
This is the author’s final accepted version.

There may be differences between this version and the published version. You are advised to consult the publisher’s version if you wish to cite from it.

[http://eprints.gla.ac.uk/144640/](http://eprints.gla.ac.uk/144640/)

Deposited on: 23 August 2017
Age-related changes in testicular histology

Developmental changes in the histological structure of the testes, and testosterone profiles in male guinea fowls (Numida meleagris)

Iddriss I. Abdul-Rahman\textsuperscript{a}, Frederick Y. Obese\textsuperscript{b}, Ian A. Jeffcoate\textsuperscript{c}

\textsuperscript{a}Department of Veterinary Science, Faculty of Agriculture, University for Development Studies, P. O. Box TL 1882, Nyankpala Campus, Tamale, Ghana.

\textsuperscript{b}Department of Animal Science, School of Agriculture, University of Ghana, P. O. Box LG 226, Legon, Ghana.

\textsuperscript{c}Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bearsden Road, Glasgow G61 1QH, Scotland, UK.

Abstract

Owing to the paucity of information on the reproductive biology of guinea fowls, a study involving a total of 66 males was conducted, and documented the developmental changes in histological structure of the testes of guinea cocks from hatching until adulthood. Changes in testosterone synthesis during sexual development were also determined. Age-related changes were analysed using univariate analysis for completely randomised design and means separated using

\textsuperscript{*}Corresponding author. Tel: +233 244985023/204536769

Email: ai.iddriss@yahoo.co.uk/ibniddriss@uds.edu.gh
Age-related changes in testicular histology

Tukey's test/Kruskal-Wallis test and medians separated by Mann-Whitney U-test.

Total germ cell population per testis and testicular histological morphometric parameters increased significantly (p<0.0001) from 12 weeks of age (WOA), and stabilised between 20 and 24 WOA. Peripheral testosterone concentrations increased gradually from 4 WOA, and peaked at 20 WOA. Correlations among all the testicular morphometric parameters were positive and highly significant (p<0.01). Similarly, significant (p<0.05) positive correlations existed between testicular weight and testicular sperm production, tubular diameter, Sertoli cell population, tubular length and peripheral testosterone concentration. Testicular sperm production was positively correlated with meiotic index (p<0.01) and round spermatids population (p<0.05). The correlations between peripheral testosterone concentrations, tubular diameter and Sertoli efficiency were also significant (p<0.05) and positive. Testicular morphometric parameters stabilized between 20 and 24 WOA, while peripheral testosterone concentrations showed two patterns of secretion, initial and final phases of increasing and decreasing testosterone secretions, respectively, and may be implicated in the development of histological structures of the testes and spermatogenesis.

Keywords: Guinea cock; histology; sexual development; testosterone; testis

1. Introduction

Avian testes are surrounded by a fibrous capsule that includes connective tissue and contractile fibers [1]. They contain interstitial tissue and seminiferous tubules, which are the site of spermatogenesis and, in developed testes, make up most of the testicular mass. Interstitial tissue includes Leydig or interstitial cells, the main source
Age-related changes in testicular histology

of testicular androgens [2, 3, 4, 5, 6]. The testes in some bird species are of identical
sizes (e.g., tree swallow, Tachycineta bicolor, [7]; chicken, Gallus domesticus, [8]),
but many species show testicular size asymmetry, with one testis normally being
larger in adulthood than the other [9, 10].

The testis of the mature bird is organized into discrete, easily discernible
cellular associations and functional compartments. However, during embryonic and
early post-hatch development this organization is less apparent [11]. The post-hatch
development of the fowl’s testis can be divided into three distinct phases: (1)
proliferation of spermatogonia and the somatic cells that support spermatogenesis
(Sertoli, peritubular myoid, and interstitial cells); (2) differentiation and the
acquisition of functional competence by somatic support cells; and (3) spermatogonial
differentiation resulting in the initiation of meiosis. While the boundaries of these
phases are not clearly defined, this three-step process results in functional
seminiferous tubules that can maintain spermatogenesis when the appropriate
hormonal cues are present [11].

The growth and histological development of the testes of White Plymouth
Rocks has been described by Kumaran and Turner [12, 13]. Their account serves as a
general description of the sequence of changes that occur in the seminiferous tubule
during the sexual maturation of the male bird. However, they reviewed observations
made on other breeds and emphasized that interbreed differences are to be found in
the relation between age of a male and a particular histological structure displayed in
the seminiferous tubule. For instance, spermatids appeared at about 12 WOA in the
exotic breed of guinea cock [14], compared to 20 WOA [15] in the local breeds.
Kumaran and Turner [12, 13], however, noted that in general, light breeds mature
earlier than heavy breeds.
Age-related changes in testicular histology

Several androgens and other steroids have been found in the fowl’s testis [16]. Testosterone is considered the most important mammalian testicular androgen and has been identified in the extracts of testis of fowls and other birds [17]. Driot and associates [18] described changes occurring in plasma testosterone concentrations in the domestic fowl during sexual development. The authors noted three stages including i. a stationary phase, observed in cockerels less than 12 weeks old, ii. an augmentation phase lasting approximately 12 to 22 WOA, and ii. an adult phase, consisting of marked fluctuations in testosterone concentrations. Testosterone in the male is essential for spermatogenesis, maintenance of the excurrent duct and secondary sexual attributes, the expression of specific behaviours, and, altering the pattern of GnRH secretion [11].

