Age-related changes in the gross anatomy of the reproductive organs, and associated steroid hormone profiles in male and female guinea fowls (*Numida meleagris*)

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A B S T R A C T

Owing to the paucity of information on the reproductive biology of guinea fowls, a study involving a total of 132 birds was conducted, and this documented the developmental changes in the gross anatomy of the reproductive organs of males and females from hatching until 32 weeks of age (WOA), and associated steroid hormone changes. Testicular anatomical biometric traits increased significantly ($p < 0.0001$) from 8 WOA, and stabilised between 16 and 20 WOA, while peripheral testosterone concentration peaked at 20 WOA. Correlations among all testicular biometric characteristics were strong and positive ($p < 0.0001$). Similarly peripheral testosterone concentrations strongly ($p < 0.01$) and positively correlated with all the testicular anatomical biometric traits. In the female guinea fowl, the ovary and oviduct were discernible and measurable at hatching. Significant ($p < 0.0001$) increases were seen in ovarian and relative ovarian weights, and oviducal weights and lengths between 24 and 28 WOA. Plasma 17β-oestradiol decreased gradually to a very minimum at 16 WOA, and then began to increase gradually until 28 WOA when it plateaued. Peripheral progesterone concentrations on the other hand increased gradually from 4 WOA and peaked at 12 WOA, and then fluctuated considerably thereafter. Correlations among ovarian/oviducal anatomical parameters were strong ($p < 0.0001$) and positive. Similarly, peripheral oestradiol concentrations strongly ($p < 0.0001$) and positively correlated with all ovarian/oviducal anatomical parameters. Testicular anatomical biometric traits stabilised between 16 and 20 WOA, coinciding with peak peripheral testosterone concentrations, while ovarian/oviducal parameters recorded huge increases between 24 and 28 WOA, and may be under the influence of oestradiol.

1. Introduction

The male bird possesses paired reproductive tracts lying along the dorsal body wall (Kirby & Froman, 2000). Each tract consists of a testis, weighing between 0.32 and 2.0 g, and 14 and 60 g in guinea (Awotwi, 1975) and domestic (Lake, 1971) cocks, respectively, depending on the breed, an epididymis, and a highly convoluted deferent duct running alongside the ureter (Lake, 1971). In terms of testicular mass, Awotwi (1975) identified 4 phases of testicular growth in the local guinea fowls (*Numida meleagris*) These include periods of rapid growth, spanning between 8 to 16 WOA; slow growth (20–28 WOA); rapid growth (28–32 WOA); and finally, almost static growth after 32 WOA. Brillard's (1986) report on the guinea fowl, however, demonstrated only 2 phases: initial phase of rapid growth (8–20 WOA) and one of static growth after 20 WOA. Bennet (1947) found a definite relationship between testes size and age of White Leghorns up to the 6th month of age (sexual maturity). This suggests factors other than age may play a role in the determination of gonad size after the attainment

of sexual maturity in the White Leghorn. It has also been shown that the weight of the testes of the fowl varies according to breed (Kumaran & Turner, 1949a, 1949b).

Age-related differences in testis size may result from younger birds secreting less gonadotropins than older birds (Ketterson & Nolan, 1992; Silverin, Kikuchi, & Ishii, 1997), but whether testicular sensitivity to these hormones is also age-dependent is unknown. Age-dependent differences in mean and maximal testis size may account for lower plasma testosterone in younger adult males compared with older males (Deviche & Sharp, 2001; Deviche, Wingfield, & Sharp, 2000; Sorenson, Nolan, Brown, Derrickson, & Monfort, 1997). Testes in testosterone-treated hypophysectomized immature quail remain undeveloped, but increase in size when birds also receive luteinizing hormone (LH) and, especially, follicle stimulating hormone (FSH) (Brown & Follett, 1977). This observation provides no evidence for a role for testosterone in regulating testicular development. However, 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 (Brown & Follett, 1977).

The normal, functional gonad of the hen is the left ovary. It is an irregularly shaped, pinkish organ which is situated on the left side of the abdominal cavity close to the median line (Deol, 1955). A right ovary is present as a rudiment at hatching, but this decreases in size and persists throughout life only as an inconspicuous vestige (Hodges, 1974). In the guinea fowl, Awotwi (1975) reported a steady increase in ovarian weight from 0.04 g at 4 WOA to 30.5 g at sexual maturity (36 WOA); this regressed to 2.7 g in non-breeding-hens (Abdul-Rahman, Obese, Robinson, Awumbila, & Jeffcoate, 2016a). The reports of Romanoff and Romanoff (1949) indicated that at day old, the ovary of a chick weighed 0.03 g. This increased to 0.32 g, 2.66 g, 6.55 g and 38.0 g at 3, 4, 5 months and after pullet had laid the first egg, respectively. Chaikoff, Lorenz, and Entenman (1941) observed that this steady increase in ovarian weight decreased to about 5 g after the hen had ceased laying. Similar observation was made by Hafez and Kamar (1955) in Fayomi chicks; these authors, however, noted that the developmental changes in this organ were more marked just prior to sexual maturity than at any other time.

The increase in progesterone concentrations 6–4 h before ovulation (Bacon et al., 2002; Etches, 1990) is predominantly the result of increased secretion by the largest preovulatory follicle (Etches, 1990). Similarly, preovulatory follicle secretion of oestradiol increases in each of the four largest follicles 6–3 h prior to ovulation and is greatest in the third- and fourth-largest follicles. Overall, however, the majority of oestrogen produced by the ovary originates from prehierarchal follicles (Johnson, 2000). Oestrogens, together with progesterone, are required for priming of the hypothalamus and pituitary in order that progesterone can induce LH release (Wilson & Sharp, 1976).

Several studies have documented age-related variation in testis size, with older adults generally having larger testes than younger adults (Deviche et al., 2000; Graves, 2004; Hill, 1994; Laskemoen, Fossoy, Rudolfsen, & Lifjeld, 2008; Morton, Peterson, Burns, & Allan, 1990; Selander & Hauser, 1965). A preliminary study by Awotwi (1975) reported age related changes in testicular and ovarian mass in the guinea fowl, these were not detailed, and involved small sample size (Awotwi, 1975). Steroid hormone synthesis in these birds during sexual development has also not been reported. Besides, there is a general paucity of information on the reproductive system of guinea fowls. The objective of the present study, therefore, was to document the developmental changes in gross anatomy of the reproductive organs of male and female guinea fowls from hatching until adulthood (32 WOA), and associated steroid hormone changes.

