

The morphological and histological study of chicken left ovary during growth and development among Hy-line brown layers of different ages

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ABSTRACT Chicken ovaries are known to develop asymmetrically and only the left ovary fully develops. Although both have been greatly investigated, a gap in scientific reports is still felt between 2-mo-old and sexual maturity. In this study, we aimed at investigating the changes in components that occur during growth to analyze the morphohistological correlation between the left ovary and the follicle development at different age stages in *Gallus domesticus*. The ovaries were harvested from 60 chickens aged 1 and 3-wk-old, 1, 2, 3, and 4-mo-old (n = 10 per age group), then fixed in AAF solution. Hematoxylin-and Eosin protocol was used to stain the tissue for microscopic observations. Results revealed that the left ovary exhibited an ovarian tissue, a site of follicular growth that displayed various shapes from

smooth to greatly indented as the follicles differentiated. Atretic follicles at various regression stages were noticed frequently as the chicks grew in age from 3-wk-old onward along with their differentiation. Rete ovarii, remnants from the male homologs were observed throughout the whole study showing epoöphoron, connecting rete, and gland-like structures that tend to diminish with age. The feature of the left ovary is closely related to the follicular developmental stage, and the bigger and differentiated the follicles are, the more indented and irregular its epithelium appears. Atresia is a normal physiological process that we observed throughout the whole study. Also that, rete ovarii do not spontaneously arise in the ovary but it develops and grows in juvenile chicken as well as in adult ones.

Key words: chicken ovary, histology, morphology, different age stages, hy-line brown

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INTRODUCTION

The ovary is normally a paired female reproductive organ (in mammals), which is the site of follicular growth and hormone secretion (estrogen and progesterone). Apart from in prey birds, pair anlagen takes place, and only the left genital primeval, grows more towards functional organs (Jacob, 2007). The left and right ovaries have been found in avian species belonging to 16 orders, which were usually thought to possess a single ovary. Although 2 oviducts occur barely if not predominantly in prey species, in the Brown Kiwi that possesses two functional ovaries, only the left oviduct is known to receive the oocyte from the left and the right ovaries (King and McLelland, 1975). In the domestic fowl, the left and right ovaries originally start to grow about 72 h following the beginning of incubation (Ishimaru et al.,

2008). Both oocytes and spermatogonia usually come from the development of Primordial germ cells (PGCs), known to be the first germ cell (GC) population established during growth which, in birds in contrast to other species, are transported to the gonadal ridge through the blood circulatory system (Tagami et al., 2017). At an early stage of growth before sexual differentiation, chicken embryonic ovaries develop symmetrically with no notable left-right asymmetry shown (Smith et al., 2008). King and McLelland (1975) reported that more of the germ cells that unequally invade the gonadal region at an earlier embryonic stage and some of which further migrate from the right to the left gonad increase the disparity in favor of the left gonad. The lack of secondary cords in the right ovary's epithelium forces it to stop growing and consequently develops a medulla without cortex, making the disparity between the left and right ovary seen from d 8 of incubation onward (Romanoff, 1960; Stahl and Carlson, 1973).

The histological and stereological changes occurring in the left and right ovaries have been greatly investigated including estrogen receptor alpha changes in localization during growth from pre-hatch in an early embryonic

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stage to about 1-mo-old post-hatched chicken (Gonzalez-Moran, 2011; 2014). Mohamed et al. (2016) reported gross anatomical, histological as well as stereological findings in post-hatch development of the left ovary from 1-day-old to 1-mo-old chicken. Laying hens' ovary has also been investigated histologically and used as a model in the study of human Epithelial ovarian cancer (Apperson et al., 2017).

Even though the left and right ovaries have been investigated up to a certain extent, no reports have been found showing the correlation between follicular growth and morphological features of the left ovary. A great gap is yet to be filled on the anatomo-morphological and histological aspects of the left ovary, especially from 2-mo-old age to sexual maturity in chicken. Literature search toward this direction did not land in the finding of reports detailing these aspects of the subject. Hence, this study was conducted to investigate and correlate the morphological and the histological changes that occur in post-hatched chicken's left ovary during growth and development from 1-wk-old to 4-mo-old.

MATERIALS AND METHODS

Ethics Statement

All experimental protocols used in this present study were reviewed and approved by the Animal Ethical and Welfare Committee (AEWC) of Gansu Agricultural University (Approval number. 2019-044). We hereby confirm that no endangered or protected animals have been involved in all experimental procedures.

Animals

A total of sixty healthy Hy-line Brown female chicks obtained from a local Poultry farm that specializes in chicks breeding around the suburb of Lanzhou in Gansu Province/ China were used in this study. The animals were divided into age groups: 1 and 3-wk-old, 1, 2, 3, and 4-mo-old. Each age group was composed of ten specimens.

Sample Preparation for Anatomical Observations

The chicks were first weighted and slaughtered, followed by the opening of their abdominal and thoracic cavities through dissection to observe and record the growth and location of the ovary and the surrounding organs in the abdomen. The ovaries were then carefully harvested; their weights and measurements (length, width, and thickness) were recorded.

Sample Preparation for Microscopic Observations

The harvested ovaries were immediately placed into the AAF solution for fixation just after recording all the

needed parameters. Samples were processed and immerse in wax blocks then cut into 5 to 7 μm sections, stained with Hematoxylin and eosin (H.E.) techniques (Mayer, 1937).

Data Collection

The follicles' diameters and rete ovarii data were collected using ImageJ 1.53a software for windows (Schneider et al., 2012). For that purpose, histological micrographs of 200X magnification were randomly selected and used to collect the data of 20 follicles in all age groups studied. Rete ovarii's diameters, epithelium thickness, and lumen diameters were collected using the morphometric method used for the male testis seminiferous tubules (Neves et al., 2002). Thirty rounded to oval tubules were randomly selected to collect the data in all studied groups using 400X magnification photomicrographs.

Statistical Analysis

Statistical analyses were performed using SPSS 25.0 for windows (SPSS Inc., Chicago, IL). Descriptive statistics were used to calculate the mean and the standard deviation for each age group to assure that the data met the requirements of the variance analysis. The results were analyzed by one-way ANOVA and Tukey's multiple comparison test was used to determine the difference between age groups. The Paired-Samples *t* test was used to calculate the mean and standard deviation of the follicle diameters to evaluate the difference between age groups. Data were presented as means \pm standard deviation and considered significant when $P < 0.05$.

RESULTS

Anatomical Observations of Chicken Ovaries at Different Ages

One-Week-Old Chick. The 1-wk-old chick postmortem examinations revealed that the left and right ovaries were connected to the backbone on the coelomic surface above the two kidneys, on either side of the dorsal aorta (Figure 1A). The disparity in size between them was well marked in favor of the left ovary whose anterior end was larger than the posterior one. Data about the left ovary's weight, length, width, and thickness are presented in (Table 1). The left ovary exhibited an ovarian tissue that had a curvature facing the right kidney and displayed various forms and shapes. The left ovary had a smooth appearance; its color was clear pink and yellow-creamy at its anterior end near the caudal lobe of the left lung (Figure 1B).

