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Postembryonic developmental changes in ovarian histology and associated 17β -oestradiol and progesterone profiles in guinea hens (*Numida meleagris*)

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POSTEMBRYONIC CHANGES IN OVARIAN HISTOLOGY

Postembryonic developmental changes in ovarian histology and associated 17β -oestradiol and progesterone profiles in guinea hens (*Numida meleagris*)**Ibn Iddriss Abdul-Rahman and Ian Jeffcoate**

Abstract: There is a paucity of information on the reproductive biology of guinea hens. A study was conducted to document postembryonic developmental changes in the ovarian histology and associated progesterone and 17β -oestradiol profiles until 32 weeks of age (WOA). Highly significant increases ($p < 0.001$) were noticed in oocyte and follicular diameters beyond 16 WOA. Other ovarian follicular morphometric traits showed inconsistent increases until 16 WOA. The first phase of yolk deposition occurred between hatching and 20 WOA, the second phase between 20 and 26 WOA, and the third and final phase between 27 and 28 WOA. Peripheral 17β -oestradiol concentrations increased from 20 WOA until it peaked at 28 WOA, while peripheral progesterone concentrations fluctuated considerably during sexual development. Both oestradiol and progesterone concentrations were negatively and moderately

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correlated ($p < 0.01$) with oocyte nuclei diameter, granulosa and theca layer heights. However, oestradiol concentrations were positively and strongly correlated ($p < 0.001$) with oocyte and follicular diameters, and moderately with progesterone concentrations. Three phases of yolk depositions were found in the guinea hen, with the final phase terminating at 28 WOA, at a much smaller oocyte diameter than in the domestic chicken. Oestradiol might play a role in yolk deposition in this species.

Key words: yolk deposition, oogenesis, folliculogenesis, guinea fowl.

Introduction

The post-embryonic development of the oocyte can be divided into three major phases based on the type of material deposited in the yolk (Marza and Marza 1935) or the rate of increase in size (Romanoff 1931); a period of slow growth characterized by the deposition of yolk consisting mainly of neutral fat, lasting for months or even years, and terminates when the oocyte is about 3 mm in diameter (Romanoff 1931). An intermediate phase when some yolk protein is added, lasting about 60 d, and terminating when the oocyte is 6 mm diameter, and the final period, 7 - 11 days prior to ovulation in the fowls (Gilbert 1971), ducks and pigeons (Johnson 2000), and 16 days in the penguins (Grau 1982). During this time the main mass of yolk is added and the oocyte increases in weight from about 0.5 to 19 g. The phase of rapid growth of an ovum is variable between birds, but usually within birds it remains fairly constant within a day.

Increasing levels of oestrogen was reported to be associated with initiation of vitellogenic stage of ovarian follicular (Chapman et al. 1994) and oviducal

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development (Renema et al. 1997; Buchanan et al. 2000), lasting 12-14 days in turkey hens (Bacon et al. 1972). Increasing peripheral progesterone concentration was also noted about 4 days prior to oviposition of the first egg in the same species (Yang et al. 1997). The increase in progesterone is predominantly the result of its secretion by the largest preovulatory follicle (Etches 1990).

Fertility and hatchability are significant constraints to guinea fowl production in Ghana (Adu-Aboagye et al. 2020). Guinea fowl eggs exhibit low hatchability than chicken eggs mainly because of egg size and shell thickness (Nwagu 1997; Yamak et al. 2016). Yet there is little research attention directed at guinea fowl eggs and its biology. For instance, phases of ovarian growth and yolk deposition in this species has not been documented. Understanding yolk formation, ovarian maturation, and ovulation, with subsequent emphasis on how these processes may become dysfunctional leading to poor egg quality (Reading et al. 2018), and consequently, lower fertility and hatchability will require some investigation. A major factor influencing egg quality is proper yolk formation, as birds are oviparous and the developing offspring are entirely dependent on stored egg yolk for nutritional sustenance. These maternally derived nutrients consist of proteins, carbohydrates, lipids, vitamins, minerals, and ions that are transported from the liver to the ovary by lipoprotein particles including vitellogenins (Reading et al. 2018).

