Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2018.15070.1332

Testicular Morpho-histometry and Semen Quality of Three Strains of Chickens

Ochai DE[1], Kalla DJU[1], Saleh B[2] & Mancha YP[1]

[1]Department of Animal Production, Abubakar Tafawa Balewa University Bauchi, Nigeria
[2]Department of Animal Science, Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria

Poultry Science Journal 2018, 6(2): 173-179

Keywords
Bantam
Isa brown
Sperm motility
FUNAAB-alpha
Testicular morphology

Corresponding author
Babangida Saleh
bbsaleh75@gmail.com

Article history
Received: July 23, 2018
Revised: October 8, 2018
Accepted: October 30, 2018

Abstract

A study was conducted to compare the testicular morphology and semen quality of three strains (FUNAAB-Alpha, ISA brown and Bantam) of chickens. A total of 45, thirty four week old cocks were used for the six months study. Semen samples were collected weekly by abdominal massage technique and semen volume, colour, pH, motility, percentage live, normal and sperm concentration were evaluated. Testes and Vas deferens were collected from all the cocks at the end of the experimental period and their gross morphological data recorded. Strain influenced ($P < 0.05$) gross testicular morphology. Weights of paired testes were 23.98, 23.23, and 11.80 g for the FUNAAB-alpha, Isa brown and Bantam, respectively. Testicular weight (paired, left, and right) and lengths were consistently lower ($P < 0.05$) for the Bantam. Testicular width was similar among the three strains. Gross motility was similar for the FUNAAB- alpha (79.82%) and ISA brown (81.18%) while the Bantam (54.63%) had the lowest ($P < 0.05$) mean. Sperm concentration was highest ($P < 0.001$) for the Bantam while the other two had similar values. It was concluded that the FUNAAB- alpha compared favourably with the ISA brown but was superior to the Bantam in semen quality and testicular morphology.

Introduction

Poultry, particularly chickens are genetically diverse, so it is important to compare their reproductive performance in order to identify populations and individuals of particular merit. According to Abdurehman and Urge (2016), the factors that are most responsive to genetic and environmental dynamics in poultry reproduction are fertility and hatchability and these components in turn govern the profitability of any poultry enterprise (Peters *et al*., 2008). Fertility and hatchability are influenced by the semen quality hence the need to evaluate semen characteristics in order to establish the reproductive potential any cock (Peters *et al*., 2004). Information on the influence of Strain on reproduction in chickens provides a useful guide for farmers and researchers for improved reproductive performance.

Donoghue and Wishart (2000) noted that several factors including breed and strain, age of rooster, body weight, diet, lighting schedule and season influence semen production in cocks. Similarly, Anderson (2001) observed that there are many factors (internal and external) that influence the male and also affect semen production. It has been reported that there are individual as well strain and breed differences in semen production and quality of cocks (Getachew, 2016). Sonseeda *et al.* (2013) reported that breed had an effect on semen production. Ameen *et al.* (2014) similarly confirmed that breed had significant influence on semen production in cocks.

Please cite this article as: Ochai DE, Kalla DJU, Saleh B & Mancha YP. 2018. Testicular Morpho-histometry and Semen Quality of Three Strains of Chickens. Poult. Sci. J. 6(2): 173-179.

Avian semen quality is often defined by determination of its volume, colour, concentration, motility, viability and morphology of spermatozoa (Giza, 2016; Santiago-Moreno *et al.,* 2016). It is well established that to select a good breeding cock, semen evaluation is essential as it gives a reflection of its reproductive performance (Cheng *et al.,* 2002; Partyka *et al.,* 2012). Sperm quality varies between individuals and samples and is highly correlated with fertilizing ability (Machebe and Ezekwe, 2004; Partyka *et al.,* 2012). The objective of this study was to compare semen quality and testicular morphology of three breeds of chickens.