Even though a preliminary study documented some histological descriptions of the age-related changes in the reproductive organs of male guinea fowls, these were not detailed, and only involved qualitative descriptions, and small sample size [15]. Also, the endocrine profiles associated with these changes are unknown. For example, testosterone concentrations have only been documented in breeding and non-breeding males [19]. Besides, there is a general paucity of information on the reproductive system of guinea fowls. The objective of the present study, therefore, was to determine the developmental changes in histology of the reproductive organs of guinea cocks from hatching until adulthood (32 weeks), and associated testosterone profiles.

2. Materials and methods

2.1 Experimental Site

The study was conducted at the Poultry Unit of the Department of Animal Science,
Age-related changes in testicular histology

University for Development Studies, Nyanpkala, Tamale (Ghana). Nyanpkala lies on latitude 9° 69'N and longitude 0° 83'W. Temperatures are generally high with minimum and maximum values of 22 °C and 35 °C recorded in March and December, respectively (Savannah Agricultural Research Institute (SARI, 2008) cited by Abdul-Rahman et al. [19]). Rainfall is monomial with mean annual rainfall varying from 1,000-1,500 mm and peaks from August to September, with a relatively long dry season extending from November to April. The area lies in the Guinea Savannah zone, and has nearly equal amounts of light and darkness (12L: 12D) throughout the year. The guinea fowls used in the present study are indigenous to this area, hence the name guinea fowl [20].

2.2 Animals and Management

A total of 66 local guinea cocks (Numida meleagris), of the pearl variety, were used for the study. Birds were brooded for 6 weeks [21], and then transferred to a deep litter house (floor spacing: 1.8 sq ft/bird; Lohmann LSL, Germany) until the end of the experiment. They were individually identified using tags placed through their inner wings to prevent detection by other birds and thus avoid pecking. Keets were brooded at 35°C from hatching until three WOA, and then at 32°C until six WOA [21]. Birds were then maintained at ambient temperatures of between 22°C and 35°C until the end of the experiment. Feed and water were supplied ad libitum. Day old keets were fed ground maize in flat feeders followed by a starter ration from day 2 until 6 WOA. This was followed by a grower ration from 6 WOA until 21 WOA and then a layer feed until the end of the experiment. The starter (22% crude protein and 3,000 Kcal ME/kg diet), grower (14% crude protein and 2,800 Kcal ME/kg diet), and
Age-related changes in testicular histology

breeder (17.5% crude protein and 2,800 Kcal ME/kg diet) rations were obtained from 
a commercial feed supplier (Agricare Ghana Limited, Kumasi, Ghana).

Information on lighting requirements of the local guinea fowls from hatching are 
unavailable, and those used for chicken, are usually employed. In this case, however, 
the “golden rule” to follow in designing lighting programmes for pullets [22] was 
followed. All birds received 24 h light from day old until one-WOA, and this was 
reduced to 16 h until birds were 3 weeks old. These longer light periods during the 
first 3 weeks of life were to ensure maximum feed consumption, enough to ensure 
maximum growth, initially. This was gradually reduced to a minimum of 12 h by the 
7th WOA, marking the phase of constant light [22]. Thereafter, birds were maintained 
under natural photoperiods (12L: 12D) until the end of the study.

2.3 Experimental procedure

All procedures used followed approved guidelines for ethical treatment of 
experimental animals.

A total of 56 male guinea fowls (7 per age group) were bled at 4, 8, 12, 16, 20, 24, 
28, and 32 WOA. Two ml of blood was collected into EDTA vacutainer tubes from 
the wing vein, and spun at 7100 x g for 3 min at room temperature (18-25 °C). Plasma 
was then pipetted into a 1.5 ml microcentrifuge tube and stored at –20 °C until 
subsequently analysed for testosterone.

Prior to bleeding, however, 5 birds at each age were weighed, and then following 
bleeding, were sacrificed by cervical dislocation. Their testes and reproductive tracts 
were completely freed from the adjoining ligaments and fascia, weighed and fixed in 
Bouin’s solution overnight for histology.
Age-related changes in testicular histology

2.3.1 Histological preparation, cell identification, stereological analyses and cell counts

The histological techniques used in the present study have been described previously [23-24], therefore, only a brief description is given here. The testes (with capsule intact) were each divided into 2 halves. One half of each testis was fixed in Bouin’s solution, dehydrated in absolute ethanol and embedded in paraffin wax. They were sectioned (5 µm) using microtome (Leica RM2125RT), floated onto Poly-l-lysine subbed slides (Polysine; VWR International Leuven, Germany), and stained in eosin and Mayer’s haematoxylin. Germinal cell counts were restricted to preleptotene primary spermatocyte, type I spermatocyte in prophase I and step I spermatids [25-26]. Sertoli and Leydig cell nuclei were also counted. The Sertoli cells were identified on the basis of their nuclei following the descriptions given by Zlotnik [27] and de Reviers [24], while Leydig cells were identified by their characteristic location as clusters in the interstitial region and by nuclear diameter. In all cases, the location, relative size, shape and nuclear morphology of germ and somatic cells helped in cell identification. Nuclear diameters of testicular germ and somatic cells were obtained with previously calibrated calipers (this was calibrated using graticule under immersion oil) under immersion oil, using sections from 5 males and counting 20 nuclei/cell type/male. Cell counts/transverse section were determined from 10 sections of individual seminiferous tubules/slide and 10 interstitial areas (surface area determined)/slide for Leydig cells. Germ cell counts were determined for all testes involved. The numbers of fragmented nuclei were relatively high, and partially sectioned nuclei were counted as seen, if their cell type were clearly recognizable. To compensate for possible overestimation of cell numbers under such conditions, initial
Age-related changes in testicular histology

cell counts were corrected using Abercrombie’s [28] correction factor as follows: Nc
= N × e/(e + d), where:

Nc = The corrected number of cells in the preparation
N = The number of nuclei counted/tubular section
e = The thickness of the histological preparation
d = the diameter of the nucleus of a given cell type.