Developmental changes in peripheral oestradiol and progesterone concentrations from hatching to sexual maturity have been documented in the chicken (Vanmontfort et al. 1995; Johnson and Brooks 1996) and turkey (Yang et al. 1999; 2005). The phases of yolk deposition, including period of time taken per phase of growth has also been documented in a number of avian species [fowls (Gilbert 1971), ducks and pigeons (Johnson 2000), and penguins (Grau 1982)], except the guinea fowl. The

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present study therefore sought to document phases of ovarian growth specifically in the guinea fowl and associated 17β -oestradiol and progesterone concentrations.

Materials and methods

Experimental site

The experimental birds were kept at the Poultry Unit of the Department of Veterinary Science, University for Development Studies, Nyanpkala, Tamale (Ghana). Nyanpkala lies on latitude $9^{\circ} 69'N$ and longitude $0^{\circ} 83'W$. Temperatures range between $22^{\circ}C$ and $35^{\circ}C$. The highest and lowest temperatures are recorded in March and December, respectively (Savannah Agricultural Research Institute (SARI 2008) cited by Abdul-Rahman et al. (2016)). The rainfall pattern is monomial with mean annual rainfall varying from 1000-1500 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, and has nearly equal amounts of light and darkness (12L: 12D) throughout the year.

Animals and management

The pearl variety of the local guinea hens (*Numida meleagris*) were used for the study. Birds were brooded for 6 weeks, and brooding management was as in Abdul-Rahman et al. (2018). Feed and water were supplied ad libitum. Feeding of day old keets commenced with ground maize in flat feeders (day old), followed by a starter ration (day 2 until 8 weeks of age [WOA]), grower ration (9 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; Nahashon et al. (2007)], grower [19% crude protein and 3100 kcal ME/kg diet; Nahashon et al. (2007)], and layer (17.5% crude

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protein and 2800 kcal ME/kg diet) rations were obtained from a commercial feed supplier (Agricare Ghana Limited, Kumasi, Ghana). The lighting programme followed was previously used by Abdul-Rahman et al. (2018).

Experimental procedure

A total of 56 guinea hens (7 per age group) were bled at 4-week interval, from 4 WOA until 32 WOA. Blood (2 ml) was collected into EDTA vacutainer tubes from the wing vein, spun at 7098 g for 3 min at room temperature (18-25 °C), and plasma pipetted into a 1.5 ml microcentrifuge tube. Samples were then stored at -20 °C until subsequently analysed for progesterone and 17 β -oestradiol. Prior to bleeding, five birds at each age were weighed, and following bleeding, were sacrificed by cervical dislocation. Their ovaries and reproductive tracts were completely freed from the adjoining ligaments and fascia, weighed and fixed in Bouin's solution overnight for histology. All procedures used were approved by the University for Development Studies Institutional Review Board and followed the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

Histological preparation, follicular diameters and phases of post-embryonic oocyte growth

The histological techniques used in the present study have been previously described (Abdul-Rahman et al. 2017). The ovary was divided into 2 halves. A half of it was fixed in Bouin's solution, dehydrated in absolute ethanol and embedded in paraffin wax. The embedded tissue was sectioned 5 μ m thick using microtome (Leica RM2125RT), floated onto Poly-1-lysine subbed slides (Polysine; VWR International Leuven, Germany), and stained in eosin and Mayer's haematoxylin.

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Using the ImageJ software (National Institutes of Health, USA), surface areas of primordial and primary follicles were determined and their respective diameters derived using the formula: $D = (\sqrt{\text{surface area} \times 4 / \pi})$. Primary and primordial follicular oocyte, oocyte nuclear diameters and granulosa layer thickness were also determined. In order to determine phases of postembryonic oocyte growth in the guinea fowls, the model developed for chicken by Romanoff (1931) was adopted. Changes in oocyte diameter were used to track oocyte growth from hatching until ovulation. At each age, 5-10 of the biggest follicles in each bird were specifically chosen for the estimation of the stage of yolk deposition. Oocytes were categorised as being in the first, second or third phase of yolk deposition depending on the diameter (Romanoff 1931). Oocyte diameter measurements started with primordial, primary and growing follicles at 4 WOA, and continued with growing follicles from 8 until 32 WOA, at 4 week intervals. Those in the range ≤ 0.01 mm to 3 mm were classified as being in the first phase of growth, those that fell between 3 mm and 6 mm were considered as being in the second stage of growth, while those measuring over 6 mm were considered as being in the final stage of oocyte growth, and therefore rapid yolk accumulation. In each case, the age that corresponded with the beginning and end of a stage was noted.