Materials and Methods

The experiment was carried out at poultry unit of the Federal College of Land Resources and Technology, Vom, Plateau state, Nigeria. A total of forty five, 34-week old cocks from three strains were used for the study (15 cocks each from the FUNAAB-alpha, ISA Brown, and Bantam). The FUNAAB-Alpha chickens were procured from the poultry unit of the Federal University of Agriculture Abeokuta. The Bantams were obtained from the poultry unit of the National Veterinary Research Institute, Vom, Plateau state while the Isa Brown chickens were from reputable hatchery in Awe, Oyo state, Nigeria. The birds were fed a commercial diet and water *ad libitum* throughout the six months experimental period. An approval was obtained from the Departmental Ethical Committee on Animal Use of the Department of Animal production Abubakar Tafawa Balewa University, Bauchi, Nigeria.

Semen collection and analysis

Semen samples were collected weekly by abdominal massage technique**.** Semen volume was read directly from the graduated Eppendorf tube (WHO, 2008). Semen colour was visually assessed immediately after collection and scored as follows; 1 = creamy white, 2 = between opaque and creamy-white, and 3 = opaque (Peters *et al.,* 2008). A drop of semen was evenly placed onto a pH paper (range 6 – 10), after the colour of the impregnated zone became uniform (< 30 seconds) it was compared with the calibration strip to read the pH. Individual sperm motility was subjectively determined by examining the sperm at a magnification of ×400 as described by Cheng *et al.,* (2002).

Percentages live/dead and normal/abnormal spermatozoa were evaluated using the Eosin/Nigrosin staining procedure. One drop of the stain was taken using a pipette and placed at the end of a warm slide and mixed with one drop of semen. A thin smear was made by drawing the edge of a second slide across the mixture. The stained slide was allowed to dry, and then examined using a light microscope at a magnification power of 400×. The live spermatozoa were identified as bright and not stained. Their outlines were clearly defined and their heads were bright and retractile against the backgrounds. The dead spermatozoa stained pink and outlines were not clearly defined. The percentages of live and dead spermatozoa were calculated from 200 spermatozoa which were counted in each slide from different microscopic fields. The morphologically abnormal spermatozoa were also estimated on the same smears by counting 200 spermatozoa in different microscope fields.

Semen concentration was determined with the improved Neubauer haemocytometer using the direct cell count method. Neat semen was mixed with 0.9% normal saline at the dilution rate of 1:250. The diluted semen was then picked up using a micropipette. A drop of the diluted semen was then dropped on one end of the hemocytometer and also on the other end and allowed to settle. Counting was done at a magnification of ×400. The spermatozoa's head that falls within the sub-divided smaller squares at the four edges and centre of the hemocytometer are counted. The concentration of sperm per volume was found using the formula of Peters *et al.* (2008):

C = 50, 000 x N x D.,

Where:

C = Concentration of semen per volume (mL),

N = Number of spermatozoa counted,

D = Dilution rate.

Gross morphology histological processing of the testes

All testes collected from the cocks were trimmed of all adhering fat and tissue and then weighed to the nearest 0.01g using an electronic scale (SF – 400). Testis length and width were measured using a digital vernier caliper to the nearest 0.01 mm. Volume was obtained using Archimedes principle. The Vas deferens was trimmed of all adhering tissues and the paired weight obtained using an electronic scale while length was measured to the nearest millimeter with the aid of a ruler.

Testes were taken from nine cocks (three from each strain) in the experiment for histological observations. They were fixed in 10% formal saline for one week and thereafter processed for routine paraffin histological sectioning. The tissues were dehydrated through graded concentration of ethanol (70%, 90%, absolute ethanol) and cleared in xylene. The tissues were pre-impregnated in xylene paraffin wax in the oven and embedded in pure paraffin wax. The organs were sectioned at 7 µm thickness and tissues were stained with Haematoxylin and Eosin (H&E) for light microscopic examinations (Bancroft and Stevens, 1972; Baker and Silverton, 1985).