2. Materials and methods

2.1. Experimental site

The study was conducted at the Poultry Unit of the Department of Animal Science, 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, Unpublished). Rainfall is monomial with mean annual rainfall varying from 1000 to 1500 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. The zone has nearly equal amounts of light and darkness (12L:12D) within a 24 h period throughout the year. The guinea fowl used in the present study is native to this location, and breeds more intensely between April and August during rainy season, and declines when the season is receding between August and October (Abdul-Rahman, Robinson, Obese, Jeffcoate, & Awumbila, 2016b).

2.2. Animals and management

A total of 132 local guinea fowls (*Numida meleagris*), of the pearl variety, were used for the study. Birds were brooded for 6 WOA (Teye & Gyawu, 2002), and then transferred to a deep litter house 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 (Teye & Gyawu, 2002). 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 3000 Kcal ME/kg diet), grower (14% crude protein and 2800 Kcal ME/kg diet), and breeder (17.5% crude protein and 2800 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 (Thiele, 2009) 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 WOA. 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 13 h, marking the phase of constant light, since no increase in day length is recommended until the phase of planned light stimulation is reached (Thiele, 2009). The phase of planned light stimulation was identified by the onset of lay in two female birds at 21 WOA. This could not be planned earlier because laying age in these birds vary considerably (Awotwi, 1987). At this age, lights were gradually adjusted to 14 h using incandescent bulbs (10 lx) to adjust the day length by 2 h during the dark period.

2.3. Experimental procedure

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

A total of 112 (56 per sex) guinea fowls (14 per age group; 7 per sex) 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 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 in males, and oestardiol and progesterone in females.

Prior to bleeding, however, 10 birds at each age (5 per sex) were weighed, and then following bleeding, were sacrificed by cervical dislocation. Their testes/ovaries and reproductive tracts were completely freed from the adjoining ligaments and fascia, weighed and measurements taken.

2.3.1. Gross anatomical and peripheral testosterone measurements in the male

Testicular length, width and height were measured using calipers and recorded to the nearest 0.1 mm. Testicular volume was estimated using the formula for a prolated spheroid as follows: $V = 4/3\pi \, (1/2\,L)(1/2\,W)^2$ (Ramirez-Bautista & Gutierrez-Mayen, 2003) and recorded to the nearest 0.1 cm³. Testicular weights were taken with a Mettler electronic scale and recorded to the nearest 0.1 mg. Relative testes weight was also calculated as the ratio of testes to body weight. Length of ductus deferens was measured as the distance from the point of attachment of the ductus to the epididymal region until it entered the connective tissue of the internal part of the cloaca on either sides of the large intestine and ureters, using a ruler and recorded to the nearest
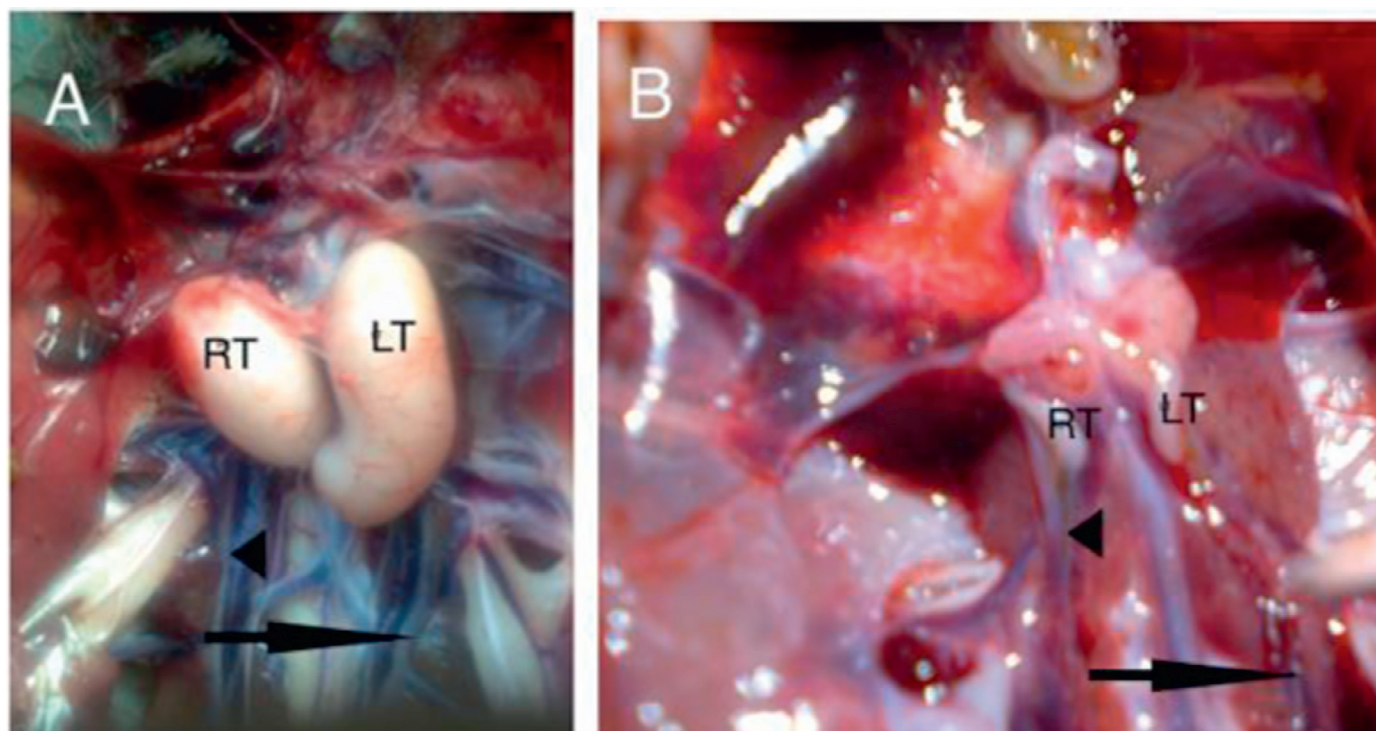
Fig. 1. Reproductive tracts of sexually mature (A) and 8 week old (B) male guinea fowls (in-situ), showing the left testes (LT), right testes (RT), left (arrowed) and right (arrow head) ductus deferens.

