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MORPHOLOGICAL EVALUATION OF THE EPIDIDYMIS AND VAS DEFERENS AND THE SPERMIOGRAM OF LIGHT ECOTYPE NIGERIAN INDIGENOUS CHICKEN (*Gallus gallus domesticus*)

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ABSTRACT

The morphology of the epididymis and vas deferens and the spermiogram of light ecotype Nigerian indigenous chicken were examined using gross anatomical, histological and sperm evaluation techniques. The results showed that the mean weight and length of the left epididymis was significantly ($p < 0.05$) greater than the right counterpart. Whereas the proximal part of the efferent ductules exhibited numerous mucosal folds with ciliated simple and pseudo-stratified columnar epithelium, the distal part of the efferent ductules, the connecting ducts and duct of epididymis lacked the mucosal folds and showed rare occurrence of cilia in the epithelium. The tunica mucosa of the vas deferens was covered by pseudo-stratified columnar epithelium, and it exhibited mucosal folds and sieve-like invaginations into the subjacent lamina propria especially in the distal part. The semen evaluation showed that testicular sperm count was 14.68 ± 1.90 million cells/gram testes with 85% motility and 95% vitality, while the percentage vas deferens total sperm abnormalities were 6.50 ± 0.65% for the left and 5.50 ± 0.65 % for the right vas deferens. It was concluded that the epididymis and vas deferens of this breed of chicken play key roles in sperm storage, seminal fluid concentration and sperm release. Moreover, they also have high reproductive potentials.

Keywords: Epididymis, Vas Deferens, Morphology, Nigerian indigenous chicken, Spermiogram.

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INTRODUCTION

The light ecotype Nigerian indigenous chicken is a very productive breed adapted for high hatchability despite harsh environmental conditions; and this may reflect an evolutionary trend to enhance its performance in the wild [1]. Survival and propagation of different species of animal and man are the major functions of the reproductive system [2,3,4]. Components of the male reproductive tract in avian species include the testes, epididymis and vas deferens, all of which are intra-abdominal organs in birds.

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The male reproductive tract have been shown to play the important roles of spermatogenesis, sperm nutrition, sperm surface modification, sperm storage and passage, as well as fluid and calcium re-absorption; and so are indispensable in avian reproduction [5,6,7]. Assessment of sperm morphology, sperm mortality and sperm concentration could serve as a reliable indicator for predicting the fertilizing ability of spermatozoa in a given species or breed [8,9].

The testes are usually considered the most strategic component of the avian reproductive tract, and most studies have focused on the morphological features of the testes [10,11,12]. Testicular asymmetry and the possible dominant role(s) of either the left or right reproductive tract of birds have been reported [13,14,15,16]. In contrast, only very little attention have been paid to the morphology of the epididymis and vas deferens [17]. Thus, the strategic roles of different segments of the epididymis and vas deferens in avian species are not well understood. This underscores the need to investigate the functional morphology of the avian epididymis and vas deferens to identify their roles in spermatozoa viability and male fertility. The aim of the present study was to evaluate the morphological features of the epididymis and vas deferens, as well as the sperm parameters of the light ecotype Nigerian indigenous chicken.

MATERIALS AND METHODS

Experimental animals

Ten apparently healthy light ecotype Nigerian indigenous chickens (900-1000 gram body weight) were used for the study. The birds were obtained from local markets in Nsukka Local Government Area of Enugu State, Nigeria and were slaughtered for human consumption. Testes, epididymis and vas deferens were dissected and used for sperm evaluation, gross anatomical and histological studies.

Gross anatomical evaluation.

The weights and lengths of the left and right epididymis and vas deferens were determined using a weighing balance and a meter rule respectively, while the volumes of the left and right epididymis and vas deferens were determined by the fluid displacement method [18].

Testicular sperm count.

The tunica albuginea was removed from the testis and 500 grams of the testicular parenchyma was thoroughly minced and homogenized in 2 ml of phosphate-buffered saline (PBS, pH 7.4). A 1:10 dilution of the homogenate was made in sperm dilution fluid containing formalin and gentian violet stain. Spermatozoa were counted using a haemocytometer (Weber, England). Testicular sperm count was expressed as number of sperm cells per gram of testes.

