stallion; Arewa; Ilorin

INTRODUCTION

Management of stallions is concerned with maintaining a healthy horse that meets the expectations of a breeding animal (Lemma *et al.*, 2015). Stallion breeding soundness examination is a fundamental segment

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of the reproductive activity of a stallion which has a direct influence on the overall equine reproduction (Davies and Mina, 2015). This is because a well-proven stallion can serve at least more than five mares per year (Storer, 2002). Testicular biometrics involves the measurement of testicular length, width, height, circumference, volume and the gonadosomatic index. Evaluation of these parameters is very crucial in stallions' selection for breeding. This is because it is an indirect determining factor of their reproductive potential. Matured stallions' testes are averagely capable of producing approximately 18–20 million sperms per gram of testicular tissue per day (McCue, 2014; Rua *et al.*, 2017). Testicular biometrics is indicative of testicular parenchymal tissue which determines sperm output through spermatogenesis. The process of spermatogenesis is being controlled by the male sex hormones through the hypothalamic-pituitary-testicular (HPT) axis (Dwyer and Quinton, 2019). The gonadotropic hormones (luteinizing hormone and follicle-stimulating hormone) stimulate the Leydig and Sertoli cells respectively. While the Leydig cell produces testosterone, the Sertoli cells nourish and support the developing spermatozoa. Research performed in the last three decades has provided a great deal of scientific information on normal testicular size, spermatogenic efficiency (including sperm production rates), and sperm output in stallions(Blanchard *et al.*, 2008).

However, the relationship between the testicular biometrics and the serum level of reproductive hormones is yet to be established in stallions. In addition, there is a dearth of information on the testicular biometrics of Arewa stallions in Ilorin, Nigeria. The aim of the present study was therefore to determine testicular biometrics of Arewa stallions in Ilorin and evaluate their relationship with the serum levels of reproductive hormone(Davies and Mina, 2015; Lemma *et al.*, 2015).

MATERIALS AND METHODS

Geographical Location

This study was conducted in Ilorin, Kwara State, Nigeria. Ilorin is the capital of Kwara State and one of the largest cities in the North-central part of Nigeria(National Bureau of Statistics, 2017). Ilorin is located on latitude 8.4799 North-Central, Nigeria. It is situated 320 meters above sea level. Horses are one of the important heritages of the monarchies (emirates) in the northern part of the country including the Ilorin emirate dated back to the pre-colonial era (Law, 2018). These include cultural activities like the Durbar festival where different horse skills are displayed. Also, horse racing and polo game are sports that involved the use of horses, horses are also kept as exotic pets. Ilorin is advantageously located and serves as a gateway between the Northern and

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Southern areas of the country which makes it accessible to all parts of the country by air, road, and rail transportation (Abiodun and Gbenga, 2016).

In Nigeria, Arewa and their crosses with Arabian, Dongola and Sudanese breeds are the major breeds of horse in the country, which are conventionally grown in Northern Nigeria and are used for cultural ceremony, draft, transportation, sports, and research purposes. Their phenotype varies depending on their pedigree but they usually have black, brown colors with white markings on the head and limbs (Ihedioha and Agina, 2013; Garba *et al.*, 2015; Agina and Ihedioha, 2017).

Experimental Stallion

Healthy forty-three healthyArewa stallions crossbred (362.10±40.25kg) between the age of 5-18 years old (7.95±2.62 years) were considered for this study. They were from different stables owned by the Emir; the royal chieftaincies, Nigeria Army Sobi Barracks, and the Nigeria Police Force (mounted troop) within Ilorin metropolis, Nigeria. All the procedures which include blood collection, body weight measurement, and testicular measurement were done ethically under physical restraint. The ethical review committee of the Faculty of Veterinary Medicine, University of Ilorin, approved and endorsed the research study with approval reference number: UERC/FVM/2021/011.