Statistical Analysis

Data were analysed as a Completely Randomized Design using the linear function of Statistics 9. Where means differ, least square difference (LSD) was used to separate them.

Results

The effect of strain on gross testicular morphology of chicken is shown in Table 1. Live weight for FUNAAB-alpha, Isa brown, and Bantam cocks were 2899, 2199, and 1021g, respectively. Strain significantly ($P < 0.05$) influenced live weight of the cocks. Testicular weight (paired, left, and right) were consistently lower ($P < 0.05$) for the Bantam while that of the FUNAAB-alpha and ISA brown were similar. Testes length (left and right) were also lower ($P < 0.05$) for the Bantam although right testicular length of the Bantam was similar to that of the ISA brown. Testicular width was similar among the three strains. Testicular volume was consistently lower ($P < 0.05$) for the Bantam. The FUNAAB-alpha had the highest ($P < 0.05$) weight and length of vas deferens. The Gonadosomatic index was lower for the FUNAAB-alpha and highest for the Bantam while the ISA brown was similar to both ($P < 0.05$).

Table 1. Effect of chicken strain on gross testicular morphology

Parameter	FUNAAB – alpha	ISA brown	Bantam	±*SEM*
Live weight (g)	2899^{a}	2199^{b}	1021^{c}	122.32*
Paired testes weight (g)	23.98^{a}	23.23^{a}	11.80^{b}	4.31*
Left testis weight (g)	12.82^{a}	11.79^{a}	5.80^{b}	2.20*
Right testis weight (mm)	11.16^{a}	11.43^{a}	6.00^{b}	2.13*
Left testis length (mm)	41.26^{a}	41.16^{a}	34.03^{b}	1.99*
Right testis length (mm)	43.25^{a}	39.85^{ab}	33.88^{b}	2.95*
Left testis width (mm)	20.63	20.33	17.11	1.88^{NS}
Right testis width (mm)	19.61	20.97	17.56	1.83^{NS}
Volume left (mL)	13.33^{a}	10.67^{a}	6.00^{b}	2.06*
Volume right (mL)	11.33^{a}	9.67^{ab}	7.00^{b}	1.92*
Volume paired (mL)	24.00^{a}	20.33^{ab}	13.00^{b}	3.66*
Weight of Vas deferens(g)	2.96^{a}	2.89^{a}	1.53^{b}	0.20*
Length of Vas deferens (cm)	14.00^{a}	11.97^{b}	9.00^{c}	0.62*
Gonadosomatic index	0.80^{b}	1.05^{ab}	1.16^{a}	0.14*

$^{a-c}$ Means within the same row bearing different superscripts differ significantly ($P < 0.05$).
SEM: standard error of mean; NS: not significant ($P > 0.05$); *: Significant ($P < 0.05$).

Table 2. Effect of strain on semen quality of chickens

Parameter	FUNAAB – alpha	ISA brown	Bantam	±*SEM*
Volume (mL)	0.73	0.34	0.64	0.35^{NS}
Colour	CW	CW	CW	NA
pH	6.64^{ab}	7.00^{a}	6.52^{b}	0.14*
Gross Motility (%)	79.82^{a}	81.18^{a}	54.63^{b}	5.89*
Sperm Concentration ×10^{9}/ml	0.24^{b}	0.27^{b}	0.56^{a}	0.09***
Percent live (%)	79.82^{a}	81.68^{a}	54.63^{b}	9.21*
Percent normal (%)	67.72	62.50	59.63	5.16^{NS}

$^{a-c}$ Means within the same row bearing different superscripts differ significantly ($P < 0.05$).
SEM: standard error of mean; NS: not significant ($P > 0.05$); *: Significant ($P < 0.05$); ***: Significant ($P < 0.001$); NA: Not analysed; CW- creamy white.