Sperm motility

Sperm motility was determined using the sperm diffusion method as previously described by Seed et al. [19]. Briefly, the caudal part of the vas deferens was sectioned and immersed in drops of pre-warmed phosphate-buffered saline (PBS; pH 7.4, 37 °C) on a clean glass slide for 2 minutes to facilitate sperm diffusion into the buffer. The tissues were removed, and the sperm incubated for 5 minutes until adequate dispersion was achieved. Sperm motility (%) was evaluated by examining the sample at x400 magnification using a phase-contrast microscope (Motic B3; Motic, Carlsbad, CA, USA) equipped with a stage slide-warmer at 37°C (TCS-100; Amscope, Ivrine, CA, USA). A total of 200 sperm cells were counted and the number of motile sperm cells was recorded and expressed as a percentage of the total.

Sperm vitality

Sperm vitality was determined after staining with eosin-nigrosin vital stain. Equal volumes of the sperm suspension from the caudal part of vas deferens and eosin-nigrosin stain were mixed for 30 seconds. Thin smears of the sperm-eosin-nigrosin mixture were made on clean microscope slides and air-dried. Live sperm (unstained head) and dead sperm (markedly pink-stained head) were identified using light microscopy (Motic B3; Motic, Carlsbad, CA, USA) at x1000 magnification. A total of 200 sperm cells

were counted and the number of live sperm cells recorded and expressed as percentage vitality of the sample. All micrographs were captured using Moticam 2.0 image system (Motic, Carlsbad, CA, USA).

Sperm morphology

Wet mounts of the sperm suspension from the caudal part of the vas deferens were evaluated for sperm morphology and structural abnormalities at x400 and x1000 magnifications using phase-contrast microscopy (Motic B3; Motic, Carlsbad, CA, USA). A total of 200 sperm cells were counted and the number of abnormal sperm cells was expressed in percentage as sperm total abnormalities (STA).

Histological preparation

Sections of the left and right epididymis and vas deferens were fixed in Bouin's fluid for about 36 hours. Thereafter, the fixed tissues were dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. About 5 – 7 µm thick sections of the embedded tissues were obtained using a rotary microtome and mounted on clean glass slides for staining with haematoxylin and eosin (H&E). Photomicrographs were evaluated and captured using a Moticam Images Plus 2.0 digital camera (Motic Group Ltd, China 1999-2004) attached to the Motic binocular light microscope.

Data analysis

Data obtained were presented as mean ± SEM. The data were statistically analyzed using independent sample t-test. Statistical significance was defined as $p < 0.05$.

RESULTS

Gross anatomy of male the reproductive tract

The male reproductive tract of the light ecotype Nigerian indigenous chicken consisted of paired testes, epididymis, vas deferens and a cloaca. The epididymis lacked distinct head, body and tail, but its proximal end was attached to the dorso-medial aspect of the testis (Fig. 1). The distal end of the epididymis was continuous with the vas deferens, which extended along the dorso-medial wall of the abdominal cavity to empty into the urodeum via a small papilla. The initial segment of the vas deferens was relatively narrow, but its distal portion widened towards its point of union with the cloaca (Fig. 1). The mean weight and mean length of the left epididymis were significantly ($p < 0.05$) greater than those of right epididymis, but there was no significant difference ($p > 0.05$) between the mean volumes of the left and right epididymis (Table 1). The mean weight, length and volume did not differ significantly ($p > 0.05$) between the left and right vas deferens (Table 1).

Spermiogram

There was no significant difference ($p > 0.05$) between the left and right mean testicular sperm count, sperm motility, sperm vitality and sperm abnormality (Table 1). The highly elongated spermatozoa of the light ecotype Nigerian indigenous chicken were filiform-shaped and approximately 97.92 µm in length (Table 1). They exhibited vermiform (long and narrow) heads, which were approximately 14.50 µm in length (Figs. 2a, 2b). Their tails were smooth and elongated (sauropsid type). Heads without tails, small heads and coiled tails were the commonly observed sperm abnormalities (Fig. 2a).