Three-Week-Old Chick. The left ovary of the 3-wk-old chick showed various shapes and sizes as well as an increase in weight and (Table 1). The ovarian tissue displayed a flat, spreading feature that began to show superficial indentations on its smooth tissue, while some ovaries displayed a posterior end that folded up; its pink

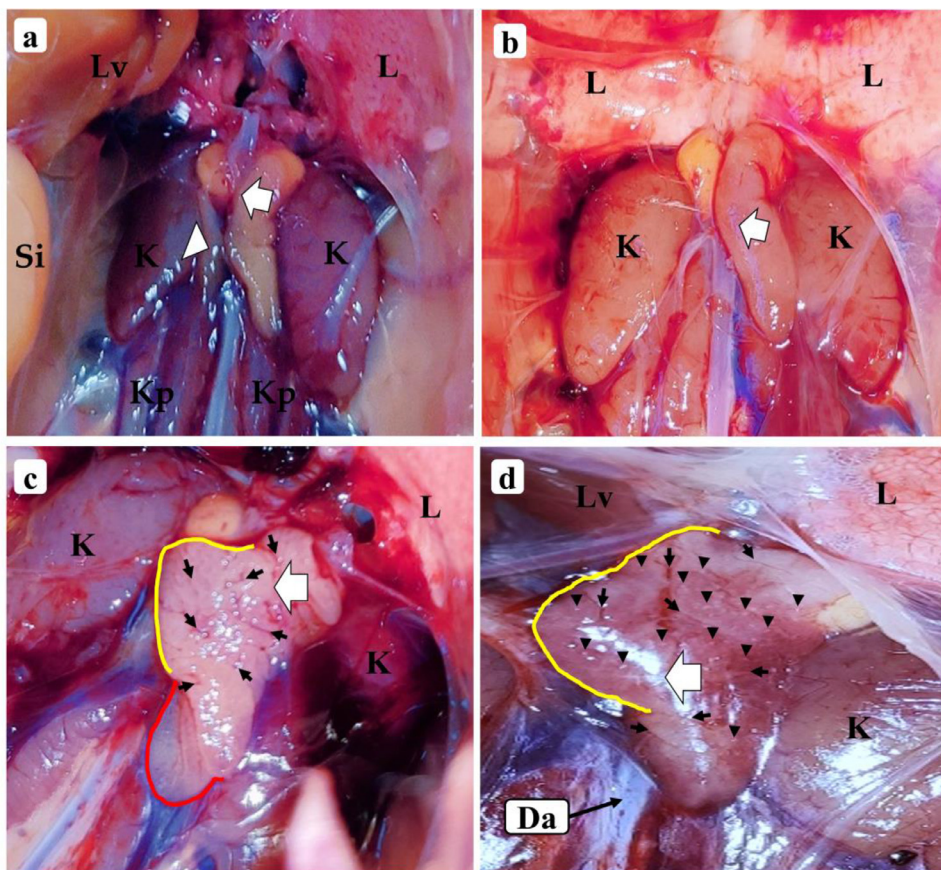


Figure 1. Morphological photographs of Chicken ovaries from 1-wk-old to 1-mo-old. (A, B) One-wk-old, (C) 3-wk-old, and (D) 1-mo-old chick: Left ovary (White arrow), right ovary (white arrowhead), inner portion of the liver (Lv), small intestine (Si), anterior lobes of kidneys (K), posterior lobes of kidneys (Kp), caudal lobe of the left lung (L), ovarian indentations (black arrows), the left ovary spreading curvature (yellow line), left ovary's posterior end folding up (red line), dorsal aorta (Da), white dots on the left ovary surface (black arrowheads).

color deepened slightly (Figure 1C). The spreading of the left ovary's curvature partially covered the right kidney (Figure 1C).

One-Month-Old Chick. The 1-mo-old chick left ovary's curvature facing the right kidney spread further and the ovary now overlapped both left and right kidneys at their inner anterior portions facing the dorsal aorta. The ovary's weight and measurements are

presented in (Table 1). The left ovary whitened slightly, particularly at the top of its anterior section (Figure 1C), while its middle part looked deep red and exhibited various sizes and shapes. It appeared more vascularized; white dots announcing the appearance of granules formed on its smooth tissue showing a structural change in the oocytes growing within the ovary.

Table 1. Ovary's weight and morphometric parameters

Parameters	Weight (g)	Length (mm)	Width (mm)		Thickness (mm)
			Ae	Pe	
1 wk	***0.020 ± 0.003	***6.663 ± 1.43	2.08 ± 0.41	1.35 ± 0.15	1.59 ± 0.33
3 wk	***0.09 ± 0.04	***10.79 ± 1.36	***4.09 ± 1.14	1.53 ± 0.53	2.24 ± 0.76
1 mo	***0.12 ± 0.02	***11.16 ± 1.67	***5.46 ± 1.45	1.86 ± 0.64	2.26 ± 0.46
2 mo	***0.23 ± 0.05	***14.71 ± 0.85	***6.95 ± 1.85	3.35 ± 1.96	3.34 ± 0.51
3 mo	***0.29 ± 0.10	***15.99 ± 2.09	***7.56 ± 1.73	3.42 ± 1.35	3.22 ± 0.71
4 mo	***0.56 ± 0.23	***17.61 ± 2.50	***10.3 ± 2.75	***4.79 ± 2.00	3.81 ± 1.08

Abbreviations: Ae, Anterior end, Pe, Posterior end.

Statistical difference: *** $P < 0.00$; values without asterisks: $P > 0.05$.

Table 1 shows the weight, length, width, and thickness evolution of the left ovary at various stages of growth. We observed a slow growth phase in the ovary between 1-wk and 1-mo age. The ovary then showed a fast growth phase with a significant increase in weight that almost doubled between 1 and 2-mo-old and continued to increase gradually to the end of the study ($P = 1$). The ovary's length was significantly different at all ages studied (*** $P = 0.00$) whereas the width of its anterior and posterior ends did not show significantly different in the 1-wk-old chick ($P = 0.1380.138$) but appeared significantly different throughout the rest of the study (*** $P = 0.00$) except in the comparison with the thickness of the ovary ($P = 0.138$ to $P = 0.933$). All parameters were different from each other (*** $P = 0.00$) at all ages observed as demonstrated by One-way ANOVA test results ($F(4,45) = 136.778$, *** $P = 0.00$) for the 1-wk-old chick, ($F(4,45) = 218.767$, *** $P = 0.00$) for the 3-wk-old chick, and ($F(4,45) = 275.364$, *** $P = 0.00$) for the 1-mo-old chick, ($F(4,45) = 170.012$, *** $P = 0.00$), for the 2-mo-old chick. ($F(4,45) = 200.163$, *** $P = 0.00$), for the 3-mo-old chick, and ($F(4,45) = 117.657$, *** $P = 0.00$), for the 4-mo-old chick. A Tukey multiple tests revealed a constant increase in parameters at all ages observed.