0.1 cm. Finally, qualitative changes including change in colour of the testes were observed and recorded. Total reading for a parameter per testis was presented as average for the 2 testes (i. e left testis reading + right testis reading/2).

The testosterone assay had been previously validated for guinea fowl (Abdul-Rahman et al., 2016b). The assay was a RIA using tritiated tracer (Amersham Int., Amersham, Bucks, UK) and a procedure as originally described by Sheffield and O'Shaughnessy (1989). The 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.

2.3.2. Gross anatomical, peripheral progesterone and 17β-oestradiol measurements in the female

Ovarian and oviduct weights were taken on a Mettler electronic scale and recorded to the nearest 0.001 g and 0.01 g, respectively. Relative ovarian weight was calculated from the ratio of ovarian to body weight. Length of oviduct was also measured with a ruler/flexible tape and recorded to the nearest 0.1 cm. At sexual maturity, lengths and widths of the various regions (identified on the basis of differences in widths of the various regions, Romanoff & Romanoff, 1949) of the oviduct were taken, namely, the infundibulm, magnum, isthmus, vagina and uterus. Also, numbers and diameters of visible oocytes in breeding birds were determined. Oocytes were categorised into F1 to F7 for yellow follicles, F1 denoting the biggest yellow follicle and F7 the smallest. White follicles were also categorised into small (<1 mm–3 mm), medium (>3 mm–6 mm) and large (>6–8 mm) follicles (Romanoff & Romanoff, 1949). Also, qualitative changes including change in colour and shape of the ovary were observed and recorded.

As with testosterone, 17β-oestradiol assay had also been previously

validated for guinea fowl (Abdul-Rahman et al., 2016a). Using the Oestradiol Maia kit (RADIM diagnostics, USA), all samples were evaluated in one assay, and intraassay CV was 9.8%. The assay sensitivity was 5.2 pg/ml. For progesterone concentrations determination, no validation was required since it had been previously measured in the guinea fowl (Onyeanusi, 2007). The Coat-A-Count® Progesterone kit (Siemens Healthcare Diagnostics, USA) was used for determining progesterone concentrations. The plasma progesterone concentrations in all samples were determined in one assay, and the intra-assay CV was 7.1%. The sensitivity of the assay was 0.08 ng/ml.

2.4. Statistical analysis

Data were analysed using the SPSS software, version 20 (IBM, 2011). The data were evaluated for normality of variance and homogeneity using the Shapiro-Wilk's W and Levene's tests, respectively. Age-related changes in gross anatomy of reproductive organs of male and female, and associated peripheral steroid hormone concentrations 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). Correlations among most of the variables measured were also determined where appropriate. All comparisons were done at 5% level of significance.

3. Results

3.1. Gross anatomy of male reproductive tract and peripheral testosterone profiles

The paired reproductive tracts of the guinea fowl consist of testes and highly convoluted vas deferens. After hatching, the testes and vas deferens are not discernible until about 4 WOA. At this age, the testes are seen as thickenings on the upper parts of their respective vas

Table 1
Developmental changes in testicular anatomical traits and peripheral testosterone concentrations in male local guinea fowls.

Testicular Anatomical trait {Median (Interquartile range)}		AGE (Weeks)							
		4	8	12	16	20	24	28	32
Testicular weight	(mg)	–	5.0 (2.8–7.8)[e]	38.5 (23.0–91.5)[d]	94.5 (82.5–133.5)[c]	192.5 (131.5–241.3)[b]	170.5 (86.5–304.5)[bc]	367.5 (226.1–428.6)[a]	351 (246.0–408.5)[a]
Gonadosomatic index (GSI	(x10^{-4})	–	0.8 (0.3–1.0)[c]	1.0 (1.0–3.0)[b]	3.0 (3.0–4.0)[a]	4.5 (2.8–5.5)[a]	4.0 (3.0–8.0)[a]	6.0 (4.0–7.0)[a]	5.0 (4.0–7.0)[a]
Testicular volume	(Cm^3)	–	0.01 (0.005–0.014)[d]	0.04 (0.04–0.13)[c]	0.13 (0.11–0.20)[bc]	0.25 (0.16–0.31)[ab]	0.21 (0.12–0.38)[ab]	0.38 (0.23–0.49)[a]	0.38 (0.30–0.46)[a]
Testicular width	(mm)	–	1.9 (1.6–2.1)[d]	3.6 (3.3–4.9)[c]	4.8 (4.7–5.7)[b]	6.1 (5.3–6.9)[ab]	6.2 (4.7–7.4)[ab]	7.2 (6.3–7.9)[a]	7.4 (6.4–7.8)[a]
Testicular height	(mm)	–	1.3 (1.0–1.5)[e]	3.0 (2.6–0.9)[d]	4.0 (3.7–4.1)[cd]	4.9 (4.1–5.5)[b]	4.4 (3.6–6.3)[bc]	6.0 (5.3–6.8)[a]	6.0 (5.9–6.8)[a]
*Testicular length	(mm)	–	4.6 ± 0.6[d]	7.4 ± 0.5[c]	9.9 ± 0.5[b]	12.0 ± 0.6[ab]	11.1 ± 0.5[ab]	13.2 ± 0.6[a]	13.3 ± 0.5[a]
*Testosterone concentration	(ng/ml)	0.09 ± 0.04[c]	0.12 ± 0.04[bc]	0.19 ± 0.04[abc]	0.26 ± 0.04[ab]	0.28 ± 0.04[a]	0.16 ± 0.04[abc]	0.17 ± 0.037[abc]	0.18 ± 0.037[abc]

Means/Medians (Interquartile range) within a row having no superscript in common are significantly ($p < 0.05$) different. *Mean ± SEM. –: Testis appeared just as a thickening at this age and could not be detached for any measurement.

deferens, but cannot be detached for weighing or good histological section. The vas deferens can, however, be measured at this age. In this study the testes were first detached for measurement at 8 WOA. They were bean shaped and creamy in colour (Fig. 1).