This correction determines the number of cells with nuclei effectively present in the preparation.

Total number of cell (Nt): Total cell numbers for germ and Sertoli cells per testis were determined using the formula Nt = Lt × Nc / e

Where Lt = Length of seminiferous tubules (estimated below), and e and Nc defined as in the above. Total Leydig cell numbers were determined in relation to the interstitial area occupied by the cells, and expressed as number of cells per 1000 µm² of interstitial area.

2.3.2 Dimensions of Seminiferous tubule (ST)

Total length of seminiferous tubule (Lt) was estimated based on the formula Lt = Vr × (100-C) •10^{-1}/S [23, 29], where: Vr = percentage of testicular tissue occupied by the ST as measured by a modification of the Chalkley’s [30] technique. This was determined by taking a picture of an entire cross section of each testis under the light microscope at ×4 magnification. Each cross section therefore yielded several pictures depending on the size of the cross section. Each picture was subsequently opened with previously calibrated ImageJ software (National Institutes of Health, USA), and grids 50 µm apart were superimposed on the entire image. With a pencil tool plug-in, the grids on each image were grouped into 25 points grids (as obtained with 25-point
grid graticule) and each field labeled, in ascending order, until the entire cross section was covered. Forty fields were then randomly chosen per cross section and counted as in the Chalkley’s [30] technique. Points that fell on the tubes (including the basement membrane) were considered as tubular while those that fell outside the tube were considered as non-tubular. This also represents the ratio of tubular to non-tubular tissue [14]. Vr is expressed as a percentage of testicular tissue occupied by seminiferous tubules. From this therefore, Vr could be determined according to the formula: 

\[ Vr = \frac{TW \times \% \text{tubes}}{p} \]

where TW = testis weight (g), p = specific gravity of the testis (in guinea fowls p = 1.05g/cm³, as in the male chicken, [23]). \% tubes = (number of ST points within the eye piece/total number of points of the eye piece) 

100. C = the histological contraction of the testes, is given by (Volume of fresh tissue - Volume of embedded tissue/Volume of fresh tissue) x100 [29]. For guinea fowls, C in both immature and mature birds was estimated as 33.4±13.1 [14]. S = mean area of a transverse section of ST. The ImageJ software (National Institutes of Health, USA) was used to measure the surface area of the tubules directly instead of deriving it from the diameter. Tubules tended to elongate with age, and diameters may therefore not be accurate when measured directly. Nonetheless, in tubules with minimum and maximum diameter differences not exceeding 20% [14], diameters and surface areas were measured in order to compare apparent diameters (diameters measured directly) to actual diameters (diameters derived from the surface area using the formula \( D = \sqrt{\text{surface area} \times \frac{4}{\pi}} \)). LT was expressed in meters (m).

2.3.3 Sertoli efficiency and quantitation of spermatogenesis

Other parameters estimated were ratio of round spermatids to Sertoli cells, Sertoli efficiency (total number of germ cells beyond the spermatogonia stage, supported by
Age-related changes in testicular histology

Each Sertoli cell) and meiotic index. Meiotic index, which measures the rate of spermatogenesis, was expressed as a theoretical ratio based on the mean ratio for 5 males, and was calculated as follows: given that each type I spermatocyte should provide 4 round spermatids during meiosis (MI = 4), and that ultimately, the actual ratio of type I spermatocytes to round spermatids is dependent on the life span of each cell type, %MI is therefore given as 100 (Number of round spermatids/life span of round spermatids)/4(number of type I spermatocyte/life span of type I spermatocyte) [31]. The life spans of primary spermatocyte and round spermatid in the guinea fowl (Numida meleagris) are 4.5 and 2.5 days, respectively, as obtained from BrdU observations and reported by Hein et al. [32].

Total reading for a parameter per testis was presented as average for the 2 testes (i.e left testis reading + right testis reading/2).

2.3.4 Testicular sperm production

A total of ten 32-week old guinea cocks were involved. A fragment of testis (of volumes ranging between 28.3 mm$^3$-265 mm$^3$) from each testis was weighed (fwt), homogenised in 0.25M sucrose (1:200; testes: sucrose), and elongated spermatids (el) and testicular spermatozoa (tspz) were counted using haemocytometer (10 replicates per testes). Results for each male were estimated as follows:

\[ \text{TSP/male} = \text{right TSP + left TSP} = \frac{(\text{el} + \text{tspz})}{\text{fwt}} \times \text{testicular weight} \] [31]

2.3.5 Testosterone assay

The testosterone assay had been previously validated for guinea fowl [19]. The assay was a RIA using tritiated tracer (Amersham Int., Amersham, Bucks, UK) and a procedure as originally described by Sheffield and O’Shaughnessy [33].
Age-related changes in testicular histology

testosterone antibody was obtained from Guildhay Antisera, Surrey, UK. The
detection limit was 0.06 ng/ml, and intra-assay coefficient of variation was 9.5%.
Cross reactivity with androstenedione and androstanediol were 0.3% and 3.9%,
respectively. The assays were performed after sample extraction using diethyl ether in
duplicate of 50 μl aliquots. Peripheral testosterone concentrations in all the samples
assayed were determined using the standard curve generated by the Assayzap
software (Biosoft®, USA). All samples were evaluated for testosterone in one assay.

2.4 Statistical analysis
Data were analysed using the SPSS software, version 20.0 [34]. Age-related changes
in histology of the reproductive organs and testosterone profiles in male guinea fowls
were analysed using univariate analysis for completely randomised design, and means
separated using tukey’s test. Where variances were not homogenous, Kruskal-Wallis
test was used instead and medians separated using Mann-Whitney U test. Data were
presented either as mean±standard error of mean or median (Interquartile range). All
comparisons were done at 5% level of significance.