Histology of the epididymis

Histological examination showed three types of ducts in the epididymis of the light ecotype Nigerian indigenous chicken namely, efferent ductules, connecting ducts and epididymal duct (Fig. 2c). The efferent ductules continued from the rete testis and were highly convoluted. The proximal part of the efferent ductules was characterized by numerous mucosal folds covered by ciliated simple columnar and pseudo-stratified columnar epithelium, while the distal part showed no mucosal folds, but exhibited a covering of ciliated pseudo-stratified columnar epithelium (Figs. 2c, 2d, 3a). The connecting ducts were numerous, but smaller in size than other ducts of the epididymis (Fig. 3b). They lacked mucosal folds and the epithelial type was ciliated pseudo-stratified columnar epithelium (Fig. 3b). The epididymal duct was

very tortuous and larger than other ducts of the epididymis. It had a pseudo-stratified columnar epithelium, but cilia rarely occurred in the epithelium (Fig. 2c). Numerous spermatozoa were found within the lumen of all segments of the epididymis.

Table 1: Morphometric features and spermiogram of the epididymis and vas deferens of light ecotype Nigerian indigenous chicken.

Parameters	Mean weight of vas deferens (g)	Mean length of vas deferens (cm)	Mean weight of epididymis (g)	Mean length of epididymis (cm)	Mean volume of epididymis (ml)
Left	0.33 ± 0.05^{a}	9.58 ± 0.39^{a}	0.14 ± 0.01^{a}	1.80 ± 0.10^{a}	0.27 ± 0.07^{a}
Right	0.25 ± 0.02^{a}	9.58 ± 0.17^{a}	0.10 ± 0.02^{b}	1.40 ± 0.07^{b}	0.20 ± 0.00^{a}

Parameters	Mean volume of vas deferens (ml)	Mean testicular sperm count $\times 10^{6}$ per gram testis	Mean sperm motility (%)	Mean sperm vitality (%)	Mean total sperm abnormality (%)
Left	0.87 ± 0.19^{a}	14.68 ± 1.90^{a}	86.00 ± 2.27^{a}	95.75 ± 0.63^{a}	5.50 ± 0.65^{a}
Right	0.90 ± 0.10^{a}	13.91 ± 1.29^{a}	83.75 ± 1.38^{a}	94.50 ± 1.55^{a}	6.50 ± 0.65^{a}

Different superscripts in a column indicate significant differences ($p < 0.05$) between the left and right

Fig. 1: Photograph of the reproductive organs of Nigerian indigenous chicken showing epididymis (black arrow), cranial (arrow head) and caudal vas deferens (white arrow).