Testicular Measurement

Testicular measurements were carried out as previously described by (McCue, 2014). Then, the measurement was used to determine the testicular volume. Each of the testes was measured separately using a digital caliper (Shanghai shenhan®) to determine the height, length, and width of the testis(Kavak *et al.*, 2003; McCue, 2014).

The stallion testicle has an ellipsoid shape because stallion testis closely resembles the three-dimensional shape of an ellipsoidal shape(Love *et al.*, 1991; Blanchard *et al.*, 2008). Thus, the testicular volume (in cubic centimeters) of each testis was determined using the following formula:

Testicular volume (cm^3) = $4/3 \times \pi \times Length\ (cm)/2 \times Width\ (cm)/2 \times Height\ (cm)/2$

Testicular volume (cm^3) = $0.5233 \times Length\ (cm) \times Width\ (cm) \times Height\ (cm)$

The total testicular volume (TTV) was calculated by summing the right and left testicular volume (Kavak *et al.*, 2003; McCue, 2014);

Total testicular volume (TTV) = $TVr + TVl$

Testicular Weight and Gonadosomatic Index

Testicular weight for each of the stallion was calculated from the formula for a prolate spheroid. given as: $4/3 \times \pi \times a^2 \times b$ where a is half the diameter of the testis at its widest

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prolate spheroid and b is half its length (Almeida*et al*., 2006; Eljarah *et al*., 2017). Thus,

Testicular weight(cm^3) = $4/3 \times \pi \times a^2 \times b$

The gonadosomatic index (GSI) was calculated for each of the stallion using the formula: GSI = *(Testicular weight/Stallion weight)* ×*100* (Almeida*et al*., 2006).

Blood Collection and Serum Preparation

Procedure for blood collection was done (under physical restraint) through venipuncture of the jugular vein. Five milliliter of blood sample was collected aseptically from each of the horses and dispensed into a sterile plain bottle for hormonal analysis. Blood samples collected from each of the stable were transported to the laboratory under a regulated temperature using an insulated cold box (12°C-20°C). This is to prevent the effect of excessive wavering of the environmental temperature that may be encountered during the period of transportation (Ihedioha and Agina, 2013; Barrelet and Ricketts, 2015).

Hormonal Assay

Selected reproductive hormones which have major role in the hypothalamic-pituitary-testicular (HPT) axis of stallion were analyzed using enzyme-linked immunoassay kit (Calbiotech®), for testosterone, prolactin, Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), estradiol,and Thyroid-Stimulating Hormone (TSH) to determine the serum level of each hormone.

Statistical Analysis

All data collected were summarized and tabulated using Microsoft® Excel 2019 (Redmond, VA, USA). Data analysis was done using Statistical Package for Social Sciences (SPSS) version 2016 (IBM Corp., Armonk, N.Y., USA). The data analysis done includes Primary Testicular parameters (LTW, LTL, LTH, RTW, RTL, and RTH) and the Derived Testicular parameters (TTV, TTW, and GSI) which were analyzed to check for the descriptive statistics i.e., mean, standard error of mean, minimum and maximum of each sample; which was presented in tables.

Pearson correlation coefficients between serum level of the hormones with the Primary testicular parameters as well as with the Derived testicular parameters.

RESULTS

Results of the testicular biometrics of crossbred Arewa stallions

The mean, standard deviation, minimum, and maximum of the primary and derived testicular parameters were presented in Table 1. The mean of left testicular width, length, and height were 5.01, 6.86, and 10.96 cm. While the mean of right testicular width, length, and height were 5.10, 6.69, and 10.99 cm. The mean oftotal testicular volume (TTV), the total testicular weight, and the gonadosomatic index (GSI) were 400.80 cm^3, 1196.80 cm^3, and 301.20 % respectively.

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Table 1. Primary testicular and derived biometric parameters of Arewa stallions.