3. Results
3.1 Testicular histology
The testes of the guinea fowl were contained in a covering, the tunica albuginea. The
capsule did not give off septa, and therefore no separation of testes into lobules was
seen in any of the birds. The seminiferous tubules were not separated by true septa,
but rather only fine strands of connective tissues passed inwards from the tunica to
separate the tubules. Occasionally, larger amounts of connective tissue were found
surrounding a blood vessel passing towards the tunica. In the testes of a mature
Age-related changes in testicular histology

breeding male guinea fowl, there were 4 germ and 2 somatic cell types. The germ cell
types were spermatogonia, primary spermatocytes, secondary spermatocytes and
round spermatids, which lined the basement membrane in a stratified manner. Three
different types of spermatogonia were seen in mature testes which could be
distinguished based on heterochromatin appearance and distribution, and nuclei
diameter. The somatic cells were Leydig and Sertoli cells.

At 8 WOA, only spermatogonia and Sertoli cells were present in the
seminiferous tubule of the birds, and the tubular lumen was absent or poorly
developed. These cells lined the basement membrane. There were no changes in the
tubular epithelium until at 12 WOA when both round and elongated spermatids (in
some samples) were visible. At this age, the lumen was generally well formed, but
tubules were widely separated by abundant interstitial tissue. By 16 WOA fully
formed spermatozoa could be found in both the tubular lumen and ductuli efferentes
of the epididymis, marking the onset of sexual activity. At this age, the interstitium
had decreased considerably in size and Leydig cells had become organized into
compact groups lying in the angular areas between adjacent seminiferous tubules
(Figure 1).

Age-related changes in testicular histological morphometric traits are shown in
Table 1. Round spermatid population size in the seminiferous tubules increased
significantly (Kruskal-Wallis $X^2 = 183.003$, df = 5, $p < 0.0001$) between 12 and 20
WOA. Cumulatively, the increase in round spermatid population size between week
20 and 28, and 24 and 32 were significant ($p < 0.05$). Type I spermatocyte population
size on the other hand remained constant between 12 and 16 WOA, and saw
significant (Kruskal-Wallis test $X^2 = 169.975$, df = 5, $p < 0.0001$) increases thereafter
until 20 WOA, dipped at 24 weeks, and increased ($p < 0.05$) until 32 WOA. Total germ
Age-related changes in testicular histology

cell numbers in the seminiferous tubule increased significantly (Kruskal-Wallis test $\chi^2 = 186.147$, df = 5, p < 0.0001) between 12 and 20 WOA. It remained constant thereafter until 24 weeks of age and then increased significantly (p < 0.05) between 24 and 32 WOA. Sertoli cell population size in the tubule also increased significantly (Kruskal-Wallis test $\chi^2 = 214.116$, df = 6, p < 0.0001) between 8 and 20 WOA. This was followed by a significant decrease (p < 0.05) at 24 weeks and thereafter, a significant rise at 28 and 32 WOA.

Number of round spermatids per Sertoli cell increased significantly (Kruskal-Wallis test $\chi^2 = 142.834$, df = 5, p < 0.0001) between 12 and 24 WOA. The value then dropped (p < 0.05) between this age and 28 WOA, and rose (p < 0.05) again to the level similar to that observed at 24 weeks, between 28 and 32 WOA. Similarly, total number of germ cells supported by each Sertoli cell differed (p < 0.0001) among age groups. It decreased significantly (p < 0.05) between 12 and 16 WOA, then increased (p < 0.05) cumulatively between 16 and 24 WOA. This was followed by a dip (p < 0.05) at 28 weeks and finally, a significant rise (p < 0.05) at 32 WOA.

Meiotic index, which is an indication of the rate of cellular death during the first and second meiotic divisions increased significantly (Kruskal-Wallis test $\chi^2 = 141.059$, df = 5, p < 0.0001) between 12 and 24 WOA. This was followed by a highly significant drop at 28 WOA, and finally, a significant rise (p < 0.05) between 28 and 32 WOA. The highest value was at 24 WOA {83.5 (71.3-95.8)%) and the lowest {6 (0-17.5)%)} at 12 WOA.

Both apparent and actual seminiferous tubular diameters exhibited the same pattern of growth between 8 and 32 WOA. Significant increases were recorded in apparent (Kruskal wallis test $\chi^2 = 189.885$, df = 6, p < 0.0001) and actual (Kruskal-Wallis test $\chi^2 = 206.497$, df = 6, p < 0.0001) seminiferous tubular diameters between 8
Age-related changes in testicular histology

and 24 WOA. From this point onward, there were no significant increases in both cases, however, there were cumulative increases (p<0.05) in both parameters between 24 and 32 WOA. Actual tubular diameter was significantly bigger (p<0.05) than apparent tubular diameter \(526.6 \pm 576.0 \mu m\) vs \(383 \pm 419.9 \mu m\). Relative volume of seminiferous tubule in the testes increased significantly (Kruskal-Wallis test \(X^2 = 348.574\), df = 6, \(p < 0.0001\)) between 8 and 20 WOA. It then stabilised for the next 8 weeks before increasing at 32 WOA. Seminiferous tubular length, on the other hand, significantly (Kruskal-Wallis test \(X^2 = 623.228\), df = 6, \(p < 0.0001\)) increased between 8 \(2.5 \pm 5.0\) m and 20 \(9.8 \pm 10.5\) m WOA, followed by a dip (p<0.05) at 24 WOA. It then increased (p<0.05) between 24 and 32 WOA. Testicular sperm production in the adult breeding guinea cock averaged \(9.9 \pm 10^7\) (8.5 \(\pm\)10^7 - 18.0 \(\pm\)10^7)

The Sertoli cells were located on the basement membrane. The Leydig cells had spherical nuclei and occurred as clusters in the interstitial region. They possessed prominent nucleoli. In the guinea fowls, the Sertoli cells were quasi-circular in most cases, and were significantly bigger (p<0.05) than the Leydig cell nuclei \(4.3\pm.07 \mu m\) vs \(3.0\pm.07 \mu m\).