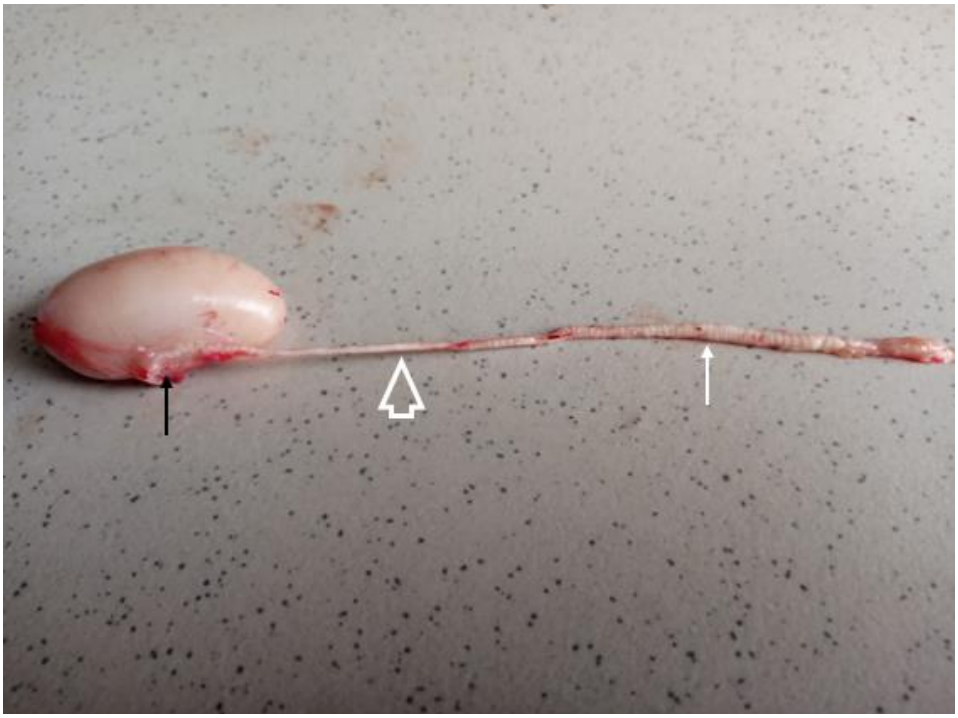

Fig. 1: Photograph of the reproductive organs of Nigerian indigenous chicken showing epididymis (black arrow), cranial (arrow head) and caudal vas deferens (white arrow).