The histological photomicrographs of the testis of the various strains are shown in figures 1 to 3. There were no histological differences among the strains. The effect of strain on semen quality of chickens is shown in Table 2. No effect of strain was recorded for semen volume. Semen pH was higher ($P < 0.05$) in the ISA brown while the Bantam had the lowest. The pH for the FUNAAB-alpha was similar to the others. Gross motility was similar for the FUNAAB-alpha and ISA brown while the Bantam had the lowest ($P < 0.05$) mean. Sperm concentration was highest ($P < 0.001$) for the Bantam while the other two had similar values. Percentage live was similarly lower ($P < 0.05$) for the Bantam compared to the other strains percentage of normal sperm cells was similar for all the strains.

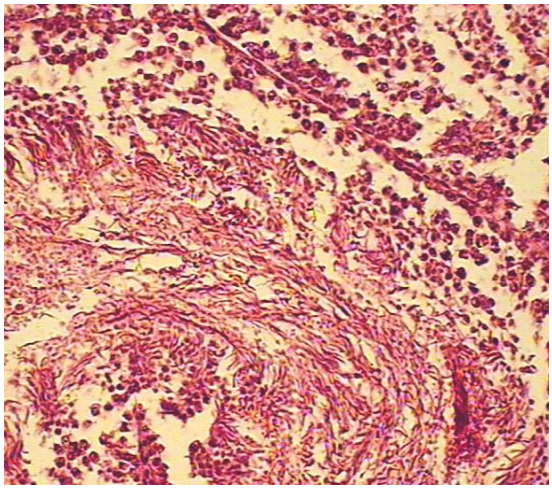

Figure 1. Photomicrograph of FUNAAB-alpha (450 µm) testis showing normal features with lots of spermatozoa in the seminiferous tubules.

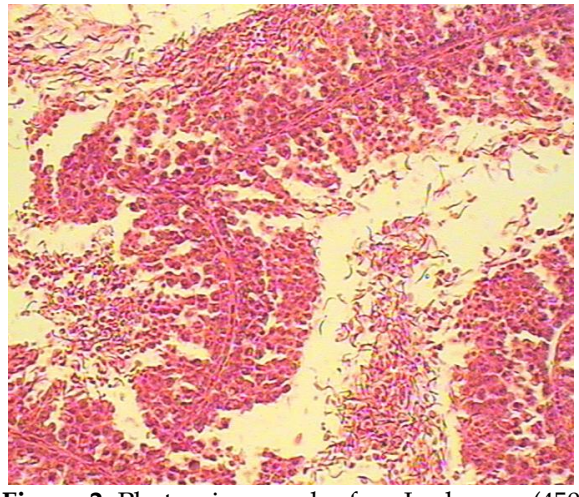

Figure 2. Photomicrograph of an Isa brown (450 µm) testis showing normal spermatogenic cell series.

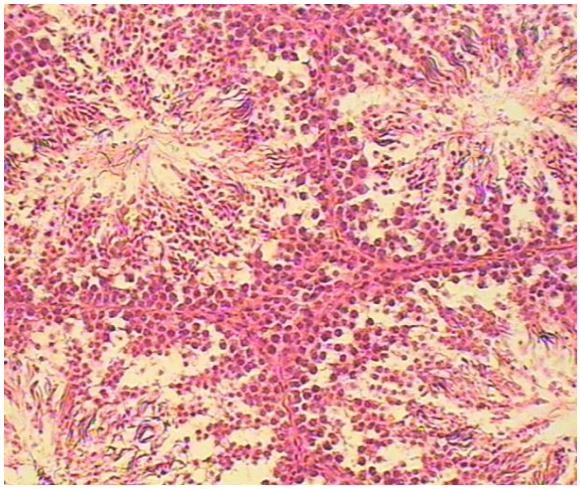

Figure 3. Photomicrograph of a Bantam (450 µm) testis showing normal seminiferous tubules with normal spermatogenic cell series.