Correlations among all the testicular morphometric parameters were positive and highly significant (p<0.01). Similarly, significant correlations existed between testicular weight and testicular sperm production, actual tubular diameter, Sertoli cell population, tubular length (p<0.01) and Sertoli efficiency (number of round spermatids per Sertoli cell and total number of germ cells per Sertoli cell) (p<0.05). The correlations between testicular weight and all the parameters except Sertoli efficiency were positive. Testicular sperm production was not correlated with any of
Age-related changes in testicular histology

the testicular morphometric parameters except meiotic index (p<0.01) and round spermatids population (p<0.05). These were positively related to testicular sperm production (Table 2).

3.2 Changes in peripheral testosterone concentration

Generally, no significant increases were recorded in peripheral testosterone concentrations measured monthly. Testosterone concentrations, however, tended to increase from 4 to 20 WOA when it peaked. Testosterone levels at sexual maturity (16 WOA) were significantly higher (p<0.05) than the levels in 4-week old birds. Similarly, the peak testosterone concentrations at 20 weeks were higher (p<0.05) than the concentrations at 4 and 8 WOA. Testosterone concentration decreased after 20 WOA to a level similar to that seen at 12 WOA and remained at that level until the end of the study (Figure 2).

Correlation between testicular weight and peripheral testosterone concentration was positive and highly significant (p< 0.0001). Similarly, there were significant (p<0.05) positive correlations between testosterone concentrations and actual tubular diameter, total number of germ cells per Sertoli cell and number of round spermatids per Sertoli cell (Sertoli efficiency) and tubular length (Table 2).

4. Discussions

4.1 Changes in the histology of the testes

In agreement with the observations made by Awotwi [15] and Brillard [14] in the local and exotic breeds of guinea fowls, respectively, the testes of a growing male guinea keet could only be detached for decent histological sections from 8 WOA. At this age, the seminiferous tubules had poorly-formed lumen or none at all; only
Sertoli cells and spermatogonia lined the basement membrane, and abundant interstitial tissue separated the tubules. Puberty, characterized by the presence of primary and secondary spermatocytes and round spermatids in the tubular lumen, was attained at 12 WOA in the birds studied by Brillard [14]. The author noted that elongated spermatids were seen in the tubular lumen of a few birds. The results of the present study confirm this earlier report by Brillard [14]. Awotwi [15], however, found only primary spermatocytes at 12 weeks and secondary spermatocyte at 16 WOA, an indication of late attainment of puberty in those birds. Guinea fowls used in this study attained sexual maturity at 16 WOA when fully formed spermatozoa were present both in the tubular lumen and the lumen of excurrent duct system. This was earlier than the 20 weeks reported by Awotwi [15] in the same breed. This result is not surprising considering the fact that the processes of spermatogenesis started earlier in the birds used in this study than those in the study by Awotwi [15]. The differences in the time of sexual maturity between the 2 flocks of birds may be attributed to possible differences in management, as management factors including feeding [35] and photoperiod [36] have been cited to alter dramatically the onset of meiosis and sustained spermatogenesis.

Seminiferous tubular diameter was measured in two ways during the present study. The actual seminiferous tubular diameter (estimation method developed during this investigation) was much larger than the apparent diameter (conventional method of tubular diameter estimation). This indicates that tubular diameters are usually underestimated using the conventional method of measurement. Another disadvantage of the conventional method is that not all tubules are given equal chances of being selected for measurement since the tubule has to be quasi-circular in order to be considered. Where a software package is employed for area measurement from which
Age-related changes in testicular histology

the diameter is determined, all these problems are avoided. Even though the use of the apparent tubular diameter underestimates the diameter of the tubule, it is still reflective of the true situation when comparing across groups or conducting trend analysis, as evidenced by the relationship between the trends of age-related variations in the two tubular diameters in the present study. The use of the actual tubular diameter approach is particularly useful when estimating tubular diameters in a situation where transverse sections of seminiferous tubules tend to elongate in growing animals, making it difficult to obtain the number of tubules required for the estimation of tubular diameters and other tubular parameters.

Several quantitative histological changes occurred in the testes of male guinea fowl during the period before sexual maturity. Both the apparent and actual seminiferous tubular diameters increased from 74.4 μm and 87.2 μm, respectively, at 8 weeks to 326.8 μm and 387.7 μm, respectively, at 20 WOA. Tubular length also increased from 2.5 m at 8 weeks to 9.8 m at 20 weeks. These reflected in massive increase in the relative volume of the seminiferous tubules. These figures tended to plateau after 20 WOA. Brillard [14], therefore, defined 20 weeks as the beginning of adulthood in the guinea fowl. The fluctuations seen after 20 weeks was attributable to the fact that these birds attained sexual maturity during the minor breeding season, and this may have influenced subsequent readings. The modifications seen in the seminiferous tubules led to early onset of spermatogenesis and rapid development of the spermatocytes population between 8 to 12 WOA (0 to 0.503 x 10⁸). Round spermatids were also present in all samples analysed at 12 WOA. It increased from this age and tended to stabilise from 20 WOA. Puberty in these birds therefore commenced from 12 WOA. A similar observation was made by Brillard [14]. This study, found some type I spermatocyte at 8 WOA, however, this was not noticed in
Age-related changes in testicular histology

The Sertoli cells were quasi-circular in the guinea fowl. This is in agreement with the earlier observation by Brillard [14]. Sertoli cell population increased even during adulthood and was linearly correlated with total germ cell numbers. This is consistent with the report of Brillard [14] in the exotic breeds of guinea fowls. The author evoked 2 hypotheses to explain the increase in Sertoli cell population during adulthood in the guinea fowl. First, even at sexual maturity, a low level of mitotic activity may persist among the Sertoli population. Secondly, some undifferentiated Sertoli cells might remain in the testes after sexual maturity. These cells could play the role of reserves proliferating and differentiating slowly during adulthood.