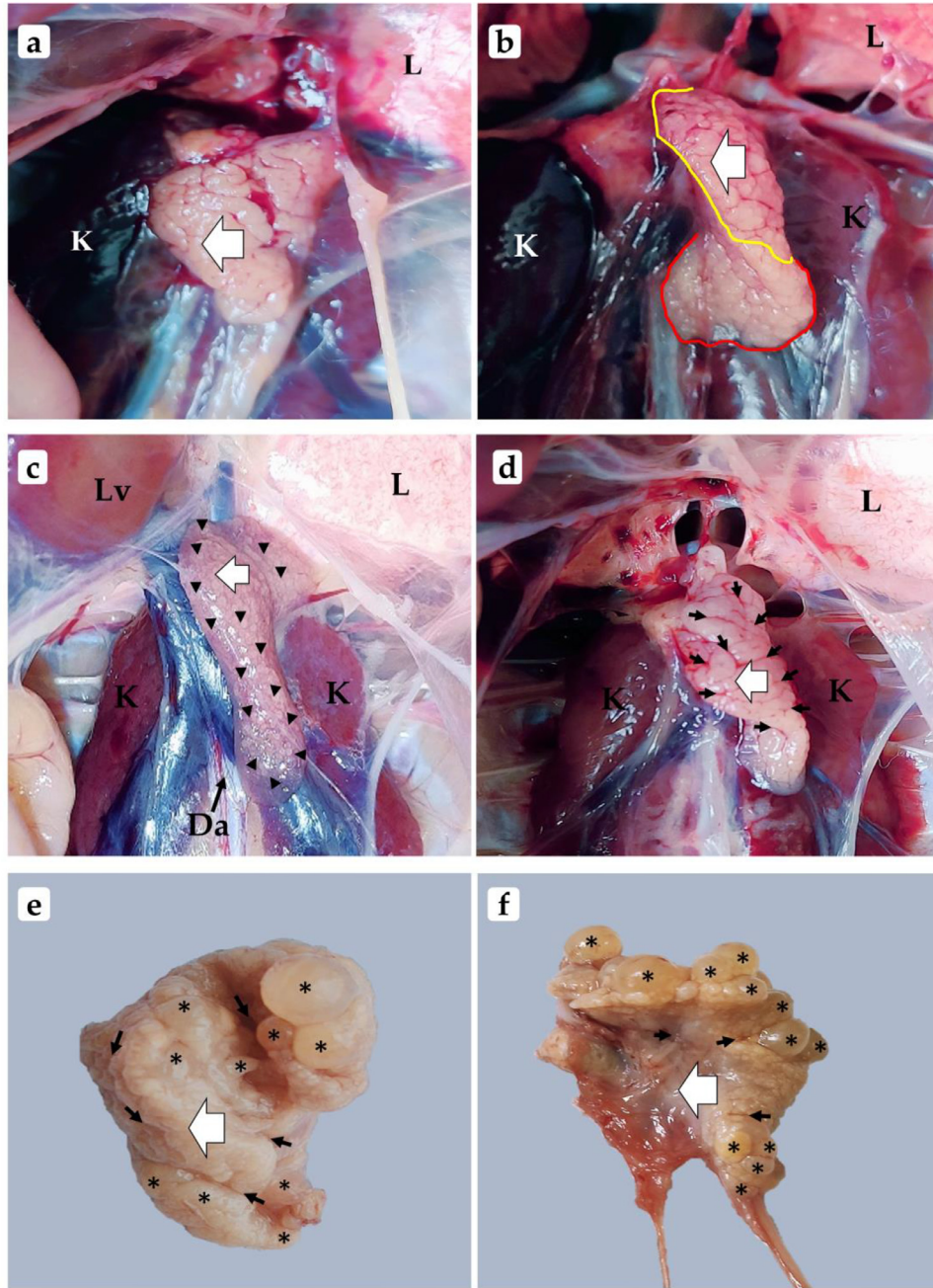


Figure 2. Morphological photographs of Chicken ovaries from 2-mo-old to 4-mo-old. Two-mo-old (A, B), 3-mo-old (C, D) and 4-mo-old chick (E, F): left ovary (white arrow), anterior lobe of the left and right kidneys (K), caudal lobe of the left lung (L), spreading curvature of the left ovary (yellow line), left ovary's posterior end folding up (red line), dorsal aorta (Da), evolving granules on the ovarian tissue (black arrowhead), ovary's indentations (black arrows), Bulged follicles on the ovarian tissue (*).

Two-Month-Old Chick. The left ovary of the 2-mo-old chick showed a great expansion of its curvature facing the right kidney which covered it furthermore (Figure 2A). The left ovary weight, length, width, and thickness are presented in (Table 1). The white dots that formed on the ovarian tissue of the 1-mo-old chick appeared to have gradually evolved, making it show more ovarian indentations and made its unevenness noticeable at sight. The left ovary became yellow creamy and displayed white dots on top of the granulations (Figure 2B). This could be interpreted as a further structural change within oocyte cytoplasm following what was observed at the preceding age.

Three-Month-Old Chick. At this stage of growth, the anterior end of the left ovary had a connection with the caudal end of the left lung; its color became yellow creamy uniform and displayed several white granules on its ovarian tissue (Figure 2C). The most noticeable were the granulations on the ovarian tissue that gradually enlarged especially at its anterior end, and displayed a more compact and much more vascularized feature. Ovarian indentations grew deeper and increased its unevenness with a complete loss of its smooth appearance (Figure 2D). The left ovary's weight and measurements are presented in (Table 1).

Four-Month-Old Chick. The left ovary displayed an anterior end that grew significantly larger; its connection to the caudal end of the left lung and the inner part of the liver was well marked. The weight and measurement parameters of the left ovary are presented in (Table 1). The granulations formed on the ovarian tissue at 3-mo-old developed significantly, fast-developing follicles: translucent in their aspect, grew bigger due to yolk accumulation in the oocyte's cytoplasm and finally bulged the ovarian tissue (Figures 2E, 2F). The ovary showed deeper indentations that took over the whole ovary.

Histological Observation of Chicken Ovaries at Different Ages

One-Week-Old Chick. The microscopic observations of the 1-wk-old chick left ovary from the outside inward generally revealed a germinal epithelium, a cortex, and a medulla (Figure 3A). The germinal epithelium appeared thin, composed of a single layer of cuboidal epithelial cells. The cortex looked dense, rich with primordial follicles in a state of stasis, organized in clusters, and enclosed within its epithelium (Figure 3D). Table 2 displays the follicle diameters. A narrow medulla occupied the middle part of the ovary displaying blood vessels, avian red blood cells, as well as the lacunar channels. The cortex and medulla showed no clear separation between the two regions.

Three-Week-Old Chick. The left ovary of the 3-wk-old chick revealed a thin germinal epithelium; the ovarian cortex grew further, showing follicles that began to differentiate and swell thereby diminished the medullar space. Few follicles revealed a circular nucleus, whereas many only showed a compact cytoplasm. The pregranulosa cells began to show cuboidal features with the basal lamina and the perivitelline membrane noticed

(Figure 3E). The larger growing follicles progressively moved at the middle-down part of the cortex while the slow-growing ones remained at the tip of the germinal epithelium and still looked enclosed within the ovarian cortex (Figure 3B). The follicle diameters are presented in (Table 2). The lacunar channels appeared to have developed further with blood vessels noticed more than avian red blood cells among the lacunae.

One-Month-Old Chick. The 1-mo-old chick left ovary exhibited a thin germinal epithelium; the cortex was swollen because of growing follicles that differentiated into Primary follicles (Figure 3C). From inside outward the fast-developing follicles were the germinal vesicle, the yolk-laden cytoplasm, the perivitelline membrane, a layer of granulosa cells, the basal lamina, and the theca layers which were not easily noticed at this age (Figure 3F). The slow-growing follicles still appeared enclosed within the cortex. Table 2 displays the follicle diameters. The medulla appeared narrower with the lacunar channel that showed well-developed structures.