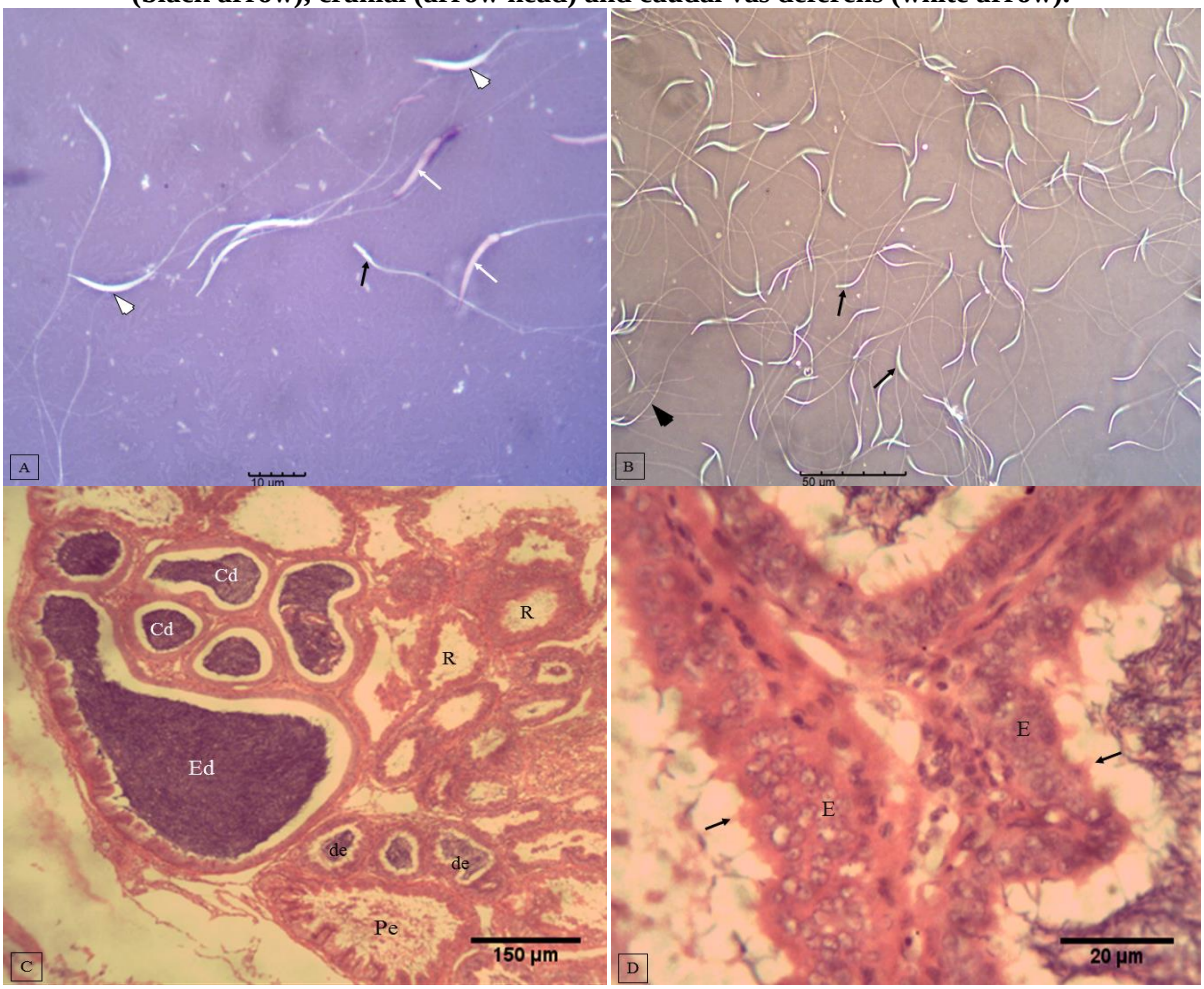

Fig. 2: A, Spermatozoa of Nigerian indigenous chicken showing dead spermatozoa (white arrows) and live spermatozoa (arrow heads). Note the small-headed sperm cell (black arrow). Eosin & Nigrosin stain x1000. B, Spermatozoa of Nigerian indigenous chicken showing phase contrast micrograph of the filliform-shaped sperm cells (arrows) with vermiform heads (arrows) and elongated tail (arrow head). x400. C, The epididymis of Nigerian indigenous chicken showing proximal (Pe) and distal (de) efferent ductules, rete testes (R), connecting ducts (Cd) and duct of epididymis (Ed). H&E stain x40. D, Efferent ductules of Nigerian indigenous chicken showing apical cilia (arrows) and pseudo-stratified columnar epithelia (E). H&E stain x 400.

Histology of the vas deferens

The vas deferens of light ecotype Nigerian indigenous chicken showed three tissue layers namely, tunica mucosa, tunica muscularis and tunica serosa (Fig. 3c). The tunica mucosa exhibited mucosal folds and a lining of pseudo-stratified columnar epithelial cells. The epithelial lining of the proximal two-thirds of the vas deferens was largely non-ciliated (Figs. 3d, 4a-b), while the distal one-third showed tall mucosal folds and ciliated pseudo-stratified columnar epithelium with sieve-like invaginations into the subjacent lamina propria (Figs. 4c-d). The tunica muscularis contained circularly arranged layers of smooth muscle cells in the proximal part of the vas deferens, but this was thicker and organized as inner circular and outer

longitudinal layers in the distal segment of the duct (Fig. 4c). The lumen of the vas deferens contained many spermatozoa.

