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Morphology and histology of the developing ovary in Malaysian village chicken (*Gallus gallus domesticus*) chicks

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Abstract: The Malaysian village chicken (MVC; *Gallus gallus domesticus*) is an indigenous breed derived from the red junglefowl (*Gallus gallus*), exhibiting slower growth and reproductive rates. The MVC plays an important ecological role in local farming systems and represents a valuable genetic resource. However, detailed information on ovarian development in this breed remains limited. This study provides the first comprehensive morphological and histological characterization of the developing ovary in MVC chicks. A total of 20 chicks aged 1, 2, 4, and 8 weeks (n = 5 per age group) were examined. These MVC chicks possessed a single functional left ovary positioned ventromedially to the cranial lobe of the left kidney. Age-related gross morphological changes were evident in size, shape, color, and consistency. Histologically, significant ($p < 0.05$) increases in cortical and medullary thickness were observed, with follicular growth progressing from primordial to primary and more advanced stages from 2 weeks after hatching, with cortical lobulation becoming prominent by 8 weeks. The proportion of primordial follicles (approximately 88%–89%) remained high across 2–8 weeks, indicating the maintenance of the ovarian reserve. These findings provide novel insight into MVC reproductive biology and establish baseline data to guide future studies on avian reproduction, development, and conservation in indigenous chicken populations.

Key words: Anatomy, development, histology, Malaysian village chicken, ovary

1. Introduction

The Malaysian village chicken (MVC; *Gallus gallus domesticus*) is an indigenous breed widely reared under free-range conditions across rural Malaysia. It is believed to have originated from the red junglefowl (RJF; *Gallus gallus*), the wild ancestor of domestic chickens native to Southeast Asia [1]. Genetic analyses indicate close genomic and phenotypic relationships between the MVC and RJF, with MVCs exhibiting traits such as strong disease resistance, efficient foraging ability, and adaptability to tropical environments [2–4]. These genetic and phenotypic traits suggest that MVCs may retain reproductive characteristics inherited from the RJF, which could influence ovarian development and folliculogenesis. However, these adaptive traits are accompanied by slower growth, delayed sexual maturity, and lower egg production compared to commercial breeds, limiting the productivity of the MVC in intensive farming systems [5].

MVCs typically reach sexual maturity at approximately 4 to 6 months of age, depending on diet, management, and environmental factors [6,7]. Despite this slower growth, MVCs are valued for leaner meat with a distinctive flavor, which is preferred by many consumers over meat from fast-growing commercial breeds [1,8].

In female chickens, the reproductive system consists primarily of the ovary and oviduct. Unlike most vertebrates, only the left ovary persists and remains functional after hatching [9]. The avian ovary contains follicles at various developmental stages, each comprising an oocyte surrounded by a single layer of granulosa cells that provide nutrients and regulatory signals [10]. Follicle development is tightly regulated by endocrine and intraovarian factors

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[11–13], progressing hierarchically from small primordial follicles to mature tertiary follicles prior to ovulation [14].

Ovarian folliculogenesis plays a central role in reproductive performance and egg production [15,16]. At sexual maturity, a hen's ovary contains approximately 12,000 oocytes, though only a few hundred are recruited into ovulatory follicles, while the remainder undergo atresia [17,18]. Consequently, reproductive productivity depends on both the number of functional follicles and the oviduct's ability to convert ova into complete eggs [19].

Understanding the reproductive anatomy and histology of MVCs is critical for enhancing reproductive efficiency and preserving genetic traits valuable for conservation and breeding programs [20]. While ovarian morphology and follicular dynamics have been extensively studied in commercial chicken breeds [21–23], comparable data for MVCs remain scarce. Moreover, characterizing the ovarian medulla and cortex during early development could reveal how structural and follicular dynamics support the unique reproductive physiology of indigenous breeds.

Therefore, this study aimed to characterize the morphological and histological development of the ovary in MVC chicks aged 1–8 weeks, focusing on age-related changes in the cortex and medulla, and to establish baseline data that may inform future comparative and developmental studies in indigenous avian species.

2. Materials and methods

2.1. Animals

A total of 20 chicks aged 1, 2, 4, and 8 weeks (n = 5 per age group) were used in this study. The chicks were obtained from Orilui Neutral Farm (Hulu Langat, Selangor, Malaysia). All experimental protocols involving animals were approved by the University of Putra Malaysia's Institutional Animal Care and Use Committee (IACUC; Ref. No. UPM/IACUC/AUP-R028/2023).