Two-Month-Old Chick. The left ovary displayed a thin germinal epithelium; the cortex expanded further and greatly reduced the space of the medulla. It harbored follicles of different sizes at various developmental stages that appeared more differentiated (Figure 4A). The follicles at an advanced stage of growth swelled gradually (Table 2) and showed a well-organized structure with the theca layers well noticed for some of them (Figure 4G). Those follicles eventually migrated toward the outer cortex. The compact cytoplasm began to show rounded structures known as rounded granules of albuminoid with yolk spheres noted: the beginning of yolk accumulation (Figure 4D). An undifferentiated cytoplasmic zone was observed between the perivitelline membrane and the rest of the follicle cytoplasm that seemed to facilitate the transport of yolk particles into the middle part of the follicle.

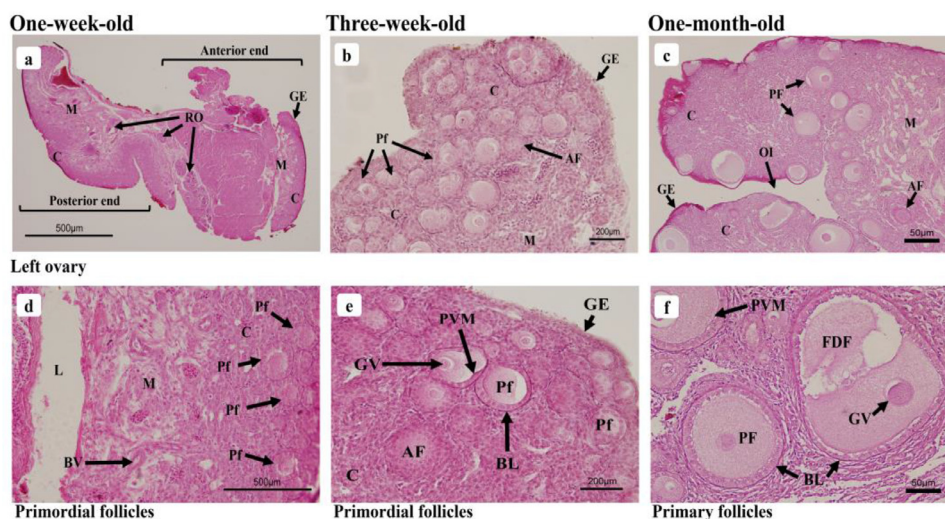


Figure 3. Histological micrographs of chicken ovaries at various stage of growth. (A, D) 1-wk-old, (B, E) 3-wk-old, and (C, F) 1-mo-old: germinal epithelium (GE), cortex (C), medulla (M), rete ovarii (RO), primordial follicles (Pf), atretic follicles (AF), primary follicles (PF), ovarian indentation (OI), lacunae (L), blood vessels (BV), perivitelline membrane (PVM), basal lamina (BL), germinal vesicle (GV), fast developing follicles (FDF).

Table 2. Follicle diameters (mean \pm SD).

Parameters	1 wk	3 wk	1 mo	2 mo	3 mo	4 mo
Follicles (μm)	***53.05 \pm 13.58	***256.20 \pm 79.81	***344.64 \pm 78.37	***543.23 \pm 88.90	**921.12 \pm 180.37	**1204.90 \pm 377.49
Min	32.22	150.78	217.59	380.35	544.93	685.73
Max	70.88	409.93	474.58	687.93	1129.72	1667.62

Statistical difference: *** $P < 0.00$; ** $P < 0.03$ to $P < 0.02$.

Table 2 displays the follicle diameters at various age stages. The Paired-Samples test showed that there was a difference (***) $P = 0.00$ in follicle diameters between the 1-wk-old and 3-wk-old age likely as it was noted between the 1-mo-old and 2-mo-old. A significant increase in diameter (***) $P = 0.029$ was then noted between 3-mo-old and 4-mo-old age.

Three-Month-Old Chick. The cortex of the 3-mo-old chick's left ovary continued to expand due to follicular development. Many of the rapidly growing follicles increased significantly in size (Table 2) and were easily noticed (Figure 4B). The rounded granules of albuminoid spread greatly from the middle section of the follicle to the perivitelline membrane with yolk spheres noticed, gradually taking over the whole cytoplasm except where an undifferentiated cytoplasmic zone was noted (Figure 4E). The number of rapidly growing follicles increased significantly and migrated towards the germinal epithelium (Figure 4H).

Four-Month-Old Chick. The cortex of the ovary appeared swollen due to follicular growth and yolk accumulation in the fast-growing follicles, which displayed denser rounded granules of albuminoid and yolk spheres (Figure 4F). The number of fast-growing follicles continued to increase, the larger ones in an advanced yolk accumulation state migrated toward the germinal

epithelium and bulged the ovarian tissue (Figure 4C). The follicle diameters are presented in (Table 2). The distinction between cortex and medulla started becoming hardly noticeable. The lacunar channel developed greatly on the entire medulla and around the fast-growing follicles (Figure 4I).

Atretic Follicles. Atretic follicles were observed among the normally growing follicles from 3-wk-old to the end of the study; displaying a follicle that exhibited a cytoplasm filled with cuboidal cells from the granulosa layer. Based on histological observation, they were classified into 2 types; (1) a non-brusting type (having few amounts of cytoplasm) and (2) a brusting type (with large amounts of cytoplasm), all related to the stage of regression they were found at. Stage 1, was about the follicles that had their perivitelline membrane destroyed, which freed the granulosa layer content into the follicle's cytoplasm (Figures 5A, 5B), stage 2, were those from which the granulosa layer's content spread within the