Proportion of the ovary occupied by follicles and general histological changes in the ovary

A modification of the Chalkley's (1943) technique, as was previously described by Abdul-Rahman et al. (2017) was used for the determination of this parameter. An image of the entire cross section of each ovary was captured at $\times 4$ magnification under the light microscope. Each cross section, therefore, yielded

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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 labelled, 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 (1943) technique. Points that fell on the follicles (including theca cells) were considered as follicular while those that fell outside the follicles were considered as non-follicular. This also represents the ratio of follicular tissue to non-follicular tissue. From this, percentage of follicles occupying the ovary was estimated as: (number of follicular points within the eye piece/total number of points of the eye piece) $\times 100$. General histological changes in the ovary were also described.

Peripheral progesterone and 17β -oestradiol measurements guinea hens

Both the 17β -oestradiol and progesterone assays were radioimmunoassay. The 17β -oestradiol assay had been previously validated for guinea fowl (Abdul-Rahman et al. 2016). All samples were analysed for 17β -oestradiol using the Oestradiol Maia kit (RADIM diagnostics, USA). The intraassay CV was 9.6%, while assay sensitivity was 5.1 pg/ml. For progesterone concentrations determination, the Coat-A-Count® Progesterone kit (Siemens Healthcare Diagnostics, USA) was used. Peripheral progesterone concentrations in all samples were determined in one assay, and the intra-assay CV was 6.9%. The sensitivity of the assay was 0.08 ng/ml.

Statistical analysis

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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 ovarian histology and associated oestrogen and progesterone profiles in guinea hens 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 $\pm$ standard error of mean or median (Interquartile range). Pearson's correlations between ovarian follicular morphometric parameters and peripheral steroid hormone concentrations were also explored. All comparisons were done at 5% level of significance.

Results

Ovarian changes

The ovary of a guinea fowl consisted essentially of an outer cortex containing the ova which surrounded a highly vascular medulla composed primarily of loose connective tissue. In keets, these two areas were distinctly separate, but as the ovary grew and matured, the distinction between the two became obscured (usually from 16 weeks) and was eventually lost (usually from 20 weeks). The surface of the cortex was covered by cuboidal germinal epithelium, and on the largest follicles, the epithelium became almost squamous. Beneath the germinal epithelium, was a layer of connective tissue, the tunica albuginea, beneath which was the stroma which was a loose connective tissue that contained several blood vessels (Fig. 1).

Within the cortex were numerous minute growing ova. The average primordial and primary follicular diameters were 0.06 (0.04-0.08) mm and 0.08 (0.07-0.09) mm, respectively. The epithelium of the primordial follicle was simple squamous, while

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that of the primary follicle was low cuboidal. Growing follicles, on the other hand, had pseudo-stratified columnar epithelium. Each ovum consisted of a centrally located nucleus or germinal vesicle containing one or more nucleoli and a diffuse chromatin content. Central vacuolation of the oocyte started in primordial follicles and by 12 WOA (in growing follicles), vacuoles began to form at the periphery, when the oocyte was 0.37 (0.34-0.40) mm. At 16 weeks old, the central and peripheral vacuoles had both intensified and merged, indicating the deposition of white yolk. Zona radiata and perivitelline layer became noticeable at this age. As early as 4 WOA, single layers of simple squamous cells began to organise around growing follicles to form theca layer and by 16 WOA they had become organised into theca interna and externa (Fig. 1).