Discussion

The variations in live weight observed in this study agrees with the report of Packard (2014) who worked with four South African native chickens and a hybrid and concluded that breed affected live weight. Similar observations were made by Ojedapo *et al.* (2008). The difference observed among the three strains of chickens for the testicular morphology is not totally unexpected since the Bantam has a lower body weight than the FUNAAB- Alpha and Isa brown. This confirms the assertion of Ubah *et al.* (2017) that cocks with high bodyweights usually have bigger testes. The testicular weight and weight of the FUNAAB –Alpha and the Isa brown were similar to those reported earlier (Sturkie and Opel, 1976; Obidi *et al.,* 2008; Chidozie *et al.,* 2010; Saleh *et al.,* 2017). The size of the testes, epididymis and vas deferens are related to semen output. Thus birds with large reproductive organs will likely produce higher quantity of semen (Urom, 2016). The gonadosomatic index which is an indication of sperm production efficiency were similar to the about 1% reported by Chidozie *et al.* (2010) for matured chickens. The normal histological features of the testes of the three strains is an indication that they can all thrive well under the Nigerian environment and are able to produce sperm cells.

In this study, there were no variations among the breeds for semen volume and colour. This is similar to the report of Malik *et al.* (2013) who observed no difference in semen volume among the red jungle fowl, domestic chicken, and bantam chicken. The observations however

contradicts the reports of Peters *et al.* (2008) who reported that strain significantly affected semen volume in seven strains of chickens reared in the humid tropic region of Nigeria. Semen volume (0.34–0.73 mL) recorded in this study was similar to those (0.29– 0.52 mL) reported by Omeje and Marine (1990), for cocks of different genetic backgrounds. Since breed is known to influence semen volume (Donoghue and ishart, 2000), the variation in semen volume observed in this study may be explained in terms of the difference in the normal processes regulating spermatogenesis and like the response of the different breeds to the abdominal massage method of semen collection (Hambu *et al.,* 2016).

Semen colour was creamy white and similar for all the strains. Hrnčár *et al.* (2013) observed that the most obvious evaluation of semen quality is colour. Peters *et al.* (2008) reported that semen colour is significantly influenced by chicken strain. The authors further noted that semen colour that deviates from creamy white may be an indication of the presence of contaminations.

The pH values in this study indicated strain difference between the Bantam and the ISA Brown but were all similar with the FUNAAB – alpha. The mean pH in this study fall within the range 6.0 to 8.0 that chicken sperm can tolerate (Siudzinska and Lukaszewicz, 2008). The pH of cock semen is 7.0 – 7.6 depending on the amount of transparent fluid present (Lake, 1981).

The assessment of sperm motility is one of the most often used parameters for semen evaluation (Malik *et al.,* 2013). The values obtained for semen motility for all 3 breeds in this study were within the range reported for normal cock semen of 40%– 80% (Malik *et al.,* 2013). The motility observed for the FUNAAB-alpha and Bantam was similar to the means 79.99% and 49.0% reported by Saleh (2017) and Malik *et al.* (2013), respectively.

The sperm concentration recorded in this experiment were lower than 1.76 to 2.66 billion/mL, 1.7 to 3.5 billion/mL and 1.83 billion/mL reported for the FUNAAB-Alpha, Leghorn, and Bantam chickens respectively, (Lake, 1966; Malik *et al.,* 2013; Saleh, 2017). The variations in sperm concentration among the strains may be attributed to strain differences and confirms the observations of Malik *et al.* (2013) who noted that genetic background of cocks affect their semen concentration directly or indirectly by affecting factors such as feed intake and body size.

Sperm morphology is recommended as one of the most essential qualitative characteristics of poultry semen (Kuster *et al.,* 2004) which can be used to ascertain the fertilizing ability of spermatozoa (Siudzinska and Lukaszewicz, 2008). The observation in this study of live sperm percentage contradicts earlier reports of Malik *et al.* (2013) and Hambu *et al.* (2016) who observed no difference among chicken breeds for live sperm. However, the similarity in normal sperm percentage corroborates their reports.