Parameters	N	Mean±SD	Minimum	Maximum
LTW (cm)	43	5.01±0.64	3.84	6.39
LTL (cm)	43	6.86±1.12	4.70	9.60
LTH (cm)	43	10.96±1.27	8.62	13.62
RTW (cm)	43	5.10±0.76	3.18	7.42
RTL (cm)	43	6.69±1.23	4.23	10.11
RTH (cm)	43	10.99±1.48	8.56	16.60
TTV (cm^3)	43	400.80±116.80	205.49	722.16
TTW (cm^3)	43	1196.80±335.00	645.54	2117.45
GSI (kg/cm^3)	43	301.20±36.59	223.30	409.35

Notes : LTW = left testicular width; LTL = left testicular length; LTH = left testicular height; RTW = right testicular width; RTL = right testicular length; RTH = right testicular height; TTV = Total Testicular Volume; TTW = Total Testicular Weight; GSI = Gonadosomatic Index

Results of the serum levels of reproductive hormones of crossbred Arewa stallions

Reproductive hormones analyzed were testosterone, FSH, LH, thyroid-stimulating hormone, prolactin, and estradiol. Table 2 shows the serum level of testosterone, LH and FSH in Arewa stallions crossbred which were 0.16±0.08 ng/mL,0.14±0.02 mIU/mL and 0.64±0.11 mIU/mL respectively. Correlation of testicular biometric parameters with serum reproductive hormones in Arewa stallions crossbred were shown in Table 3.

Although there was no significant correlation between serum concentration of testosterone and testicular biometrics ($p>0.05$), the TTW and TTV show the highest correlation coefficient. No significant ($P>0.05$) correlation between testicular biometrics and serum level of follicle-stimulating hormone,luteinizing hormone and estradiol.

A negative correlation was observed between the serum level of prolactin and testicular biometrics parameters (LTW, LTL, LTH, RTH, and TTV). The left testicular width (LTW) showed a significant ($P<0.05$) correlation with TSH serum level.

Table 4 shows that there is significant association between the left and right testicular length with the gonadosomatic index. As well as negative correlation in LTL, LTH, RTL, RTH and TTV with the gonadosomatic index (P value < 0.05).

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Table 2. Serum level of reproductive hormones of Arewa stallions

Hormones	N	Mean±SD
Testosterone (ng/ml)	43	0.16±0.08
Prolactin (ng/ml)	43	0.29±0.05
LH (mIU/ml)	43	0.14±0.02
FSH (mIU/ml)	43	0.64±0.11
TSH (mIU/ml)	43	0.09±0.02
Estradiol (pg/ml)	43	13.40±2.95

Notes : LH = Luteinizing Hormone; FSH = Follicle-Stimulating Hormone; TSH = Thyroid-stimulating hormone.

Table 3. Pearson correlation coefficients between serum level of reproductive hormones and testicular biometric parameters of Arewa stallions crossbred

Parameters/ Hormones	Testosterone		FSH		LH		Prolactin		TSH		Estradiol	
	R	p value	R	p value	R	p value	R	p value	R	p value	R	p value
LTW (cm)	0.16	0.32	0.17	0.28	0.07	0.67	-0.04	0.83	0.30	0.05*	0.03	0.86
LTL (cm)	0.13	0.39	0.26	0.07	0.15	0.32	-0.17	0.29	0.27	0.07	0.03	0. .87
LTH (cm)	0.19	0.21	0.11	0.47	0.03	0.83	-0.03	0.83	0.11	0.47	0.12	0.46
RTW (cm)	0.19	0.21	0.12	0.44	-0.28	0.07	0.15	0.35	0.12	0.46	-0.09	0.56
RTL (cm)	0.11	0.50	0.21	0.19	-0.13	0.42	0.07	0.67	-0.05	0.77	-0.07	0.66
RTH (cm)	0.17	0.19	0.05	0.77	0.09	0.55	-0.07	0.67	-0.12	0.44	0.09	0.58
TTW (cm^3)	0.25	0.11	0.22	0.17	-0.08	0.59	0.03	0.87	0.19	0.21	0.02	0.91
TTV (cm^3)	0.21	0.17	0.28	0.07	-0.03	0.83	-0.03	0.87	0.15	0.35	0.02	0.91

Notes : *Significant at $p<0.05$. LTW = left testicular width; LTL = left testicular height; RTW = right testicular width; RTL = right testicular length; RTH = right testicular height; TTW = total testicular weight; TTV = total testicular volume.