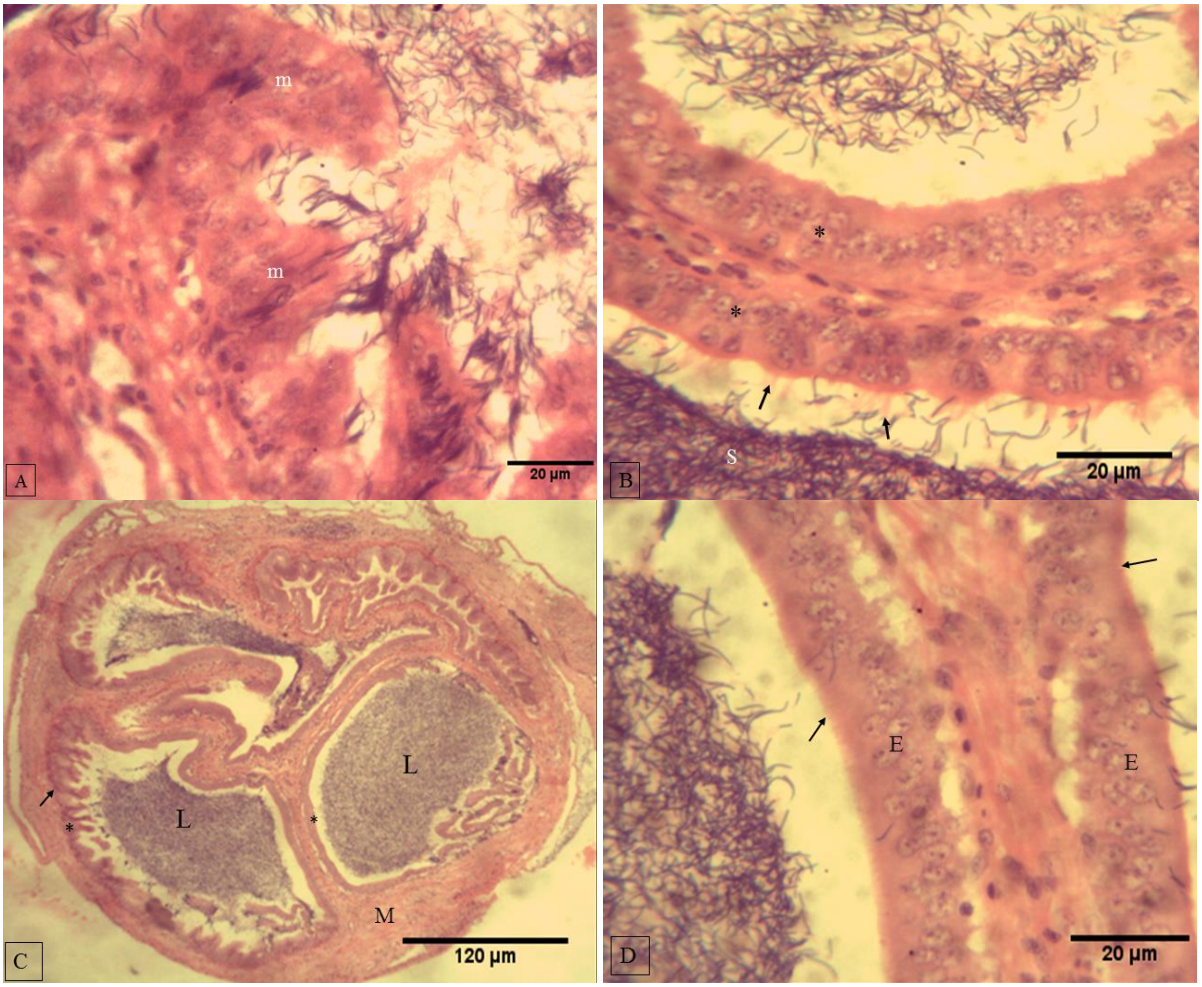

Fig. 3: A, Light micrograph of the proximal efferent ductule showing mucosal folds (m). H&E stain x400. B, connecting ducts of the epididymis showing sparsely distributed cilia (arrows), pseudo-stratified columnar epithelia (asterisks) and luminal packs of spermatozoa (S). H&E stain x400. C, Cranial part of vas deferens showing tunica mucosa (asterisks), tunica muscularis (M) and tunica serosa (arrows). Note that the luminal compartments (L) are packed with spermatozoa. H&E stain x40. D, Cranial part of vas deferens with non-ciliated (arrows) pseudo-stratified columnar epithelia (E). H&E stain x400.