Conclusion

The FUNAAB- alpha compared favourably with the ISA brown but was superior to the Bantam in semen quality and testicular morphology. This means the FUNAAB – alpha can be used in any artificial insemination programme.

References

Abdurehman A & Urge M. 2016. Evaluation of fertility, hatchability and egg quality of rural chicken in Gorogutu district, Eastern Hararghe, Ethiopia. Asian Journal of Poultry Science, 10: 111-116.

Ameen SA, Opayemi OS, Ajayi JA & Adediwura MA. 2014. Evaluation of Semen Quality of Five Different Cockerel Breed used in Poultry Industry in Nigeria. Journal of Environmental Issues and Agriculture in Developing Countries, 6: 30–36.

Anderson J. 2001. The semen of animals and its use for artificial insemination. Green World Publishers India.

Cheng FP, Guo TJ, Wu TJ, Lin TE, Ursem PJF, Colenbrander B & Fung HP. 2002. Annual variation in semen characteristics of pigeons (*Columba livia*). Poultry Science, 81: 1050–1056. DOI: 10.1093/ps/81.7.1050

Baker FJ & Silverton RE. 1985. Introduction to Medical Laboratory Technology. Butterworths, London.

Bancroft JD & Stevens A. 1972. Theory and Practice of Histological Techniques. 2nd ed. Churchill Livingston, Edinburg, London New York.

Chidozie OG, Ugochukwu N & Kenneth A. 2010. Morphometric study of the testes of the Nigerian local breed of chicken. Animal Research International, 7: 1163–1168.

Donoghue AM & Wishart GJ. 2000. Storage of poultry semen. Animal Reproduction Science, 62: 213-232. DOI: 10.1016/S0378-