The fluctuations in the total number of germ cells per Sertoli cell may be attributed to the attainment of sexual maturity in the non-breeding season and cellular deaths. The reduced meiotic rate occurring during this period may account for the fluctuating numbers of germ cells supported by each Sertoli cell. It is currently accepted that the number of Sertoli cells established during testicular development determines the rate of spermatogenesis in sexually mature animals [37-38]. This assumption is based on the fact that each Sertoli cell supports a limited number of germ cells in a species-specific manner [39-40]. Studies have shown that spermatogenic efficiency, expressed as the number of sperm produced daily per gram of testis, is usually positively correlated with the number of germ cells supported by each Sertoli cell [39-41]. This was evidenced by the positive correlation between testicular sperm production and Sertoli efficiency in the present study. Other important factors that were reported to have correlated with spermatogenic efficiency were the volume density of the seminiferous tubule, the length of spermatogenic cycle, the number of spermatogonial generations, the rate of germ cell loss during
Age-related changes in testicular histology

spermatogenesis (supported by the strong positive correlation between testicular sperm production and meiotic index in this study), the number of Sertoli cells per gram of testis and the size of Sertoli cells [40, 42]. Contrary to the reports of Franca and Godinho [43], Sertoli cell population positively correlated with actual and apparent tubular diameter, and total germ cells per testis. The average number of round spermatids per Sertoli cell and total germ cell per Sertoli cell (Sertoli efficiency) in the adult guinea fowl were 12.5 and 7.2, respectively.

Germ cell apoptosis constitutes a normal process during spermatogenesis [44] and can occur in different developmental phases. It is considered mainly to function in density regulation of spermatogonia and to eliminate cells with chromosomal damage (meiotic phase), whereas cell loss during spermiogenesis is less prominent [40]. The quantitative significance of germ cell loss becomes clear when considering that only two to three spermatozoa of 10 theoretically possible cells are produced from type A1 spermatogonia [40, 45]. In the present study, the highest percentage of cell deaths was 94% at 12 WOA, while the least was 16.5% at 24 WOA. The high initial cell deaths at 12 WOA was not surprising considering the fact that these birds attained puberty at this age, and maximal efficiency of spermatogenesis, as indicated by quality of spermatozoa produced, is not achieved until several weeks after puberty has been attained [46]. The lower percentage of cell deaths (16.5%) observed in the present study at 24 WOA indicates a more efficient spermatogenesis in these birds at this age. The significant and positive correlations between testicular sperm production and number of round spermatids per testis, meiotic index and testicular weight, was an indication that these parameters could be good predictors of spermatogenic efficiency in guinea fowls. The lack of a significant correlation between Sertoli efficiency and Sertoli cell populations with testicular sperm production was possibly
Age-related changes in testicular histology

because of the relatively small sample size of 10 birds (for testicular sperm production).

4.2 Changes in peripheral testosterone concentration

The rise in peripheral testosterone concentrations between 12 and 20 WOA in the present study may be related to the early onset of puberty in these birds. Spermatozoa were first seen at 16 WOA. It is probable therefore that the phase of rising plasma testosterone levels occurred several weeks before the onset of sexual activity in the local guinea cocks.

In the present study, the peak testosterone concentrations in sexually mature guinea cocks were low (0.284 ng/ml). Abdul-Rahman et al. [19] also reported a low peak testosterone concentration (0.471 ng/ml) in breeding males. These results were not surprising considering earlier reports that male tropical birds have low plasma testosterone concentrations, involving low amplitude cycles with possible slight variations during times of breeding [47-49]. It is thought that these low concentrations are a way of avoiding the potential detrimental effects of elevated concentrations of testosterone, since there is a trade-off between testosterone concentration and immunity [50]. Consequently, selection in the tropics may have favoured birds with low concentrations of testosterone, in line with a slow pace of life, with more resources being allocated to immune function [51]. The guinea fowl is a tropical bird [20].

The peak testosterone concentrations recorded in the present study is several fold lower than those reported in exotic breeding guinea cocks [52-53]. A possible reason for this massive difference is that the guinea fowls used in the present study are indigenous breeds, small in stature, and have not undergone any intensive...
Age-related changes in testicular histology

selection and breeding compared to their exotic counterparts. The exotic breeds are much higher in weight at all ages than the local breeds [54]. Several workers [55-57] have reported positive relationship between body and testicular weight. Positive relationship has also been reported between testes size and testosterone titer [58-60], with some authors inferring that the link is a consequence of the phenotypic integration of spermatogenic and endocrine functions of the testes [58-59]. The testicular weight reported for the exotic guinea fowl is two fold higher [14, 52-53] than that found in the indigenous guinea fowls in the present study. The lower testicular weight and corresponding lower testosterone concentrations in the indigenous guinea fowls are, therefore, not surprising.