Age-related changes in oocyte (yolk deposition) and follicular diameters (folliculogenesis) are shown in Table 1. Both increased significantly (Kruskal Wallis test $X^2 = 159.077$, $df = 9$, $p < 0.0001$) from hatching until 28 WOA. The increases, though significant, were very minimal at the onset, but huge in both instances from 16 WOA. In the oocyte for instance, highly significant increases occurred from week 16 {0.54 (0.40-0.61) mm} to 20 {3.1 (3.0-3.2) mm} when the first phase of yolk deposition was terminated (oocytes were visible to the naked eye for the first time), week 20 {3.1 (3.0-3.2) mm} to 26 {6.0 (5.9-6.1) mm} when the second stage was terminated, and finally, week 26 {6.0 (5.9-6.1) mm} to 28 {22.9 (20.7-24.5) mm} when ovulation and oviposition were achieved. Though 2 birds laid exactly at 21 WOA, all birds laid between 27 and 28 WOA. Generally, yellow follicles were visible in the ovary after 26 WOA. 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,

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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 2).

Oocyte nuclear diameter and height of granulosa layer were measured up to 16 weeks because no decent histological sections could be prepared thereafter, since ovaries were too big to fit on slides (biggest follicle in each case could not fit on slides after 16 weeks). Both oocyte nuclear diameter and granulosa layer height exhibited similar patterns of increase. Significant increases were noticed in oocyte nuclear diameter (Kruskal-Wallis X^2 test = 96.389, $df = 5$, $p < 0.0001$) and height of granulosa layer (Kruskal-Wallis X^2 test = 75.900, $df = 5$, $p < 0.0001$) from primordial to primary follicle stage. Further increases in size were seen between 4 and 8 WOA, and 12 and 16 WOA (Table 1). The height of theca layer was measured from week 8. It also showed a similar pattern of growth to oocyte nuclear diameter. The thickness of the layer was $24.3 \pm 2.5 \mu\text{m}$ at 8 weeks, stayed unchanged between then and 12 weeks ($29.1 \pm 2.4 \mu\text{m}$), and then increased significantly ($p < 0.05$) by 16 WOA ($57.4 \pm 2.5 \mu\text{m}$).

The relative volume of follicles in the ovary of guinea fowl remained stable between 4 and 8 WOA, and then increased ($p < 0.05$) between 8 and 12 WOA. From this age, no significant increases were recorded. Cumulatively, however, the increase between 12 and 24 WOA was significant ($p < 0.05$). Relative volume of follicles in the ovary generally stabilized ($p > 0.05$) after 16 WOA (Table 1). Correlations between all ovarian follicular morphometric variables except granulosa layer height and oocyte nuclear diameter were positive and highly significant ($p < 0.01$) (Table 2).

Peripheral 17β -oestradiol and progesterone concentrations in guinea hens

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Developmental changes in peripheral 17β -oestradiol and progesterone concentrations are illustrated in Figures 3 and 4, respectively. Plasma 17β -estradiol concentrations did not change monthly between 4 and 20 WOA. The concentration at 4 WOA, however, were higher ($p < 0.05$) than those at 12, 16 and 20 WOA. Plasma oestradiol concentrations increased significantly ($p < 0.05$) from 20 WOA, peaking {57.1 (36.6-103.4) pg/ ml} at 28 WOA before a decline at 32 WOA.

Peripheral 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 weeks were significantly higher ($p < 0.05$) than those at 16 and 20 WOA. Between 16 and 20 WOA, progesterone concentrations dropped significantly ($p < 0.05$) to minimal levels. This was followed by a significant ($p < 0.05$) increase between 20 and 32 WOA.

Table 3 shows the correlations between ovarian follicular morphometric parameters and peripheral steroid hormone concentrations. Peripheral oestradiol concentration was negatively correlated to oocyte nuclei diameter, granulosa layer and theca layer heights. The correlations between oestradiol concentration and oocyte and follicular diameters were rather positive. Progesterone concentration was significantly negatively correlated with all the ovarian morphometric variables measured except with peripheral oestradiol concentration, which was significantly positive. Non-significant correlation was observed between progesterone concentration and oocyte nuclei diameter.

Discussions

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In agreement with the results of Awotwi (1975) the present study noted that there were no appreciable changes in the histology of the ovary from 4 WOA except increase in size of follicles.