Developmental changes in testicular anatomical traits and peripheral testosterone concentrations in male guinea fowls are presented in Table 1. Generally, testicular weight increased significantly (Kruskal–-Wallis test X^2 = 35.506, df = 6, $p < 0.0001$) from 8 until 20 WOA, and between 24 and 28 WOA. Similarly, gonadosomatic index, which is the relative investment into testicular growth increased significantly (Kruskal–Wallis test X^2 = 26.633, df = 6, $p < 0.0001$) between 8 and 16 WOA, and remained constant thereafter. Testicular volume, on the other hand, increased significantly (Kruskal–Wallis test X^2 = 34.113, df = 6, $p < 0.0001$) between 8 and 12 WOA, and was maintained until 16 WOA. From 12 WOA onward, no statistical ($p > 0.05$) differences were noticed between any adjacent age groups, the cumulative increase between 12 and 20 WOA was significant ($p < 0.05$). Similarly, 28 and 32 week old birds had significantly ($p < 0.05$) higher testicular volume than 16 week old birds. Generally from 20 WOA, no significant ($p > 0.05$) increase in testicular volume was noticed.

A significant rise was noticed in testicular width (Kruskal–Wallis test X^2 = 33.297, df = 6, $p < 0.0001$) from 8 until 16 WOA. Even though no differences ($p > 0.05$) in width were noticed between any adjacent age groups from 16 WOA, cumulatively, the increase between 16 and 28 weeks was significant ($p < 0.05$). Testicular height, on the other hand, showed inconsistent growth pattern. This biometric trait increased significantly (Kruskal–Wallis test X^2 = 32.301, df = 6, $p < 0.0001$) between 8 and 12, 16 and 20 and 24 and 28 WOA. Testicular length showed similar growth pattern as width. A significant increase ($p < 0.05$) was noticed in length between 8 and 16 WOA. Also, the cumulative increase between 16 and 28 weeks was significant ($p < 0.05$).

Unlike other traits of the male reproductive organs, initial suspension of growth occurred much earlier in the vas deferens. This organ increased significantly ($p < 0.05$) in length between 4 and 8 WOA, remained unchanged thereafter ($p > 0.05$) until puberty (12 WOA) and increased ($p < 0.05$) again between 12 and 16 WOA. No change was recorded in the length of the duct thereafter (Fig. 2).

Peripheral testosterone concentration 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 WOA 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. Correlations among all testicular biometric characteristics were positive and highly significant ($p < 0.0001$). Similarly, correlations between all testicular anatomical characteristics, testicular weight and peripheral testosterone concentration were positive and highly significant ($p < 0.0001$; Table 2).

3.2. Gross anatomy of female reproductive tract and peripheral steroid hormone profiles

The ovary and oviduct of the guinea fowl are discernible and measurable at hatching. The ovary was visible as a whitish-cream crescent-shaped organ from hatching until about 16 WOA when it was a creamy L-shaped organ. The grape-like appearance began to manifest from 20 WOA and by 27th to 28th WOA, the ovary had completely assumed the 'grape cluster' appearance. Yellow follicles emerged after 26 WOA (Fig. 3). Except for 2 birds which laid exactly at 21 WOA, all birds laid between 27 and 28 WOA, marking the onset of sexual maturity in these birds.

Age-related changes in ovarian/oviducal anatomical parameters and peripheral steroid hormone concentrations in guinea hens are shown in Table 3. The ovary increased significantly (Kruskal–Wallis test X^2 = 56.411, df = 8, $p < 0.0001$) in weight between 1 and 4, 8–12, 12–16, and 16–20 WOA. This would coincide with the end of first phase of follicular growth. Ovarian weight then remained unchanged until 24 WOA. From this age, there was a highly significant ($p < 0.0001$) increase in ovarian weight from 0.324 (0.293–0.433) g to 17.2 (14.8–21.2) g at sexual maturity (28 weeks). Relative ovarian weight on the other hand showed a different pattern of growth. It decreased significantly ($p < 0.05$) between 4 and 8 WOA before returning to approximately the 4 week level at 12 WOA, where it remained until 28 WOA where a highly significant ($p < 0.0001$) increase in relative weight was seen.

As with the ovary, increases in length and weight of the oviduct were also not consistent. Oviduct length increased significantly (Kruskal–Wallis test X^2 = 49.513, df = 7, $p < 0.0001$) between 4 and 8, and 12 and 16 WOA, with a final increase from 9.6 (8.2–12) cm at 24 WOA to 45 (40.5–56) cm at 28 WOA. Similarly, oviduct weight did not change significantly with age until 12 WOA, with a significant cumulative increase between 8 and 16 WOA. It then increased significantly ($p < 0.05$) between 16 and 20 WOA, and again at 28 WOA from 0.640 (0.175–1.810) g to 17.4 (14.3–24.2) g.

Peripheral 17β-oestradiol concentration did not change appreciably between 4 and 20 WOA. The levels at 4 WOA, however, were higher
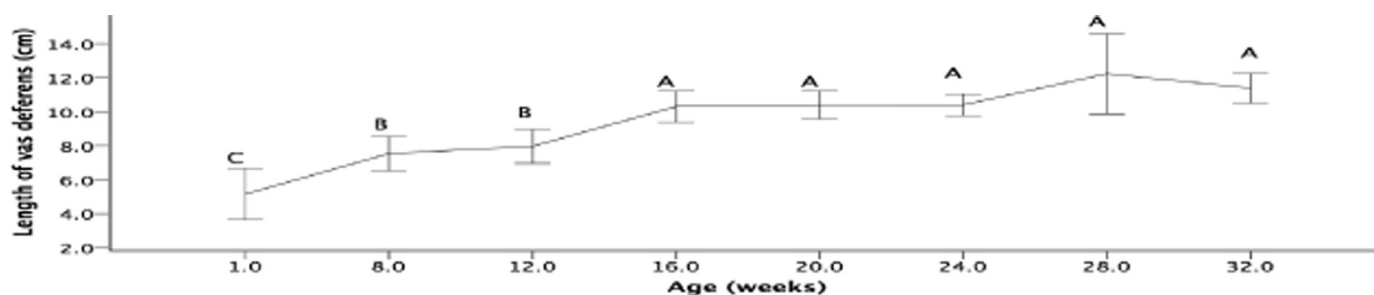
Means (+/− SEM) having different letters are significantly different (p<0.05)

Fig. 2. Age-related changes in length of ductus deferens in local guinea fowls.