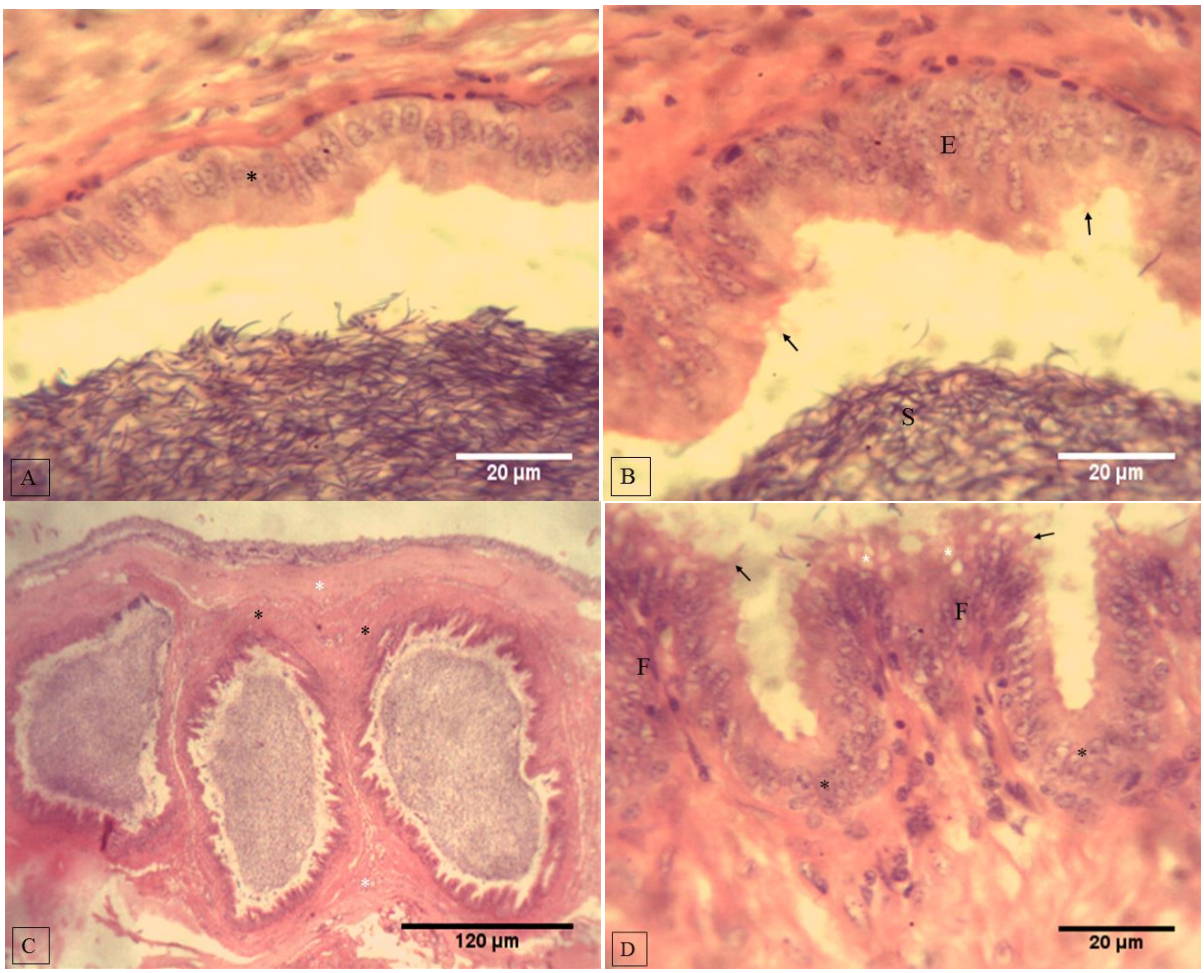

Fig. 4: A, Middle part of vas deferens of Nigerian indigenous chicken showing non-ciliated pseudo-stratified columnar epithelium (asterisk). H&E stain x400. B. Middle part of vas deferens showing pseudo-stratified columnar epithelium (E) with sparsely distributed apical cilia (arrows). Note the luminal spermatozoa (S). H&E stain x400. C, Caudal part of vas deferens showing inner circular (black asterisks) and outer longitudinal (white asterisks) smooth muscle cells. H&E stain x40. D, Caudal vas deferens with mucosal folds (F), ciliated (arrows) pseudo-stratified columnar epithelia (black asterisks). Note the sieve-like modification of the mucosal folds (white asterisks). H&E stain x400.