Several changes occurred in the follicle between the first WOA and laying. At the end of the fourth WOA, central vacuolation was seen in the cytoplasm of primordial follicles. These follicles were only 0.06 mm in diameter. This was still within the oocyte diameter range of 0.03 mm to 0.07 mm reported in 21-day old chicks (Romanoff and Romanoff 1949). The authors indicated that at this stage, the oogonia had grown mainly by an increase in protoplasm and water to produce primary oocytes; and lipid vacuoles have begun to appear in the centre of each cell about the yolk-attraction spheres. Peripheral vacuolation of the oocyte started at a much smaller diameter in the guinea hen (0.26 mm to 0.32 mm at 12 weeks) than the domestic chicken (Romanoff 1931 [1mm to 3 mm]; Mazra and Mazra 1935 [2mm to 3mm]). By 16 WOA in the guinea fowl, peripheral vacuoles had merged with central vacuoles, an indication of white yolk deposition (Romanoff and Romanoff 1949) in these birds. At this stage, the oocyte was only 0.54 mm in diameter, still much smaller in size compared to the size at which peripheral vacuolation started in the chicken oocyte.

Few changes were noticed at 20 WOA, and this included intensification of vacuolation and, therefore, white yolk deposition with a corresponding huge increase in oocyte diameter by nearly 6 fold. This marked the end of the first and beginning of the second phase of yolk deposition by Romanoff's (1931) description. Both authors, however, considered that the second phase terminated at 6 mm stage in the chicken. This was attained 6 weeks following the termination of the first phase at 26 WOA in the guinea fowl. The diameter of the oocyte increased from 3.1 mm to 6 mm. Bellairs

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(1967) reported that during the second stage, true yolk appeared, and the nucleus lay eccentrically and a large number of vacuoles formed throughout the cytoplasm.

Hierarchical follicles were first seen in the ovary of guinea fowls at the beginning of 27th WOA, (i.e nearly 2 days into 27th week) and by the end of the 28th WOA, all birds had laid for the first time, indicating that the rapid yolk deposition stage took between 12 to 14 days in the guinea fowl. Gilbert (1971) reported 14 days and 9 - 14 days for the domestic chicken, which was comparable to the 12 to 14 days found for guinea hens in the present study. In chicken, the diameter of the oocyte increased from 6 mm to 35 mm within this period (Romanoff and Romanoff 1949). In the guinea fowl, however, it grew from 6 mm to 22.9 mm between 12 to 14 days. It is therefore not surprising that the guinea fowl's egg is much smaller than those in most strains of domestic chicken (Smith 2020). Hatchability for small eggs is lower than medium and large eggs (King'ori, 2011), and this was subsequently confirmed in guinea fowls by Kyere et al. (2017). This may likely explain why guinea fowl eggs have generally lower hatchability than chicken (Yamak et al. 2016).

During the rapid yolk deposition phase, the weight of the follicle increases linearly (Gilbert 1971); hence the rate of transport of metabolites across follicular wall decreases with age (Smith 1959). In contrast, measurements of oocyte dimensions in the present study indicated that it took longer periods for significant depositions to occur initially during the rapid growth phase than approaching ovulation (i.e 3 days from F7 stage initially and 2 days from F5 stage subsequently), indicating increasing rate of transport of metabolites across the follicular wall with age. Perhaps differences exist between the guinea fowl and domestic chicken in the pattern of transport of metabolites across the follicular wall during the rapid yolk deposition stage. A further

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investigation will therefore elucidate these phenomena since the present results were obtained using diameter measurements.

Marza and Marza (1935) indicated that during the three phases of yolk formation, the depth of the follicular epithelium increases and then diminishes as the ovum grows. The author noted that during the first phase, the depth of the epithelium increased from 4 μm to 20 μm in the domestic fowl. This was comparable to the range of 2.5 μm to 16.5 μm found in the present study. The depth of the follicular epithelium decreased from 20 μm to 7 μm during the second phase of growth in the domestic fowl (Marza and Marza 1935). In the guinea fowl, however, the depth continued to increase to 19.5 μm at 16 WOA, which fell within the second phase of growth based on the descriptions of Marza and Marza (1935). Two theories could be proposed to explain the continual increase in the depth of follicular epithelium during Marza and Marza's (1935) second phase of growth in the guinea fowl. First, it was possible that in these birds the epithelial depth increased deeper into the second phase before decreasing, and since histological sections could not be made in these birds beyond this age, the entire changes occurring in the follicular epithelium during this phase could not be captured. Secondly, this age still fell within the first phase of oocyte growth described by Romanoff (1931), and therefore the follicular epithelium could have been increasing in depth, as seen in follicles of all birds during earlier phases of follicular growth (Johnson 2000).