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Table 4. Pearson correlation coefficients between Gonadosomatic index and Testicular biometric parameter of Arewa stallions crossbred.

Parameters	R	P value
LTW (cm)	0.133	0.397
LTL (cm)	-0.395*	0.009*
LTH (cm)	-0.054	0.732
RTW (cm)	0.205	0.188
RTL (cm)	-0.598	0.0*
RTH (cm)	-0.031	0.846
TTW (cm^3)	0.128	0.415
TTV (cm^3)	-0.263	0.089

Notes : *Significant at $p<0.05$. LTW = left testicular width; LTL = left testicular height; RTW = right testicular width; RTL = right testicular length; RTH = right testicular height; TTW = total testicular weight; TTV = total testicular volume.

DISCUSSION

The mean testicular length of crossbred Arewa stallions sampled was shorter (left 6.8 cm, right 6.6 cm) than in full-size horses (left 10.3 cm, right 10.8 cm) (Kavak *et al.*, 2003). This is due to the average weight size and weight of Arewa horses.

In the breeds of stallions sampled, no significant left-right differences were found in the testicular measurements which are by earlier studies of full-size horses, (Thompson *et al.*, 1979) found that the right testis was longer than the left testis. This iterates the results in this study for the mean testicular height (right 10.99cm, left 10.95). However, (Thompson *et al.*, 1979)found that the left testis was wider which is also in tandem with this study. The explanation for the left-right difference of testis size is not well fully understood (Thompson *et al.*, 1979; Kavak *et al.*, 2003).

There was no significant correlation between the testicular measurement and reproductive hormones (testosterone, prolactin, LH, FSH, and estradiol) ($p>0.05$). Although a positive and significant correlation between LH and testosterone with the testicular measurement would be expected. However, the correlation between testicular size and reproductive hormone most especially testosterone could be affected by some factors. This response may be a consequence of multiple alterations in the Leydig cell's function and seasonal variation which were not assessed in this study(Ansari *et al.*,

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2007; Rua *et al.*, 2017). Thus, this explains the non significant correlation observed in this study.

Estradiol and prolactin secretion increase simultaneously with a decrease in testosterone and LH secretion in mare which is the opposite in the hormonal study of a stallion (Thompson and Oberhaus, 2015). This repeats the negative correlation seen between both prolactin and estradiol serum levels with the testicular measurement observed from this study. Prolactin secretion could also be affected by season, dopaminergic and antidopaminergic agents, exercise and stressful stimuli, meal feeding, estrogen treatment, and antiopioidergic agents in the horse (Thompson and Oberhaus, 2015).

The significant correlation between the gonadosomatic index and testicular measurement in this study recapitulated the correlation from a study done on Arabian oryx (Eljarah *et al.*, 2017). However, there is little or no study relating the GSI with the testicular measurement in the stallion.

In this study, all stallions were adults within the age of 5 years and above. Therefore, differences in age in testicular measurement could not be observed. However, (Blanchard *et al.*, 2008)stated that the testicular measurement specifically the testicular volume has different values for different age groups. Another consideration is the fact that stallions are seasonal breeders of which the testicular size varies with the season of the year (Blanchard *et al.*, 2008; Ali and Derar, 2015) and the variation in the season was not considered in this study. Nevertheless, studies have indicated that stallions under tropical conditions, stallions show less reproductive seasonality than stallions in temperate zones (Leme, Papa and Roser, 2012).

CONCLUSION

The current findings from this study will provide valuable data and a stepping stone for future research on the reproductive status of Arewa crossed bred stallion within the country and the sub-Saharan region at large. The relationship between gonadosomatic index and testicular parameters is a good indicator in determining the reproductive status of stallion. This is a quick and reliable method to assess the reproductive status of stallion without going through rigorous sperm analysis.

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