($p < 0.05$) than those at 12, 16 and 20 WOA. Peripheral oestradiol concentrations increased significantly ($p < 0.05$) from 20 WOA, reaching a peak {57.1 (36.6–103.4) pg/ ml} at 28 WOA before a slight decline at 32 WOA. Progesterone concentrations were relatively constant between 4 and 12 WOA, and then tended to decrease ($p > 0.05$) between 12 and 16 WOA. The concentrations at 8 WOA were significantly higher ($p < 0.05$) than those at 16 and 20 WOA. Between 16 and 20 WOA, peripheral progesterone concentration dropped significantly ($p < 0.05$) to minimal levels. This was followed by a significant ($p < 0.05$) increase between 20 and 32 WOA.

The number of visible oocytes in the ovary of breeding guinea hens ranged from 92 to 177 and averaged 127. Oocytes ranging in diameter between 3 mm and below (small white follicles) were the dominant (107) while those ranging between over 6 and 8 mm (large white follicles) were the least (Table 4).

No adjacent pre-ovulatory follicles in the ovary differed significantly ($p > 0.05$) in diameter. However, a significant ($p < 0.05$) yolk accumulation occurred between F7 and F4, F6 and F3, F5 and F3, F4 and F2, and, F3 and F1. Generally, significant ($p < 0.05$) yolk depositions in the pre- ovulatory follicles took 3 days up to F7 and F6, and 2 days up to F5, F4 and F3 (Fig. 4).

Variations in the lengths and widths of the various sections of the oviduct and cloaca in sexually mature birds in breeding condition are presented in Table 5. Generally, the various sections varied in length. The longest section of the oviduct was the magnum {18.5 (16–20) cm}, forming about 40% of the entire duct. This was closely followed by the isthmus, the infundibulum and uterus, and finally the vagina, which is the shortest. The cloaca was much shorter than any other part of the duct. Similarly, the width of the various sections of the oviduct varied among themselves. In descending order, the infundibular lip was the widest, followed by the uterus, and then the magnum and vagina having approximately the same width, and finally, the isthmus. The cloaca was approximately of the same width as the uterus, but wider than the magnum, vagina and isthmus.

Correlations among ovarian and oviducal anatomical parameters were strong ($p < 0.001$) and positive. Similarly, Correlations between 17β-oestradiol concentration and ovarian/oviducal anatomical

parameters were highly significant ($p < 0.0001$) and positive. Peripheral progesterone concentrations, on the other hand, were insignificantly ($p > 0.05$) correlated with all the oviducal/ovarian anatomical parameters (Table 6).

4. Discussion

4.1. Gross anatomy of male reproductive tract

As reported by other workers (Awotwi, 1975; Brillard, 1986; Brillard & de Reviers, 1981), testes of a growing guinea cock could only be detached for measurements from 8 WOA. Testicular weight increased until 20 WOA, and remained stable thereafter. This is similar to the reports of Brillard and de Reviers (1981) and Brillard (1986) in the exotic breeds of guinea fowl. The authors indicated that this marked the commencement of adulthood in those birds. In the local guinea fowl, however, despite the stablisatioin of testicular weight from 20 weeks, fully formed spermatozoa was first seen both in the tubular lumen and epididymal region at 16 WOA (Abdul-Rahman, 2013), indicating earlier commencement of sexual activity in these birds. Several studies also reported bigger testes in older than younger birds (Deviche et al., 2000; Graves, 2004; Hill, 1994; Laskemoen et al., 2008; Morton et al., 1990). The increase in testicular weight with increasing age may be attributable to higher secretion of gonadotropins in older than younger birds (Ketterson & Nolan, 1992; Silverin et al., 1997). Gonadosomatic index, which is the relative investment into testicular growth, stablised from 16 WOA. This is possibly because this coincides with the age at sexual maturity and, therefore, no further investment into testicular growth is expected. Fluctuations were however seen in both parameters after stablisation, possibly because sexual maturity in those birds coincided with the minor breeding season.

Gribbins, Rheuberta, Colliera, Siegelb, and Severc (2008) reported increasing testicular volume with increasing spermatogenic activity and noted that this is a very good measure of spermatogenesis in animals. The increases in testicular volume noted between 8 and 12 WOA in the present study is attributable to both increased testicular parenchyma and commencement of spermatogenic activity since at this age, males

Table 2
Correlations between testicular biometric variables in guinea fowls.

	Testicular weight	Gonadosomatic index (GSI	Testicular length	Testicular height	Testicular width	Testicular Volume	Length of vas deferens
Gonadosomatic index (GSI	0.989***						
Testicular length	0.921***	0.918***					
Testicular height	0.888***	0.882***	0.927***				
Testicular width	0.906***	0.902***	0.940***	0.973***			
Testicular Volume	0.988***	0.978***	0.922***	0.882***	0.923***		
Length of vas deferens	0.497***	0.480***	0.622***	0.666***	0.661***	0.505***	
Testosterone concentration	0.563**	0.571***	0.524***	0.406**	0.465***	0.557***	0.357**