DISCUSSION

The anatomical location of the epididymis and vas deferens in the light ecotype Nigerian indigenous chicken is consistent with what has been reported for other breeds of bird [17]. The mean weight and length of the left epididymis were significantly greater than the right epididymis. This may be related to the size of their corresponding testes as reports of some previous authors indicate that the weight of the left testis is greater than the right testis in chicken [14, 16]. However, greater testicular size may not be associated with a proportional increase in sperm production [20]. Thus, there were no significant differences between the left and right mean testicular sperm count, mean vas deferens sperm vitality and mean vas deferens sperm abnormalities in the present study; suggesting equal participation of the left and right testes in sperm production at sexual maturity in the light ecotype Nigerian indigenous chicken.

It is generally believed that the excurrent ducts of the male reproductive system of birds, including the epididymis are primarily pathways for the transport of sperm cells to the cloaca [21, 22, 23]. The epididymis of the light ecotype Nigerian indigenous chicken showed three duct types namely, the efferent ductules, connecting ducts and duct of epididymis. The histological features of these ducts suggest their specialized roles. Presence of a pseudo-stratified columnar epithelium in the efferent ductules has been previously reported [24, 25], but the occurrence of mucosal folds and cilia in the proximal segment of these ductules may serve to provide a greatly increased surface area essential for absorption of fluid. It has been demonstrated that fluid absorption is a major function of the efferent ductules in birds [26, 27]. The proximal efferent ductules play the important role of sperm concentration in mammals [28]. In the present study, the distal part of efferent ductules, the connecting duct and duct of epididymis, whose lumen contain large populations of sperm cells, show no mucosal folds and may serve as passages and storage areas for the sperm cells. This suggestion is contrary to an earlier proposition that non-passerines have limited or no capacity for extra-gonadal sperm storage [5, 13]. It has been demonstrated that breed variation and higher competitive mating system [13, 22], among certain birds may influence the duration of epididymal sperm storage.

The convolution of the vas deferens in the light ecotype Nigerian indigenous chicken follows previous reports [10, 24]. However, the absence of mucosal folds and presence of a largely non-ciliated epithelium in the proximal and middle parts of the vas deferens suggest that the sperm storage capacity of these segments may be very transient. Their major role may be to serve as pathways for sperm passage. A similar role is commonly ascribed to the vas deferens of several avian species [10, 23, 29]. In contrast, the distal part of the vas deferens of the light ecotype Nigerian indigenous chicken, which showed tall mucosal folds and ciliated pseudo-stratified columnar epithelium, may take part in fluid absorption and semen concentration. The sieve-like invaginations of the mucosa of this segment of the vas deferens could be semen-holding pockets homologous to the 'seminal glomus' reported in passerines [29, 30]. The more robust organization of muscle fibres in the distal vas deferens may be an adaptation to facilitate the release of semen from the vas deferens during mating.

Spermatozoa of the light ecotype Nigerian indigenous chicken are filiform-shaped and this is similar to the spermatozoa of most galliformes [31]. The average length of the head of the spermatozoa (14.50 µm) falls within the normal range of 11 to 21 µm reported for the breed [32]. Furthermore, the testicular sperm count and the high percentages of sperm motility and vitality, as well as the very low percentage sperm abnormalities suggest that the light ecotype Nigerian indigenous chicken has a high reproductive potential. This suggestion is further highlighted by the fact that the percentage vas deferens total sperm abnormalities ($6.50 \pm 0.65\%$ for the left and 5.50 ± 0.65 % for the right) reported in this study are within the minimum range (5 to 10 %) allowed for primary sperm defects in animals [33, 34]. The sperm abnormalities recorded in this study are probably due to a defect in spermatogenesis (primary sperm defects). These abnormalities, which include small heads, heads without tails and coiled tails are among the commonly reported sperm defects in animals [35, 36], and are thought to originate mainly from defective spermatogenesis [35].

In conclusion, the morphological features of the epididymis and vas deferens indicate the important roles of these ducts in spermatozoa storage, seminal fluid concentration and facilitation of semen release during mating in the light ecotype Nigerian indigenous chicken. Moreover, the spermiogram of the light ecotype Nigerian indigenous chicken supports the idea that the breed has a high reproductive potential.

ETHICAL STATEMENT

This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (FVM-UNN-IACUC-2019-0913).

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest

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