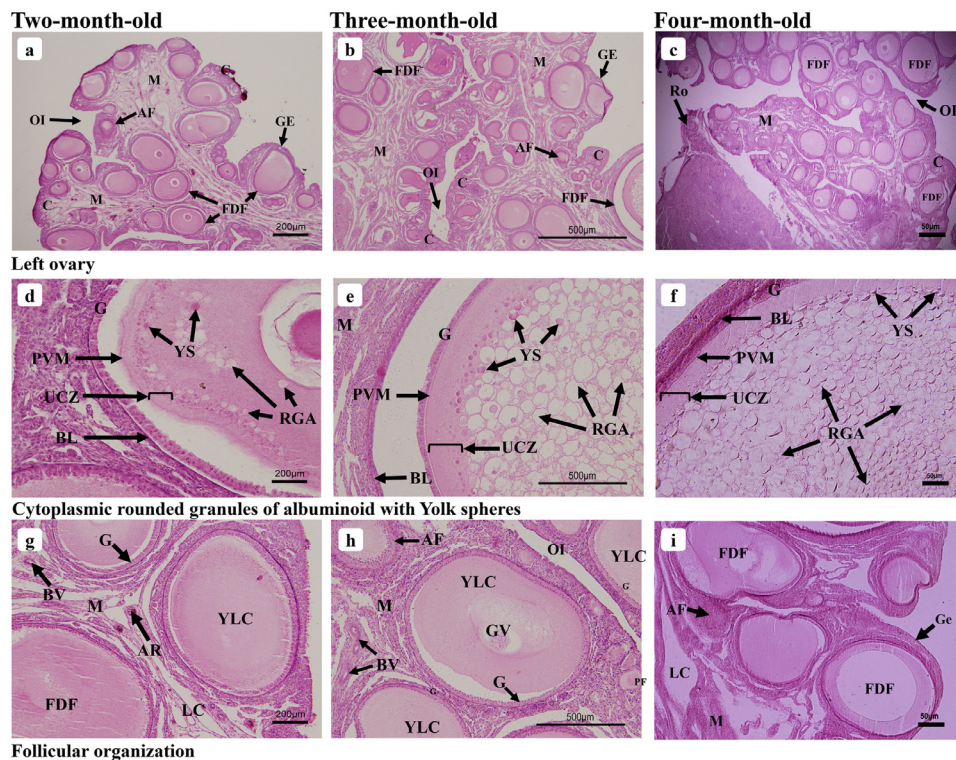


Figure 4. Histological micrographs of chicken ovaries at various stage of growth. (A, D, G) 2-mo-old, (B, E, H) 3-mo-old, and (C, F, I) 4-mo-old: ovarian indentation (OI), medulla (M), Atretic follicle (AF), germinal epithelium (Ge), fast-developing follicles (FDF), cortex (C), rete ovarii (RO), perivitelline membrane (PVM), granulosa cells (G), basal lamina (BL), yolk spheres (YS), undifferentiated cytoplasmic zone (UCZ), rounded granules of albuminoid (RGA), blood vessels (BV), avian red blood cells (AR), lacunar channel (LC).

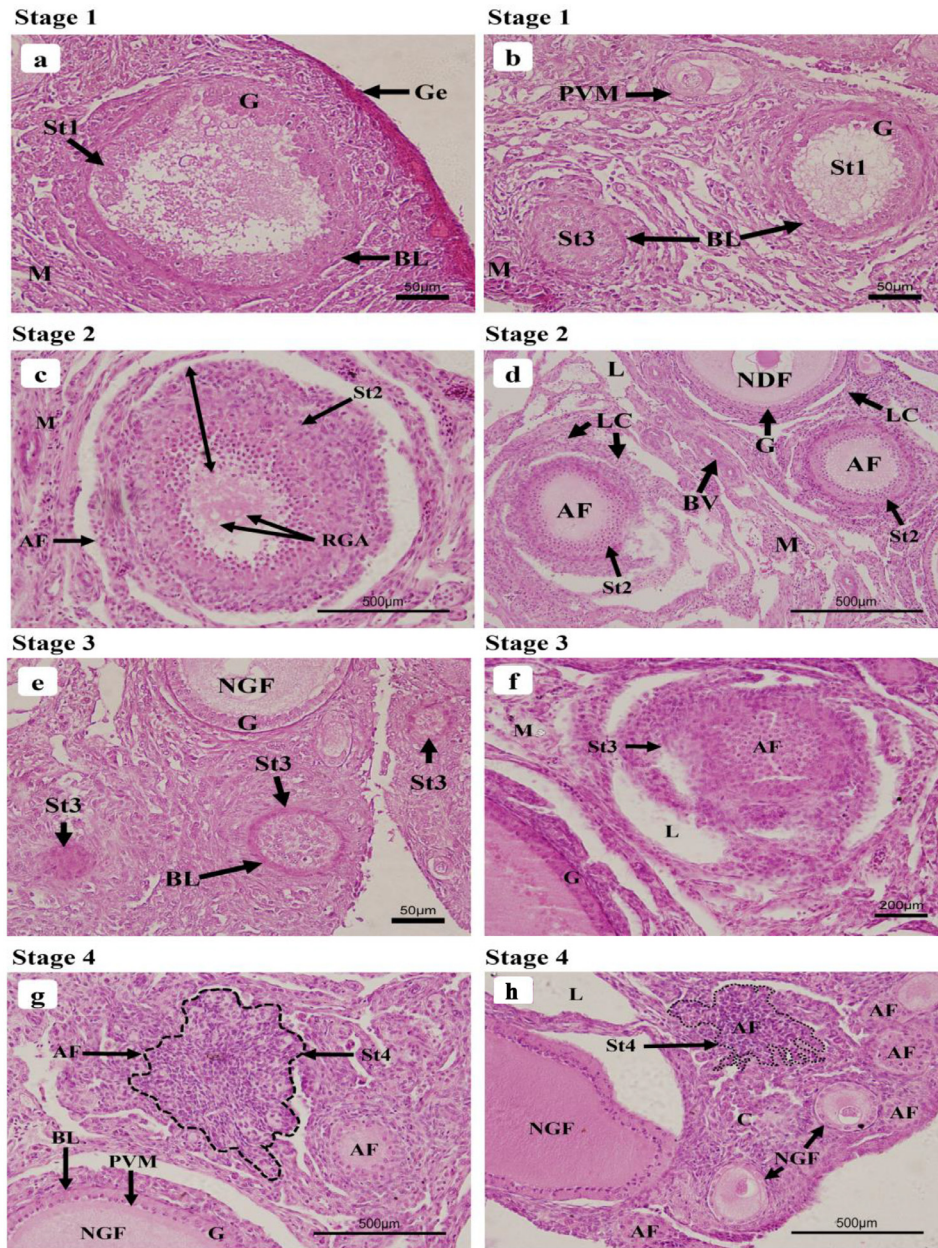


Figure 5. Histological micrographs of atretic follicles in the chicken ovaries at various stage of growth. (A, B) Stage 1, (C, D) Stage 2, (E, F) Stage 3, and (G, H) Stage 4: Stage 1 (St1), granulosa membrane (G), germinal epithelium (Ge), basal lamina (BL), medulla (M), perivitelline membrane (PVM), Stage 3 (St3), atretic follicle (AF), rounded granules of albuminoid (RGA), cuboidal cells migration towards the cytoplasm (double headed arrow line), Stage 2 (St2), luteal cells (LC), lacunae (L), normally developing follicles (NDF), blood vessels (BV), follicle delimitation (discontinuous dots), Stage 4 (St4), normally growing follicle (NGF), cortex (C).

cytoplasm (Figures 5C, D), stage 3 those shrinking or fading within the ovarian stroma (Figures 5E, 5F), and stage 4 those that exhibited a scar-like feature that gradually shrank away (Figures 5G, 5H). In Type 1, the destruction of the perivitelline membrane freed the granulosa layer's content into the cytoplasm while the basal lamina remained. The cuboidal cells then migrated toward the center of the follicle where they agglomerate and hypertrophied until they completely shrink away (Figure 5E). In Type 2, the destruction of both the perivitelline membrane and the basal lamina was observed, which freed the follicle's content into the ovarian stroma and gradually absorbed it by phagocytosis leaving a scar-like feature behind (Figures 5G, 5H). Atretic follicles were

frequently noticed as the chick grew in age with some displaying luteal cells around them (Figure 5D).