**Correlation is significant at $p < 0.01$; ***Correlation is significant at $p < 0.001$ level (2-tailed).
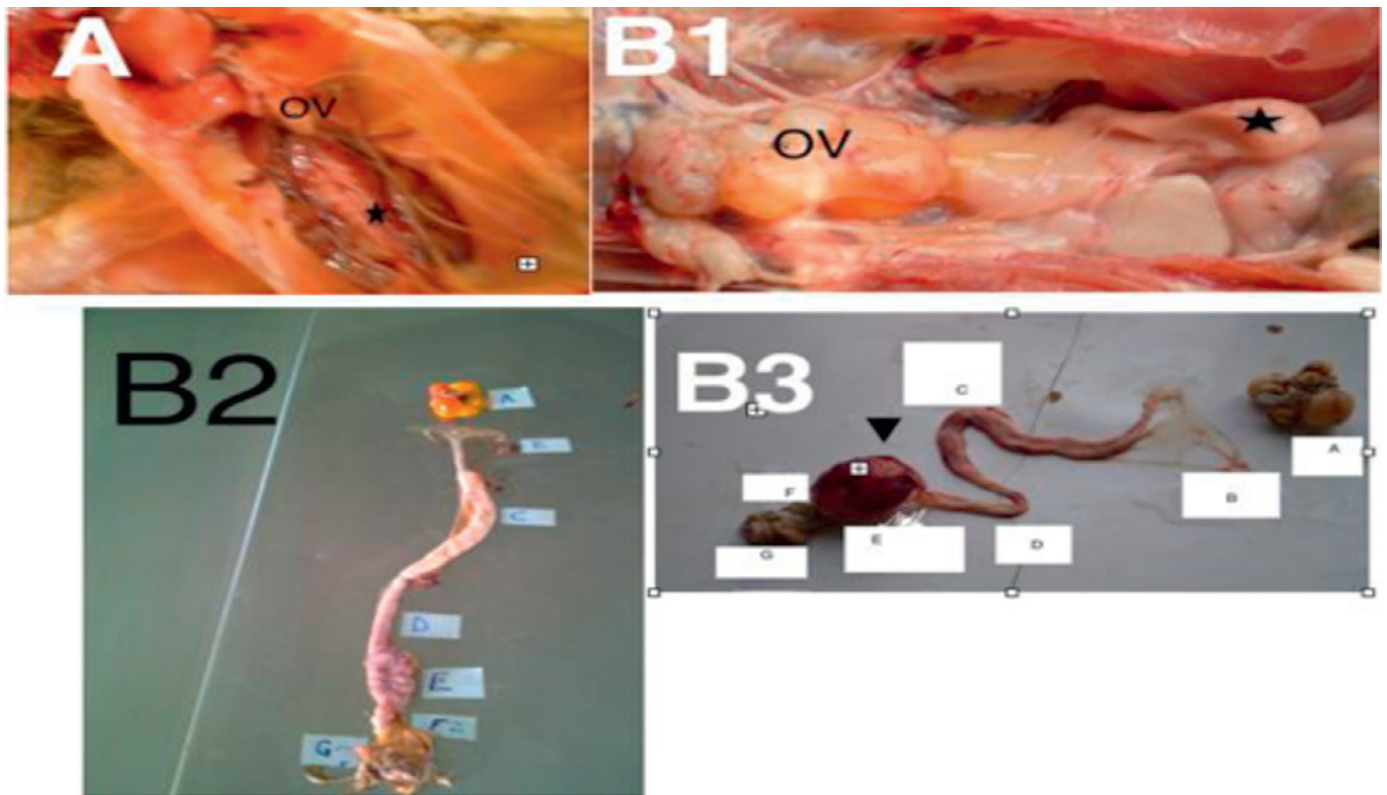
Fig. 3. Reproductive systems of a 4-week old (A) and sexually mature (B1, B2 and B3) female guinea fowl. In A and B1, note the ovary (OV) and oviduct (Star) in-situ. In B2 and B3, note the ovary (A), infundibulum (B), magnum (C), isthmus (D), uterus (E), vagina (F) and cloaca (G). Note an egg in the uterus ((arrowhead).

attained puberty and primary and secondary spermatocytes as well as round and a few elongated spermatids were visible in their tubules for the first time (Abdul-Rahman, 2013). The lack of change at 16 weeks and subsequent marginal and inconsistent increases noticed in testicular volume was not surprising, considering the fact that sexual maturity in these birds coincided with the minor breeding season and a decrease in spermatogenic activity accompanied by decreased testicular volume could be expected during periods when the testis is quiescent (Gribbins et al., 2008). Except testicular height which showed inconsistent growth pattern from 8 WOA, the other biometric traits showed a similar pattern of increase as testicular volume. Testicular volume, length, width and length of vas deferens all tended to stablise from 16 WOA. This age could therefore be considered as the beginning of

adulthood in male guinea cocks. This is earlier than the 20 weeks reported by Awotwi (1975) and Brillard (1986). It has been reported that cold temperatures can delay reproductive readiness and the onset of photo-refractoriness in male birds (Jones, 1986; Perfito et al., 2004; Silverin & Viebke, 1994; Silverin et al., 2008), while warmer temperatures can advance testicular development (Silverin et al., 2008). Temperatures in southern Ghana are lower than those in Northern Ghana (Dickson & Benneh, 1988). The study by Awotwi (1975) was conducted in southern Ghana while the present study was conducted in northern Ghana, and the variation in onset of sexual activity between the two groups of birds is, therefore, not surprising.

The phase of rising plasma testosterone concentrations in the local guinea fowl from 12 WOA coincided with the period of increasing

Table 3

Age-related changes in ovarian/oviducal anatomical paramaters and steroid hormone profiles in female guinea fowls.

Ovarian/oviducal anatomical trait {Median (Interquartile range)}		Age (Weeks)								
		1	4	8	12	16	20	24	28	32
Ovarian weight	(g)	0.02 (0.01-0.03)[f]	0.05 (0.03–0.06)[e]	0.06 (0.05–0.08)[e]	0.15 (0.08–0.16)[d]	0.22 (0.20–0.30)[e]	0.41 (0.25–0.66)[b]	0.32 (0.29–0.43)[b]	17.2 (14.80–21.20)[a]	22.3 (13.80–23.90)[a]
Relative ovarian weight	(x10^{-4})	7.0 (4.0–9.0)[b]	5.0 (3.0–7.0)[b]	3.0 (2.0–4.0)[c]	3.0 (3.0–6.0)[bc]	4.0 (4.0–5.0)[b]	7.0 (3.0–8.0)[b]	6.0 (3.0–6.0)[b]	1.5×10^{-2} (1.1–1.8)[a]	1.6×10^{-2} (1.3–2.1)[a]
Oviducal weight	(g)	–	0.04 (0.04–0.05)[d]	0.05 (0.05–0.05)[d]	0.07 (0.05–0.11)[cd]	0.11 (0.07–0.20)[c]	0.99 (0.23–2.0)[b]	0.64 (0.18–0.81)[b]	17.40 (14.30–24.20)[a]	23.60 (18.40–26.70)[a]
Oviducal length	(cm)	–	4.5 (4.0–5.6)[d]	7.4 (6.9–7.6)[c]	7.3 (5.9–8.0)[c]	8.2 (8.0–9.5)[b]	9.5 (9.0–10.0)[b]	9.6 (8.2–12.0)[b]	47.0 (44.0–49.2)[a]	45.0 (40.5–56.0)[a]
P. oestradiol concentration	(pg/ml)	–	22.6 (22.1–23.9)[b]	15.7 (9.8–30.5)[bc]	11.9 (10.2–15.3)[c]	11.9 (10.7–13.6)[c]	13.4 (12.1–16.7)[c]	24.7 (21.5–28.2)[b]	63.9 (46.5–113.2)[a]	57.1 (36.6–103.4)[a]
P. progesterone	(ng/ml)	–	0.67 (0.30–1.36)[abc]	0.84 (0.76–1.38)[a]	0.89 (0.15–0.94)[abc]	0.36 (0.30–0.57)[c]	0.08 (0.08–0.08)[d]	0.38 (0.25–0.61)[bc]	0.49 (0.23–0.49)[bc]	0.68 (0.56–1.02)[ab]

Medians (Interquartile range) within a row having no superscript in common are significantly ($p < 0.05$) different. P: Peripheral, –: The oviduct could only be detached for measurements from 4 weeks of age.