In the present study, interna and externa layers were visible when the follicle was only 0.54 mm (0.40 - 0.61 mm) in diameter. This was nearly 2 to 4-fold smaller than the size at which those layers differentiated in the domestic fowl [1mm-2mm (Deol 1955)]. This may be due to the smaller mature size of guinea fowl egg compared to the chicken (Smith 2020). Relative volume of follicles occupying the

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ovary increased while follicles increased in size and stabilised after 12 WOA. The highly significant positive correlations between follicular diameter and oocyte nuclei diameter, oocyte diameter, granulosa and theca layer heights were expected, as all the aforementioned follicular morphometric parameters increase with increasing follicular diameter, particularly during the initial phase of follicular development. Inspecting the correlational data between peripheral oestradiol concentration and follicular and oocyte diameters, the coefficient of determination (R^2) in each case exceeds 90%, implying that over 90% of the variation in follicular and oocyte diameters may be explained by variation in peripheral oestradiol concentration. Less than 10% of variation in follicular and oocyte diameters may therefore be attributed to factors other than peripheral oestradiol concentrations. The present finding is consistent with the report that increasing 17β - oestradiol concentrations in birds approaching ovulation is due to increased secretion by the largest preovulatory follicle (Etches 1990). It is therefore not surprising that massive increases were seen in oestradiol concentrations between 20 and 28 WOA, accompanied by huge increases in diameters of hierarchical follicles during this period, possibly linked to the initiation of vitellogenic stage of ovarian follicular growth (Chapman et al. 1994) seen between 27 and 28 WOA. Johnson (2000) noted that the increased oestradiol secretion is mainly attributable to increased secretion in each of the four largest follicles.

The cumulative increase in progesterone concentrations between 24 and 32 WOA may be due largely to increased secretion by the largest preovulatory follicle (Etches 1990). Previous studies in birds have, however, shown clearly that progesterone has no direct influence on the ovary. For instance, Progesterone failed to induce ovulation in birds that were hypophysectomised more than 10 hr prior to the next expected ovulation (Nakada et al. 1994). At this time, the F1 follicle may be

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insufficiently mature to respond to progesterone, or pituitary hormone secretion may be inadequate for progesterone to initiate an LH surge. These results suggest that progesterone acts on the hypothalamus or pituitary to induce ovulation and has no direct action on the ovary (Bennett, 2002).

Three phases of yolk deposition were identified, as in the chicken, namely, phases of slow (up to 20 WOA), intermediate (up to 6 weeks) and rapid (between 2-14 days) yolk depositions, and the final oocyte size is much smaller than in the domestic fowl, and may partly explain the lower hatchability found in the guinea fowl. Oestradiol levels increased from the onset of the second phase of yolk deposition and may be implicated in the increased yolk deposition witnessed between 20 and 28 WOA.

Declaration of interest

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

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Table 1: Age related changes in Ovarian histological morphometry in guinea hens