Rising plasma testosterone levels in the guinea fowls corresponded to increasing seminiferous tubular diameters and volume. Sertoli and germ cell populations also increased from 12 WOA. All these parameters did not see any significant rise after the peak testosterone concentration was attained at 20 WOA, implicating this hormone in spermatogenesis and the development of the seminiferous tubules. A role for testosterone in adult testicular function is suggested by the finding in mature hypophysectomized quail that administration of large doses of testosterone, while insufficient to maintain spermatogenesis, retards testicular regression resulting from the surgery [61]. Germ cell development started between 8 and 12 WOA when the concentrations of testosterone were low, while spermatids and spermatozoa were observed between 12 and 16 WOA when testosterone had nearly peaked. Low doses of testosterone have also been implicated in the maturation of the germinal epithelium in intact immature cockerels [62-63].

The significant positive correlations between plasma testosterone concentrations and Sertoli efficiency, actual seminiferous tubular diameter and
Age-related changes in testicular histology

Seminiferous tubular length is an indication that plasma testosterone concentrations in the local guinea fowls could be highly related to these histological morphometric parameters.

In conclusion, puberty and sexual maturity were attained at 12 and 16 weeks of age, respectively, in male guinea cocks. The pattern of testosterone secretion in the guinea cock may be divided into two, initial phase of increasing testosterone concentrations prior to 20 WOA, and a final one of decreasing peripheral testosterone concentrations after 20 WOA, and may be implicated in the development of histological structures of the testes and spermatogenesis in the guinea cock.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the article.

Acknowledgements

The authors wish to thank Iain Macmillan, Peter O’Shaughnessy and Neil Evans, all of Veterinary Biosciences, University of Glasgow, for help on histological techniques and radioimmunoassay. Funding for the project was partly provided by the commonwealth Scholarship Commission in the UK, and Association of Commonwealth Universities (CSC Ref No: 2009-378).

References

Age-related changes in testicular histology


Age-related changes in testicular histology


Age-related changes in testicular histology


Age-related changes in testicular histology


[34] IBM Corp. IBM SPSS Statistics for Macintosh, Version 20.0. 2011; Armonk, NY.


[38] Hess RA, Cooke PS, Bunick D, Kirby JD. Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli cell and germ cell number. J. Endocrinol. 1993; 132: 2607-2613.

Age-related changes in testicular histology


Age-related changes in testicular histology


[57] Sarabia FJ, Pizarro DM, Abad MJ, Casanovas IP, Rodriguez-Bertos A, Barger K. Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of
Age-related changes in testicular histology


[63] Siegel HS. Nitrogen metabolism in cockerels treated with 17-α-methyl-17-β-hydroxyandrosta-Δ1, 4-3-one (methandrostenolone). Gen Comp Endocrinol. 1964; 4: 132-143.
Figure 1: Cross section of guinea fowl testes at various developmental stages: 8 (A), 12 (B), and 20 (C) weeks old. Note Interstitial tissue (INT), Seminiferous tubule (ST), Spermatogonia (SG), Sertoli cells (arrow), primary and secondary spermatocytes (SpI and II), round (RS) and elongated (EL) spermatids, Seminiferous tubular lumen (ST. Lumen), HE x20 (Scale bar = 100 μm). Plate D shows the distal ductule efferentes of guinea cock at 16 weeks indicating the first appearance of spermatozoa (arrow head) in the lumen, HE x20 (Scale bar = 100 μm).
Age-related changes in testicular histology

Figure 2: Peripheral testosterone concentrations in guinea cocks during sexual development.
## Age-related changes in testicular histology

Table 1: Developmental changes in testicular histological morphometric traits in local guinea cocks

<table>
<thead>
<tr>
<th>Testicular morphometric trait</th>
<th>Age (weeks)</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>nSpdR (x10^4)</td>
<td></td>
<td>0.1 (0.1 - 0.6)c</td>
<td>0.5 (0.3 - 0.8)c</td>
<td>1.6 (0.9 - 2.3)c</td>
<td>1.9 (1.1 - 3.0)bc</td>
<td>2.2 (1.7 - 2.8)b</td>
<td>3.8 (2.4 - 4.8)a</td>
<td></td>
</tr>
<tr>
<td>nSpcI (x10^6)</td>
<td></td>
<td>0.5 (0.2 - 1.3)c</td>
<td>0.8 (0.5 - 0.9)c</td>
<td>1.3 (1.1 - 1.7)c</td>
<td>1.1 (0.6 - 1.6)d</td>
<td>2.0 (1.6 - 2.6)b</td>
<td>3.1 (2.1 - 3.9)a</td>
<td></td>
</tr>
<tr>
<td>spdR/Sert</td>
<td></td>
<td>0.4 (0-3.2)d</td>
<td>2.0 (1.6-2.7)c</td>
<td>4.0 (1.8-4.6)b</td>
<td>6.0 (4.8-7.4)a</td>
<td>3.9 (3.1-5.4)b</td>
<td>7.2 (5.3-8.6)a</td>
<td></td>
</tr>
<tr>
<td>Mind (μm)</td>
<td></td>
<td>7.3±0.5ed</td>
<td>5.4±0.5c</td>
<td>6.5±0.5ed</td>
<td>9.9±0.6b</td>
<td>8.4±0.6c</td>
<td>12.7±0.5a</td>
<td></td>
</tr>
<tr>
<td>øtGcPlpn: Total germ cell population</td>
<td>6.0 (17.5)c</td>
<td>(20.8-57.5)d</td>
<td>(35.7-72.2)bc</td>
<td>(71.3-95.8)a</td>
<td>(46.3-47.5)c</td>
<td>(48.2-75.6)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>øappØ (μm)</td>
<td></td>
<td>134.1</td>
<td>266.0</td>
<td>326.8</td>
<td>312.5</td>
<td>384.0</td>
<td>397.8</td>
<td></td>
</tr>
<tr>
<td>øactØ (μm)</td>
<td></td>
<td>199.2</td>
<td>335.2</td>
<td>387.7</td>
<td>451.5</td>
<td>492.7</td>
<td>501.1</td>
<td></td>
</tr>
<tr>
<td>ønSert (x10^3)</td>
<td></td>
<td>0.7 (0.4-1.6)f</td>
<td>1.5 (0.6-2.0)</td>
<td>2.2 (1.9-2.8)d</td>
<td>3.7 (3.6-4.4)b</td>
<td>3.1 (4.2-5.8)b</td>
<td>3.3 (4.3-7.1)a</td>
<td></td>
</tr>
<tr>
<td>øVr (%)</td>
<td></td>
<td>60.0</td>
<td>86.0</td>
<td>90.0</td>
<td>96.0</td>
<td>96.0</td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td>øLt (m)</td>
<td></td>
<td>2.5 (1.8-5.0)d</td>
<td>4.9 (3.9-6.8)f</td>
<td>6.1 (5.4-6.8)f</td>
<td>9.8 (9.1-10.5)f</td>
<td>8.5 (4.8-10.2)d</td>
<td>10.7 (8.3-11.7)b</td>
<td></td>
</tr>
<tr>
<td>øTW (mg)</td>
<td></td>
<td>5.0</td>
<td>38.5</td>
<td>94.5</td>
<td>192.5</td>
<td>170.5</td>
<td>365.5</td>
<td></td>
</tr>
</tbody>
</table>