Table 4
Number of visible oocytes in 10 breeding guinea hens.

	Hierarchical follicles (>8 mm)	Large white (>6 mm–8 mm)	Medium white (>3 mm–6 mm)	Small White (3 mm and less)	Total
	7	5	0	82	94
	3	1	13	108	125
	5	1	10	76	92
	5	1	7	164	177
	7	1	19	68	95
	6	1	12	102	121
	7	2	20	115	144
	6	1	19	127	153
	4	1	19	114	138
	5	0	13	114	132
Average	5.5	1.4	13.2	107	127.1

Table 5
Dimensions of the various sections of the oviduct and cloaca in 20 breeding local guinea hens.

Section of oviduct	Dimension		
{Median (Interquartile range)}	Length (cm)	As Proportion of total oviducal length (%)	Width (cm)
Infundibulum	8.1 (5.4–9.0)[c]	17.2	5.1 (3.6–6.9)[a]
Magnum	18.5 (16.0–20.0)[a]	39.4	1.7 (1.6–2.5)[c]
Isthmus	11.0 (9.5–12.0)[b]	23.4	1.2 (1.0–1.5)[d]
Uterus	6.3 (6.0–7.5)[c]	13.4	3.4 (2.9–3.8)[b]
Vagina	3.1 (2.5–3.5)[d]	6.6	1.6 (1.0–2.7)[cd]
Total length	47.0	–	–
Cloaca	2.0 (1.9–3.0)[e]	–	3.0 (2.5–3.5)[b]

Medians (Interquartile range) within a column having no superscript in common are significantly ($p < 0.05$) different.

testicular anatomical traits, and this might have occurred in response to increasing plasma LH concentrations preceding rising testosterone levels (Wilson, 1979). Chicken LH and FSH are known to promote testicular growth and development (Brown, Bayle, Scanes, & Follett, 1975), while testosterone is implicated in adult testicular function (Brown & Follett, 1977). The significant positive correlations between all the testicular gross anatomical biometric characteristics, length of vas deferens and plasma testosterone concentrations was an indication that plasma testosterone concentrations in the local guinea fowls could be highly related to the development of these structures

Testicular anatomical biometric traits stabilised between 16 and 20 WOA, and this coincided with the period when peripheral testosterone concentrations peaked. Testosterone may, therefore, be implicated in the development of testicular anatomical structures

4.2. Gross anatomy of female reproductive tract

The anatomy of the female guinea fowl observed in the present study were similar to the earlier reports of Awotwi (1975) in the same species. The ovary in the study by Awotwi (1975) assumed the grape cluster appearance between 36 and 40 WOA, contrary to the much earlier age of 27 to 28 weeks that was observed in the present study. The birds in the present study laid about 8 weeks earlier than those used in the study by Awotwi (1975). Two birds even laid as early as 21 WOA, 15 weeks earlier than the earliest onset of lay (36 weeks) reported by Awotwi (1975). The earlier onset of lay and therefore sexual maturity

Table 6
Correlations among Ovarian/oviducal anatomical parameters, and peripheral steroid hormone concentrations.

	Ovarian weight	Relative ovarian weight	Oviduct length	Oviduct weight	P. Oestradiol conc
Relative ovarian weight	0.990***				
Oviduct length	0.996***	0.965***			
Oviduct weight	0.970***	0.967***	0.961***		
P. oestradiol conc	0.743***	0.765***	0.758***	0.701***	
P. progesterone conc	0.033	0.038	0.022	0.063	0.328**

**Correlation significant at $p < 0.01$ (2-tailed); ***Correlation significant at $p < 0.001$ (2-tailed). P: peripheral, Conc: Concentration.

in the birds used in the present study may be attributed to differences in management between the two flocks of birds. For instance, the birds used in the present study were fed starter, grower and layer rations with crude protein contents of 19%, 15% and 17.5%, respectively, while those used by Awotwi (1975) were only fed starter (17%) and layer (15%) rations with lower crude protein contents. Management factors including nutrition (Wilson & Harms, 1986; Yu, Marquardt, & Hodgson, 1972) and photoperiod (Morris, 1967) could influence the onset of puberty and sexual maturity in birds.
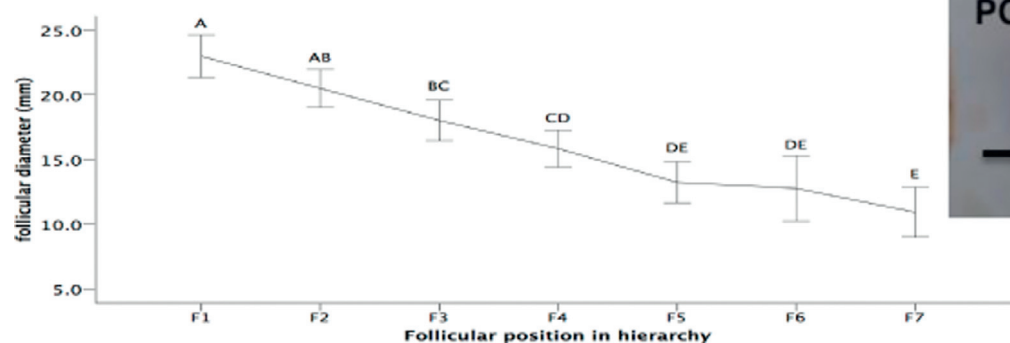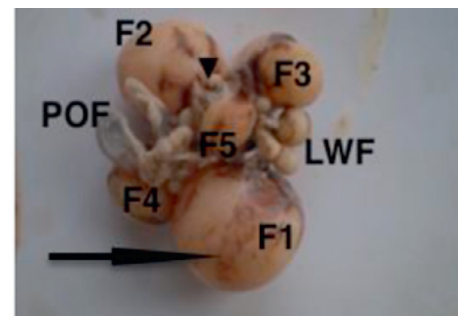

Fig. 4. Variations in the size (rate of yolk deposition) of preovulatory follicles in local guinea hens. Note that difference in size between consecutive follicles represents the rate (depth) of yolk deposition per day from one position in the hierarchy to the next. Insert: Mature ovary showing the hierarchial structure of developing follicles from F1 to F5. Note: post-ovulatory follicle (POF), large white follicle (LWF), Small white follicle (arrow head) and stigma (arrowed).