Age (weeks)	Ovarian morphometric parameters {Median (Interquartile range)}				
	Oocyte nuclear diameter (μ m)	Oocyte diameter (mm)	Follicular diameter (mm)	Granulosa layer height (μ m)	*Relative volume of follicles in the oocyte (%)
Primordial follicle	26 (19.1-29.1) ^d	0.06 (0.04-0.07) ⁱ	0.06 (0.04-0.08) ⁱ	2.5 (2.2-3.9) ^d	-
Primary follicle	32.7 (27.5-37.9) ^c	0.06 (0.05-0.08) ⁱ	0.08 (0.07-0.09) ^h	9.0 (7.0-10.6) ^c	-
4	32.7 (28.4-35.7) ^c	0.09 (0.08-0.10) ^h	0.33 (0.27-0.37) ^g	13.3 (11.0-16.1) ^b	32.4 $\pm$ 4.2 ^c
8	82.7 (64.9-95.8) ^b	0.26 (0.24-0.28) ^g	0.30 (0.29-0.32) ^g	13.5 (10.2-17.5) ^b	41.9 $\pm$ 3.7 ^c
12	82.9 (73.6-91.1) ^b	0.29 (0.26-0.32) ^f	0.37 (0.34-0.40) ^f	16.3 (11.2-19.7) ^b	59.8 $\pm$ 4.2 ^b
16	123.8 (95.4-140.4) ^a	0.54 (0.40-0.61) ^e	0.67 (0.55-0.75) ^e	19.5 (14.1-26.1) ^a	66.7 $\pm$ 3.8 ^{ab}
20	-	3.1 (3.0-3.20) ^d	3.10 (3.0-3.20) ^d	-	75.8 $\pm$ 3.8 ^{ab}
24	-	5.2 (4.60-5.50) ^c	5.2 (4.60-5.50) ^c	-	76.0 $\pm$ 4.2 ^a
26	-	6.0 (5.9-6.1) ^b	6.0 (5.9-6.1) ^b	-	-
28	-	22.9 (20.7-24.5) ^a	22.9 (20.7-24.5) ^a	-	-

Medians (Interquartile range)/means within a column having no superscript in common are significantly ($p < 0.05$) different. “-”: Parameters could not be measured beyond 16 weeks of age because at this point, follicles became too huge to fit on a slide. *Mean $\pm$ SEM

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Table 2: Intercorrelations among ovarian morphometric parameters

	Oocyte nuclear diameter	Oocyte diameter	Follicular diameter	Granulosa layer height
Oocyte diameter	0.809***			
Follicular diameter	0.716***	0.962***		
Granulosa layer height	0.156	0.263*	0.338**	
Theca layer height	0.556***	0.748***	0.810***	0.545***

*Correlation significant at $p < 0.05$ (2-tailed); **Correlation significant at $p < 0.01$ (2-tailed); ***Correlation significant at $p < 0.001$ (2-tailed)

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Table 3: Correlations between ovarian follicular morphometric parameters and peripheral steroid hormone concentrations

Ovarian follicular biometric variables	Peripheral steroid hormone concentration	
	17β-Oestradiol concentration	Progesterone concentration
Oocyte nuclei diameter	-0.361**	-0.123
Oocyte diameter	0.956***	-0.294**
Follicular diameter	0.957***	-0.362**
Granulosa layer height	-0.270*	-0.352**
Theca layer height	-0.325**	-0.441***
Peripheral oestradiol concentration		0.328**

*Correlation significant at p< 0.05 (2-tailed); **Correlation significant at p<0.01 (2 tailed); ***Correlation significant at p<0.001 (2- tailed)

Fig 1: Cross sections of guinea fowl ovary at 4 weeks (A) and follicular wall of a 3 mm follicle (B). For (A), note the cortex (C) hosting the follicles (f), and medulla (M) consisting of loose connective tissues (ct.) and blood vessels (v). HE x4 (Scale bar = 100 μ m), Insert: The ovary at a higher power showing the low cuboidal epithelium (arrowhead) and the tunica albuginea (TA). HE x10 (Scale bar = 100 μ m). For (B), from external inward, note theca externa (Te), within which are the thecal glands (L) occurring throughout, theca interna (Ti), follicular epithelium (G), zona radiata (arrowed), yolk granules (Y), migrating oocyte nucleus (N), and both central and peripheral vacuoles (V) merged. Between the membrana granulosa and zona radiata is the thin acellular zone known as perivitelline membrane. HE x x20 (Scale bar = 100 μ m)

Fig 2: Variation in size (rate of yolk deposition) of preovulatory follicles in local guinea hens. Means having no letter in common are significantly different ($p < 0.05$). Difference in size between consecutive follicles represents the rate of yolk deposition (depth) per day from one position in the hierarchy to the next.

Fig: Fig 3: Developmental changes in peripheral 17β -Oestradiol concentrations in local guinea hens. Medians (interquartile range) having no letter in common are significantly different ($p < 0.05$)

Fig: Fig 4: Developmental changes in peripheral progesterone concentrations in local guinea hens. Medians (interquartile range) having no letter in common are significantly different ($p < 0.05$)