Rete Ovary. Rete ovarii, tissues originating from the mesonephric were found on the medulla at the anterior and posterior end of the ovary and on the mesovarium the whole study (Figure 6A). Epoöphoron, round, oval to elongated seminiferous tubules-like in shape displaying scant cytoplasm and prominent black nuclei were the most abundant structures observed of the rete ovarii (Figure 6B) Most of them displayed a lumen and their tubules diameter varied from one age to another (Table 3). Aligned with them was a network of the connecting rete; epithelial structures made of irregular anastomosing epithelial tubules lined by flat to

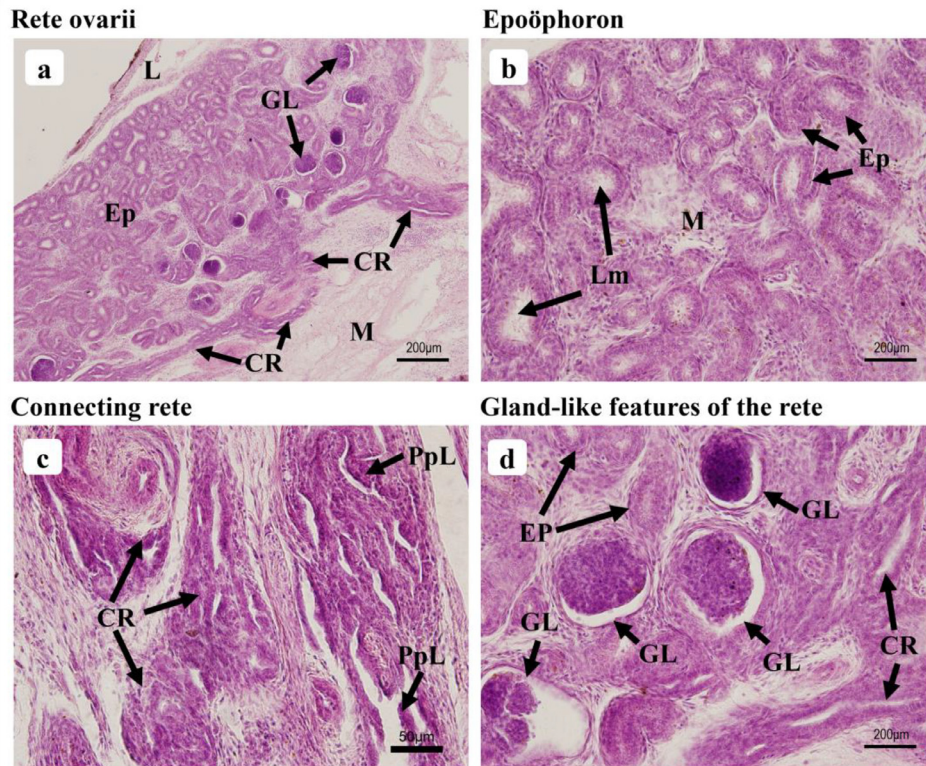


Figure 6. Histological micrographs showing chicken's rete ovarii. Lacunae (L), glande-like structures of the rete ovarii, epoöphoron (Ep), connecting rete (CR), medulla (M), Lumen (Lu), pappilae (PpL).

columnar cells displaying papillae for some (Figure 6C) that appeared elongated running along with epoöphoron structures or sometimes found isolated on the medulla. Gland-like structures, exhibiting a compact feature and lumen around them were often noticed lining between epoöphoron and the connecting rete from 1-wk-old to 2-mo-old of age (Figure 6D) that appeared to diminish from then onward as the chick grew in age.

DISCUSSION

Morphohistological Correlation Between the Left Ovary and the Follicular Development

The experimental results of this study showed that chicken left ovary morphologically displays ovarian tissue, site of follicular development, and both left and right ovary are attached to the backbone on either side of the dorsal aorta, the left ovary's basal portion dorsally

Table 3. Morphometry of the rete ovarii (mean \pm SD).

Parameters	Tubules diameter (μm)			Tubules' epithelium thickness (μm)			Lumen diameters (μm)		
	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
1 wk	***35.09 \pm 24.46	0.454	70.05	**12.73 \pm 9.06	0.15	25.67	***10.56 \pm 8.36	0.097	28.06
3 wk	**43.23 \pm 8.54	30.94	63.50	**13.80 \pm 3.05	7.28	20.56	***16.11 \pm 6.56	6.42	34.48
1 mo	**44.10 \pm 10.49	24.42	63.67	**15.14 \pm 1.20	0.29	22.7	**13.86 \pm 7.05	0.67	31.80
2 mo	***45.34 \pm 7.98	29.12	59.43	**15.20 \pm 3.47	9.32	23.66	***16.93 \pm 6.56	6.00	37.26
3 mo	***45.19 \pm 9.96	30.20	65.96	**13.37 \pm 3.16	7.07	20.56	**14.00 \pm 5.18	6.10	25.70
4 mo	**40.00 \pm 11.99	18.95	65.05	**14.65 \pm 5.96	6.10	40.69	**14.07 \pm 4.62	5.14	24.41

Statistical difference: *** $P < 0.05$, ** $P > 0.05$.

The results of Table 3 showed that there were differences between various parameters of the rete ovarii except for the tubules' epithelium thickness as demonstrated by one-way ANOVA ($F(5,174) = 2.609$, *** $P = 0.027$) for the tubules diameters, ($F(5,174) = 0.953$, ** $P = 0.448$) for the tubules' epithelium thickness, and ($F(5,174) = 3.477$, *** $P = 0.005$) for the lumen diameters.

A Tukey post hoc test revealed that almost all components of the rete ovarii were not different from each other for many of its parameters among various age groups ($P > 0.05$), except when comparing some age groups parameters to those of the 1-wk-old chick ($P < 0.05$).

A constant increase in Tubules' diameter was noted from 1-wk-old to 3-mo-old before recording a slight decrease in the 4-mo-old chicken (** $P = 0.105$ to ** $P = 1$). The tubules' diameters of the rete ovarii were only different when comparing the 1-wk-old to the 2-mo-old and the 3-mo-old chicks (*** $P = 0.042$ and *** $P = 0.047$ respectively). The epithelium of the rete ovarii's tubules displayed an increase in their thickness from 1-wk-old to 2-mo-old before decreasing slightly in the 3-mo-old chick and continued their growth in the 4-mo-old chick, displaying no statistically difference among all age groups observed (** $P = 0.541$ to ** $P = 1$). The lumen of the tubules appeared to have increased significantly between 1-wk-old and 3-wk-old of age (*** $P = 0.015$) before recording a great decrease in a 1-mo-old chicken (** $P = 0.05$). Another great increase in lumen diameters was noted in the 2-mo-old chick and appeared to be different relative to the 1-wk-old chick only (** $P = 0.003$) with parameters stabilization afterward. The comparison between other age groups showed no difference between them (** $P = 0.298$ to ** $P = 1$).

connected to the coelomic body wall (King and McLeland, 1975). Follicle development can be classified into 3 different stages: 1) slow follicle growth phase of 60 to 100 μm in diameter that lasts months to years; 2) increasingly fast growth phase, lasting for several months, consisting mainly of the deposition of yolk protein; and 3) fast growth phase in domestic fowl, ducks, and pigeons ovulation during the final 6 to 11 days prior to ovulation (up to 16 d in some penguins (Grau, 1982).