Ovarian growth during the first 24 weeks of life was slow. Even though significant increases were noticed at 4, 12, 16 and 24 WOA, these were very minimal. This period coincided with the period of slow growth of oocytes in guinea fowls. Oocyte diameters not exceeding 3 mm (Romanoff, 1931) were recorded at the end of this phase (20 weeks). This explains why very slow inconsistent and minimal increases were noticed in ovarian growth during this period. There were large increases in ovarian weight after 24 WOA. Such rapid increases are attributable to the deposition of alternate layers of white and yellow yolk (Bellairs, 1967) in the oocyte between 20 and 26 WOA (intermediate phase of yolk deposition) and yellow yolk (Gilbert, 1971) between 26 and 28 WOA (rapid phase of yolk deposition).

In the birds used in the present study, the rapid yolk deposition culminated in adult ovarian weight of 17.2–22.3 g at 28–32 WOA. This was much lower than the 30.5 to 32.1 g adult ovarian weight reported by Awotwi (1975) in the local guinea fowls. This difference may be due to the fact that the birds used by Awotwi (1975) attained sexual maturity at an older age (36 to 40 weeks), and this may be responsible for their higher ovarian weights. It has been reported that early maturity strains of birds enter lay with lower ovarian weights (Renema, Robinson, Luzi, & Feddes, 2003). Relative ovarian weight, which is an indication of investment made into ovarian growth by the guinea hen was relatively stable from 4 WOA and only saw huge increases during the intermediate and rapid yolk deposition stages. This is an indication that the guinea fowl maintains a constant level of investment into stromal tissue from about 4 WOA until puberty.

The number of visible oocytes noted in the ovaries of guinea fowls (92–177) in the present study was much lower than the range of 434 to 675 reported by Awotwi (1975). The difference in number is attributable to the fact that the birds used in the present study were much younger (28–32 WOA) than those studied by Awotwi (1975; 36–40 weeks), and since number of visible oocytes increases with age (Romanoff & Romanoff, 1949), these results were not surprising. The largest number of visible oocytes found in the domestic fowl was 3605, while the least was 586 (Romanoff & Romanoff, 1949). In the local guinea fowls, Awotwi (1975) recorded the highest of 675 and the lowest of 434, possibly because these birds were not systematically selected for intensive egg laying, as with the 1865 study which reported only 600 visible oocyte in the domestic fowl (cited by Romanoff & Romanoff, 1949). The huge increases noticed in the number of visible oocytes in the domestic breeds of chicken not only gives an indication of the enormously increased reproductive potentialities of the modern hens, but also shows the effect of selective breeding of the hen, particularly, White Leghorn for high egg production. Indicating that the local breeds of guinea fowl could also be intensively selected for improved reproductive performance.

Increases in oviducal length and weight followed a similar pattern to ovarian weight. The increases, though significant, indicate that increased weight rates were minimal until the period between 24 and 28 weeks, corresponding partly and entirely to the periods of intermediate and rapid yolk depositions, respectively. The oviduct was obviously preparing to secrete the other components of the egg following the first ovulation. Considering the fact that both the ovary and oviduct mature and function in a complementary fashion, it is not surprising that the anatomical parameters of both organs were strongly and positively correlated.

The lengths of the various sections of the oviduct in breeding guinea hens differed from those reported by Awotwi (1975). Except isthmian length, which was found to be higher in the present study (11 cm vs 7.0 cm), all other sections of the oviduct appeared to be longer in the reports of Awotwi (1975) than the present study. Infundibular lengths were, however, similar. The widths of the various sections were similar to those reported by Awotwi (1975). The differences in length between the various sections of the oviduct in the present study and the observations of Awotwi (1975) is attributable to the fact that the birds used in the present study started laying at a much younger age (28 vs 36

WOA), and their oviducts may still be going through a process of maturation to attain the full adult size.

The period between 24 and 28 WOA, which saw nearly 3-fold increase in plasma oestradiol concentrations also saw nearly 54-fold increase in ovarian weight, and coincided with the phase of rapid yolk deposition. These results are not surprising considering the fact that oestradiol induces the synthesis of Vitellogenin (VTG), a phosphoglycolipoprotein, which is a yolk protein (Shen, Steyrer, Retzek, Sanders, & Schneider, 1993). Rapid yolk deposition, could therefore, be the reason for this huge increases in ovarian weight. Oestradiol is also known to enhance growth of oviduct and promote the formation of tubular secretory glands and epithelial differentiation (Johnson, 2000). The 27- and 5-fold increase in oviducal weight and length, respectively, during this period was therefore expected. The increased oestardiol secretion between 24 and 28 WOA in the guinea hens may be attributed to increased secretion in each of the four largest follicles (Johnson, 2000). The significant negative and positive correlations noticed between ovarian/oviducal anatomical parameters and peripheral oestradiol concentrations indicate that the development of these structures could be predicted based on peripheral oestradiol concentrations in female guinea fowls.

Peripheral progesterone concentrations fluctuated considerably during sexual development. This result is not surprising considering an earlier report that peripheral progesterone concentrations varied with the physiological state of the animal (Furr, 1969). For instance, while Furr (1969) reported higher values in laying birds about 12–14 h after ovulation, Etches (1990) noted that the peak occurs 6 to 4 h before ovulation. Abdul-Rahman et al. (2016a) also reported a concentration of 5.29 ng/ml about 1–2 h prior to lay and 0.57 ng/ml post-lay in the guinea fowl. The authors indicated that this post-lay concentration was similar to that found in non-breeding birds.

Ovarian and oviducal parameters saw 2 phases of growth, initial phase of slow growth from hatching until 24 WOA, and final phase of massive increases between 24 and 28 WOA, and this may be under the influence of oestradiol. Progesterone secretion did not seem to correlate with the growth of female reproductive tract, and fluctuated considerably during sexual development.

Disclosure statement

The authors declare that there are no conflicts of interest.

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