*Mean±SEM. Abbreviations: nSpdR: Round spermatids population, nSpcI: TypeI spermatocyte population, spdR/ Sert: Round spermatids/Sertoli cell, tGM/Sert: Total number of germ cells per Sertoli cell, Mind: Meiotic index, øGcPlpn: Total germ cell population, øSert: Sertoli cells population, øØ: actual tubular diameter, øappØ: Apparent tubular diameter, øVr: Relative volume of seminiferous tubules, øLt: Seminiferous tubular length, øTW: Testicular weight.
### Table 2: Correlations among testicular morphometric characteristics, testicular sperm production and peripheral testosterone concentrations in guinea cocks

<table>
<thead>
<tr>
<th></th>
<th>nSpdR</th>
<th>nSpcl</th>
<th>spdR/Sert</th>
<th>tGM/Sert</th>
<th>Mind</th>
<th>tGcPlp</th>
<th>actØ</th>
<th>appØ</th>
<th>nSert</th>
<th>Vr</th>
<th>Lt</th>
<th>TSP</th>
<th>Testo Conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>nSpcl</td>
<td>.678***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spdR/Sert</td>
<td>.610***</td>
<td>.535***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tGm/Sert</td>
<td>.541***</td>
<td>.586***</td>
<td>.926***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mind</td>
<td>.455***</td>
<td>.204**</td>
<td>.697***</td>
<td>.448***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tGcPlp</td>
<td>.750***</td>
<td>.937***</td>
<td>.712***</td>
<td>.675***</td>
<td>.438***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>appØ</td>
<td>.582***</td>
<td>.548***</td>
<td>.607***</td>
<td>.560***</td>
<td>.488***</td>
<td>.587***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>actØ</td>
<td>.610***</td>
<td>.500***</td>
<td>.602***</td>
<td>.560***</td>
<td>.481***</td>
<td>.543***</td>
<td>.853***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nSert</td>
<td>.583***</td>
<td>.792***</td>
<td>.289***</td>
<td>.198***</td>
<td>.310***</td>
<td>.771***</td>
<td>.473***</td>
<td>.433***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vr</td>
<td>.272***</td>
<td>.240***</td>
<td>.319***</td>
<td>.236***</td>
<td>.398***</td>
<td>.281***</td>
<td>.453***</td>
<td>.529***</td>
<td>.341**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lt</td>
<td>.426***</td>
<td>.360***</td>
<td>.324***</td>
<td>.182**</td>
<td>.395***</td>
<td>.435***</td>
<td>.361***</td>
<td>.386***</td>
<td>.537***</td>
<td>.429***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSP</td>
<td>.297*</td>
<td>- .195</td>
<td>.291*</td>
<td>.258*</td>
<td>.472**</td>
<td>.171</td>
<td>-.129</td>
<td>-.105</td>
<td>.184</td>
<td>-.006</td>
<td>.195</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testo Conc</td>
<td>.156</td>
<td>-.010</td>
<td>.260*</td>
<td>.238*</td>
<td>.160</td>
<td>.107</td>
<td>.039</td>
<td>.0298*</td>
<td>.004</td>
<td>-.020</td>
<td>.239*</td>
<td>-.157</td>
<td></td>
</tr>
<tr>
<td>TW</td>
<td>.035</td>
<td>-.033</td>
<td>-.247*</td>
<td>-.403**</td>
<td>.061</td>
<td>.012</td>
<td>.212</td>
<td>.354**</td>
<td>.327**</td>
<td>.098</td>
<td>.500***</td>
<td>.459**</td>
<td>.563**</td>
</tr>
</tbody>
</table>

Abbreviations: nSpdR: Round spermatids population, nSpcl: TypeI spermatocyte population, spdR/Sert: Round spermatids/Sertoli cell, tGM/Sert: Total number of germ cells per Sertoli cell, Mind: Meiotic index, tGcPlp: Total germ cell population, nSert: Sertoli cells population, actØ: actual tubular diameter, appØ: Apparent tubular diameter, Vr: Relative volume of seminiferous tubules, Lt: Seminiferous tubular length, Testo Conc: Peripheral testosterone concentration, TW: Testicular weight