4320(00)00160-3
Getachew T. 2016. A Review Article of Artificial Insemination in Poultry. World's Veterinary Journal, 6: 25-33.
Giza, L. 2016. Semen characteristics and *in vitro* preservation in the racing pigeon (*Columba livia*): a model approach for domestic and wild pigeon species. Masters Dissertation, Faculty of Veterinary Medecine, Ghent University.
Hambu EK, Arifiantini RI, Purwantara B & Darwati S. 2016. Raw Semen Characteristics of Three Different Indonesian Local Roosters. Animal Production, 18: 165-172.
Hrnčár C, Gič D & Bujko J. 2012. The effect of eggshell quality on hatchability of pure chicken breeds. Animal Science and Biotechnologies, 45: 415–418.
Kuster CE, Singer RS & Althouse GC. 2004. Determining sample size for the morphological assessment of sperm. Theriogenology, 61: 691–703. DOI: 10.1016/S0093-691X(03)00240-1
Lake PE. 1966. A cytochemical examination of spermatozoa of the domestic fowl. Research in Veterinary Science, 7: 121–127.
Lake PE. 1981. Male genital organs. In: King, AS and McLleland, J. (eds.) Form and Function in Birds. Academic Press, London, Pp. 1 – 61.
Machebe NS & Ezekwe AG. 2004. Ejaculate characteristics of three genotypes of local cocks in the humid tropics. Journal of Agriculture, Food, Environment and Extension, 3: 33–37.
Malik A, Haron A, Yusoff R, Nesa M, Bukar M & Kasim A. 2013. Evaluation of the ejaculate quality of the red jungle fowl, domestic chicken, and bantam chicken in Malaysia. Turkish Journal of Veterinary and Animal Sciences, 37: 564-568. DOI: 10.3906/vet-1107-26.
Obidi JA, Onyeanusi BI, Rekwot PI, Ayo JO & Dzenda T. 2008. Seasonal variations in seminal characteristics of Shika brown breeder cocks. International Journal of Poultry Science, 7: 1219–1223.
Ojedapo LO, Akinokun O, Adedeji TA, Olayeni TB, Ameen SA & Amao SR. 2008. Effect of strain and sex on carcass characteristics of three commercial broilers reared in deep litter system in the derived savannah area of Nigeria. World Journal of Agricultural Sciences, 4: 487-491.
Omeje SSI & Marine BN. 1990. Evaluation of semen characteristics of adult cocks of different genetic background. *Theriogenology*, 24: 1111-1118. DOI: 10.1016/S0093-691X(05)80010-X
Packard R. 2014. Comparison of production parameters and meat quality characteristics of South African indigenous chickens. MSc thesis, Stellenbosch University.
Partyka A, Niżański W & Ochota M. 2012. Methods of Assessment of Cryopreserved Semen. In: Katkov, I. (Ed.), Current Frontiers in Cryobiology, InTech. Retrieved February 2, 2013 from: http://www.intechopen.com /books/current-frontiers-in- cryobiology /methods-of-assessment-of- cryopreserved-semen
Peters SO, Omidiji EA, Ikeobi CON, Ozoje MO & Adebambo OA. 2004. Effect of Naked neck and frizzled genes on egg traits, fertility and hatchability in local chickens. In: self sufficiency of Animal production in Nigeria. In: Proceedings of the 9th Annual conference of Animal Science Association of Nigeria. Ebony State University, Nigeria, Sept. 13-16, 2004. Pp: 262-264.
Peters SO, Shoyebo OD, Ilori BM, Ozoje MO, Ikeobi CON & Adebambo AO. 2008. Semen quality traits of seven strain of chickens raised in the humid tropics. International Journal of Poultry Science, 7: 949-953.
Saleh B. 2017. Effect of varying dietary energy and protein on reproductive performance of FUNAAB-alpha chickens. PhD Dissertation, Department of Animal Production, Abubakar Tafawa Balewa University, Bauchi. 245 pages.
Saleh B, Kalla DJU, Mbap ST, Doma UD & Girgiri AY. 2017. Effects of varying dietary energy and protein levels on gross morphology and histology of testes of breeder FUNAAB – Alpha cocks. Nigerian Journal of Animal Production, 44: 25–33.
Santiago-Moreno, J, Esteso1, MC, Villaverde-Morcillo, S, Toledano-Diaz, A, Castaño1, C, Velázquez, R, Lopez-Sebastian, A, Goya, AL & Martínez, JG. 2016. Recent advances in bird sperm morphometric analysis and its role in male gamete characterization and reproduction technologies. Asian Journal of Andrology, 18: 882–888. DOI: 10.4103/1008-682X.188660
Siudzinska A & Lukaszewicz E. 2008. Effect of semen extenders and storage time on sperm morphology of four chicken breeds. Applied Poultry Research, 17: 101–108.
Sonseeda P, Vongpralub T & Laopaiboon B. 2013. Effects of Environmental Factors, Ages and Breeds on Semen Characteristics in Thai Indigenous Chickens: A One-year Study. Thailand Journal of Veterinary Medicine, 43: 347-352.

Sturkie PD. & Opel H. 1976. Reproduction in the male, fertilization, and early embryonic development. In: Sturkie, P. D. (Ed.), Avian Reproduction (3rd Ed.), Springer- Verlag, New York.

Ubah SA, Muhammad SA, Obudu CE & Ogunbodede MA. 2017. Semen Quality of Cockerel Breeders Gallus domesticus in Two Climates in Nigeria. Biomedical Sciences, 3: 21-27. DOI: 10.11648/j.bs.20170301.14.

Urom SMOC. 2016. Hormonal profile and morphometric weight of reproductive organ of Nigerian indigenous cocks treated with clomiphene (Clomid®). Nigerian Journal of Agriculture, Food and Environment, 12: 15–18.

WHO (World Health Organization). 2010. WHO laboratory manual for the examination and processing of human semen. 5th ed., World Health Organization [WHO] Press. pp. 286.