The left ovary of the 1-wk-old chick displays a feature that appears smooth and regular; clear pink in color and yellow-creamy around the caudal lobe of the lung. It estimates 6.66 mm (~ 0.7 cm) long with a broad anterior end (**Ae**): 2.08 mm (~ 0.21 cm), a thin posterior end (**Pe**): 1.35 mm (~ 0.14 cm) wide, and a thickness of about 1.55 mm (~ 0.16 cm). Mohamed et al. (2016) estimated the 1-wk-old left ovary in Alexandria breed about 1 cm long and 4 mm wide; this difference may be due to breed variation. Histologically, the cortex and the medulla appear as 2 well-differentiated regions at 1-day-old (Gonzalez-Moran, 2005). The cortex of the 1-wk-old chick harbored primordial follicles resulting from the differentiation of primary oocytes that remain in the diplotene stage of meiotic prophase I (Gonzalez-Moran, 2007; Johnson, 2014). This agrees with Mohamed et al. (2016) and Gonzalez-Moran (2011) on the fact that the 1-wk-old chick left ovary histologically displayed a thick cortex and narrow medulla without clear tunica albuginea in between. The primary oocytes that fail to be enclosed by a layer of pregranulosa cells are predestinated to undergo apoptosis (Johnson, 2014). Many of the primordial follicles ~ 0.05 mm in diameter remain associated loosely as quiescent clusters until sexual maturity (Diaz et al. 2011). At this age, the primordial follicles are in a state of stasis, which results in showing no morphological influence on the left ovarian tissue and the reason why it appears smooth and regular.

The 3-wk-old chick's left ovary is 10.79 mm (~ 1.1 cm) long, 4.10 mm (~ 0.4 cm) Ae wide, 1.53 mm (~ 0.15 cm) Pe wide, and 2.24 mm (~ 0.22 cm) thick. Data about this age group is not available in most scientific reports, as researchers generally focus their attention on the 1-wk-old and 1-mo-old chicken during their investigations. At this age, few follicles start to enlarge then move backward at the inner cortex while the slow-growing ones remain at the top of the cortex, similarly as noticed in a 7-day-old mouse ovary (Byskov, 1975). The factors involved in the activation of primordial follicles from quiescent to actively growing follicles are yet to be widely studied in avian species (Johnson, 2014). An internal, perivitelline membrane (made of glycoproteins ZP1, ZP2, ZP3, and ZPD of the zona pellucida) develops between the granular cells and the oocyte and will eventually promote acrosome exocytosis and fertilization, at the same time acts as a microbial contamination barrier (Rodler et al., 2012) At this stage of growth, the pregranulosa cells gradually develop, showing cuboidal features meanwhile few follicles enlarge, causing the ovarian tissue to start showing indentations.

The present study estimates the 1-mo-old left ovary about 11.16 mm (~ 1.12 cm) long, 5.45 mm (~ 0.55 cm) Ae wide, 1.86 mm (~ 0.19 cm) Pe wide, and 2.26 mm (~ 0.23 cm) thick and agrees with Mohamed et al. (2016) who estimated the left ovary to be 1 cm long and 0.5 cm wide in Alexandria breed. The 1-mo-old chick ovarian cortex displays primordial follicles that differentiate into primary follicles (Johnson, 2007). In this study, the primary follicles display from inside outward, a germinal vesicle, a yolk-laden cytoplasm, a perivitelline membrane, a granulosa layer made of cuboidal cells, a basal lamina, the theca interna, and the theca externa which coincide with Al-Saffar and Ab.Abood (2014) findings on the Mallard duck's ovary at day 19 post-hatch. The oocyte remains arrested in the diplotene of prophase I of meiosis and grow simultaneously with the follicle that increases by 300-fold in a period of 2 to 3 wk in the mouse (Lintern-Moore and Moore, 1979). Also, a proliferation in RNA contents and protein synthesis is noted by 300-fold and 38-fold, respectively (Wassarman and Albertini, 1994). In the mouse, the proliferation of granulosa cells produces multiple layers of cells with the formation of secondary or preantral follicles. At this stage, theca cells made from the ovarian stromal cell, fibroblast-like cells then become connected to the follicle whereas, in chicken, the granulosa layer remains as a single layer of cells throughout its entire life (Hirshfield, 1991; Johnson, 2014). Our findings are controversial to those of Mohamed et al. (2016) and agree with Gonzalez-Moran (2011) who stated that "the two regions of the theca were not differentiated yet". The follicles' differentiation with an increase in size and a change of their cytoplasmic components appear on the ovarian tissue as white dots and an increase of ovarian indentations.

The left ovary of the 2-mo-old measures 14.71 mm (~ 1.5 cm) long, 5.95 mm (~ 0.6 cm) Ae wide, 3.35 mm (~ 0.34 cm) Pe wide, and 3.34 mm (~ 0.33 cm) thick. Well-organized follicles display luteal or interstitial cells. This finding comes in agreement with Phillips et al. (1985) who related these cells' functions to the production of steroids. The primary follicles enlarge further, "the cytoplasm of the oocyte contains the nutritive yolk that is made of rounded granules of albuminoid substances embedded in the cytoplasm" (Mann and Mann, 2008). The formation of yolk protein starts in the liver and is mainly controlled by gonadotropin and steroid hormones. From the liver, it is supplied to the ovary through the blood. The plasma-borne precursors vitellogenin (a phosphoglycolipoprotein) and very low density lipoprotein (plays a role in the transportation of triglycerides, phospholipids, and cholesterol) pass through the basement membrane via intervals between granulosa cells to the plasma membrane of the oocyte after being released by capillaries within the theca layer (Shen et al., 1993)

The number of fast-growing follicles significantly increases with many follicles displaying rounded granules of albuminoid in the cytoplasm and a few yolk spheres close to the undifferentiated cytoplasmic zone.

All these changes cause the ovarian tissue to show deeper indentations and greatly increase its unevenness.

The 3-mo-old left ovary estimates 15.99 mm (~1.6 cm) long, 7.56 mm (~0.76 cm) Ae wide, 3.42 mm (~0.34 cm) Pe wide, and 3.34 mm (~0.33 cm) thick. "In more developed follicles, granulosa cells range in appearance from columnar to pseudostratified columnar to polyhedral. Cytoplasm is palely basophilic to amphophilic and can be granular, particularly in granulosa cells surrounding more developed follicles since these cells have a role in transporting yolk into the follicle" (King and McLelland, 1975; Gilbert, 1979; Johnson, 2007; Johnson and Woods, 2009). Further transportation across the plasma membrane, vitellogenin, and very low density lipoprotein become located in yolk spheres where phosvitin, lipovitelline, triglycerides, cholesterol, or cathepsin D proteolytic processing takes place (Retzek et al., 1992). Those follicles then move toward the germinal epithelium where they begin to bulge the ovarian tissue and externally appear as white granules causing the left ovary to lose its smooth appearance to a completely granulated one.

The 4-mo-old chick's left ovary displays many fast-developing follicles in an advanced yolk accumulation state and estimates 17.61 mm (~1.76 cm) long, 10.26 mm (~1.03 cm) Ae, 4.79 mm (~0.48 cm) Pe, and 3.81 mm (~0.38 cm) thick. These results are similar to King and McLelland (1975) who reported the left ovary to weigh about 0.5 g from hatch to about 4-mo-old, which in this study was ~0.56 g. For most of the growth phase, lipids and protein deposition in the developing follicle are nearly done in the same ratio, yet considerably more lipids are integrated during the last fast-growth phase. Deposition of yolk in the maturing follicle has been proposed to end 24 h before ovulation. The ultrastructure of follicle development has been well outlined (Wyburn et al., 1965; Rothwell and Solomon, 1977; Perry et al., 1978a; 1978b, and Gilbert et al., 1980). The follicles that have bulged the ovarian tissue can be seen exhibiting a translucent feature.

This study comes in addition to the works that were previously done, highlighting some great events happening in the chicken's left and right ovaries during growth. Among which, the 8-day-old chicken embryo, when sexual differentiation definitively takes place and the left and right ovaries show morphological asymmetry between them (Gasc, 1978); the 13-day-old chicken embryo, with the hypothalamic-pituitary-gonadal axis that becomes functional (Woods and Weeks, 1969; Jenkins and Porter, 2004); 1-day-old chicken, with the left ovary displaying 2 well-differentiated regions: cortex and medulla (Gonzalez-Moran, 2005); and 4-wk-old chicken, with primordial follicles becoming primary follicles in the left ovary (Johnson, 2007). In this study, the 3-wk-old left ovary that begins to show ovarian indentations on its tissue due to primordial follicles enlargement and differentiation, 2-mo-old chick ovary, that shows clear particles of rounded granules of albuminoid and yolk spheres; 3-mo-old left ovarian tissue that loses its smooth appearance and becomes more uneven showing

white granules on its tissue due to follicular growth and yolk accumulation in the cytoplasm; and the 4-mo-old, when follicles finally bulged the ovarian tissue.

Atresia

Follicular atresia is a normal physiological process that takes place in the ovary throughout female reproductive life, at all stages as follicles develop (Gosden and Spears, 1997). Atresia is well investigated in chicken, mostly in those that have already reached sexual maturity (Gilbert et al., 1983; Gupta et al., 1988; Waddington et al., 1985). The fate of an activated primordial follicle is whether to enter the growing follicle pool or to become atretic. Approximately 99% of follicles disappear through programmed cell death due to apoptosis of granulosa cells. (Hirshfield, 1991; Matsuda-Minehata et al., 2006). This is in agreement with the present work findings, atretic follicles displayed a cytoplasm filled with the degenerating granulosa cells that appeared compact gradually taking over the whole cytoplasm. In some cases, the follicles displayed many luteal cells on the theca interna surrounding them. Luteal cells are types of stromal endocrine cells: large with clear, vacuolated cytoplasm displaying small, dark, dense, and centered nuclei. These cells are distributed throughout the ovarian cortex and on the theca interna of fast-growing follicles. Some refer to them as theca gland (Hodges, 1974; Baumel et al., 1993; Apperson et al., 2017) and Phillips et al., 1985 related these cells function to the steroids' production. Some studies in mammal's ovary (Gosden and Spears, 1997; Hirshfield, 1991; Matsuda-Minehata et al., 2006) demonstrated that antral follicle atresia may occur in case of insufficient or excess of several hormones or growth factors including LH, FSH, estrogens, androgens, and insulin-like growth factors, as well as their corresponding receptors. However, the signals and intracellular pathways involved in determining whether a follicle becomes atretic at any period of its growth or survives until the ovulation are still unknown, but many pro-and antiapoptotic molecules are likely involved (Matsuda-Minehata et al., 2006)

Rete Ovarii

Rete ovarii, are features originating from the mesonephric tissues. They are found in 3 parts in female animals: the intraovarian rete, the connecting rete, and the extra ovarian rete or epoöphoron. The components of these 3 parts vary among species and individuals (Apperson et al., 2017). The rete ovarii were observed on the left ovarian medulla throughout the whole study and the mesovarium. This fall together with (Apperson et al. (2017)) findings on the laying hen ovary. The connecting rete epithelial cells are ciliated in contrast to those of the intraovarian rete, which are not (Apperson et al., 2017).

Extraovarian rete ovarii or epoöphoron, seminiferous-like structures exhibited a scant cytoplasm with

prominent black nuclei and tended to diminish as the chick grew in age, only displaying rounded to elongated structures. In the laying hen, epoöphoron can be found completely outside in the mesosalpinx or mesovarium or the hilar section of the ovarian medulla (Apperson et al., 2017). In female animals, the rete ovarii derives from the same embryonic mesodermal similar to the mesonephric tissues in males. For this, a homologous comparison of their components can be done between them: the epoöphoron is the epididymis remainder, the connecting rete tubules are the ductus deferens remainder, and intraovarian rete tubules are the rete testis remainder (Gilbert, 1979; Baumel et al., 1993; Fleming et al. 2006). The epoöphoron observed in this actual work, in the medulla of the left ovary, appeared few in the 1-wk-old chick before increasing significantly in number in the 3-wk-old chick then regressed slightly on the medulla of the 1-mo-old chick. A similar observation was reported in a 16-day-old mouse fetus, with most extra ovarian tubules that disappeared or were regressing, and only displayed the most cranial ones, which showed no change in their length, and were located between the Wolffian duct remainder and the connecting rete's reticulum which, afterward were only presented as a single coiled tubule that laid in the periovarian connective tissue in a 7-day-old mouse (Byskov, 1975). "The rete ovarii plays a decisive role in early follicle formation because removal of the rete from the ovarian tissue before follicles are formed prevents follicular organization as well as follicle formation. Their complete removal before follicle formation in mice causes an intransient absence of follicles" (Byskov, 1975). The rete ovarii also plays an important role as oocytes, the first meiosis initiator. The mesonephric sex cords are considered to be the intraovarian rete tubules predecessors which in other species investigated displays PGCs. Through observations in young mice and embryonic chicks, it is believed that meiosis only commences in GC located in mesonephric cords that are as well aligned with the ovarian surface epithelium (Byskov, 1975; Gilbert, 1979). Tissues coming from mesonephric, such as the rete ovarii, are normal components of the reproductive tract of female animals of most, if not all, mammalian species (Fleming et al. 2006; Wenzel and Odend'hal, 1985), adult hens (Gilbert, 1979; Baumel et al., 1993; Barnes et al. 2008), other avian species (Gilbert, 1979; Barnes et al., 2008), as well as in young chicken from 1-wk-old to 4-mo-old as reported in this study.

CONCLUSION

This study shows that the feature of the left ovary is closely related to the developmental stages of the follicles, and the bigger and differentiated the follicles are, the more indented and irregular its epithelium appears. The primordial follicles are in a very slow-growth phase from 1-wk-old to 3-wk-old and become primary follicle at 1-mo-old of age with the formation of theca layers and the start of yolk accumulation. From 1-mo-old to 4-

mo-old, follicles enter the fast-growth phase and are in an advanced yolk accumulation state and bulge the ovarian tissue. The results also indicate that atresia is a continuous process that occurs throughout the chick's life from the phase of growth onward which ends in the resorption of the follicle within the ovary's epithelium for the slow-growing follicles and with a scar-like left behind by the fast-developing follicles. Furthermore, the rete ovarii do not remain unchanged as medullary components but they appear to show slight changes in their parameters as the chick grows as observed for the gland-like structures that tend to diminish with age.

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DISCLOSURES

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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