2.2. Macroscopic evaluations

All chicks were euthanized via cervical dislocation. The chicks were placed in dorsal recumbency. The feathers covering the abdomen were removed. The abdominal viscera were exposed by creating an incision along the caudal margin of the keel. The anatomy of the ovary and the surrounding tissues were examined in situ. The ovary was then removed and the weight, length and width were recorded.

2.3. Tissue processing and staining

The ovaries were fixed in 10% phosphate-buffered formaldehyde solution overnight, rinsed, and preserved in 70% alcohol until processing. The fixed ovaries were processed in an automated tissue processor (Leica TP1020, Leica Biosystems, Nussloch, Germany). Processed tissue was embedded in paraffin, sectioned at a thickness of 4 μm, deparaffinized, and stained with hematoxylin and eosin (H&E), toluidine blue, and Masson's trichrome. The H&E staining gave an overview of the cellular and tissue structure of the ovaries. Meanwhile, toluidine blue and Masson's trichrome were used to determine the distribution of the follicles and connective tissue, respectively.

2.4. Histological evaluations

Histological assessment was performed using a microscope equipped for digital photomicrography (Nikon Eclipse Ci, Nikon Corporation, Tokyo, Japan). For each replicate, three randomly selected fields were photographed at 200× and 400× magnification. The thickness of the ovarian cortex and medulla was measured by averaging three measurements taken from three random areas for each replicate. Primordial and primary follicles were counted under 400× magnification. For each replicate, three counts were taken from three microscopic fields and the mean percentage of each follicle type was calculated. For the size of the follicles, the diameter of 200 randomly selected follicles was measured. The diameter for each follicle was the average of two diameter measurements, taken from two axes. Measurements were performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.5. Statistical analyses

Data are presented as mean ± standard deviation. Continuous variables were analyzed using one-way analysis of variance (ANOVA) and categorical variables were analyzed using the chi-square test. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics 27 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Macroscopic findings

3.1.1. Gross morphology

In situ examination revealed that the MVC chicks had a single left ovary. In general, there were apparent changes in the anatomical positioning, size, and morphology of the ovaries with age (Figures 1a–1d). The ovary of 1-week-old chicks was located at the craniomedial surface of the left kidney's anterior lobe and possessed a creamy, fan-shaped structure. It was bounded by the caudal base of the left lung and the caudal part of both the ventriculus and isthmus. The caudomedial border of the ovary was attached to the craniomedial side of the cranial lobe of the left kidney (Figure 1a). The 2-week-old ovarian tissues exhibited a slightly pinkish color with a flat and smooth surface and an infolded caudal end. The ovary's curvature was expanded and slightly covered the right kidney (Figure 1b). This curvature overlapped with both kidneys at their inner cranial portions, facing the dorsal aorta in 4-week-old chicks. At this age, the caudolateral part of the ovary

formed a narrow and pointed projection. The caudomedial border of the ovary's curvature also possessed a concave surface (Figure 1c). The ovary of 8-week-old chicks was slightly larger and thicker than the 4-week-old chick's ovary. The ventral surface had a lobulated surface with prominent vascularization. The ovary's curvature was also expanded and covered more area of the right kidney (Figure 1d). Overt differences in the morphology of the developing ovaries, including size, shape and color, were further confirmed upon ex situ examination (Figures 1e–1h).

3.1.2. Morphometric analysis

The length of the MVC chicks' ovaries showed a significant increase with age ($p < 0.05$, $p \leq 0.001$) (Figure 2a). The ovaries of 4-week-old (9.9 ± 2.41 mm) and 8-week-old (10.7 ± 1.20 mm) chicks were significantly longer than those of the ovaries of 1-week-old (6 ± 1.77 mm) and 2-week-old (5.4 ± 1.19 mm) chicks. Similarly, the width of the ovaries differed significantly as age increased ($p < 0.05$, $p \leq 0.001$) (Figure 2b). The width of 4-week-old ovaries (5.2 ± 1.04 mm) was significantly greater than that of 1-week (3.5 ± 0.5 mm) and 2-week (3.5 ± 0.79 mm) ovaries, but less than that of 8-week ovaries (7.2 ± 1.10 mm). The relative weight of ovarian tissues demonstrated a similar pattern, increasing significantly with age ($p < 0.05$, $p = 0.026$) (Figure 2c). A significant difference was observed between the ovaries of the 1-week-old (~0.06 ± 0.03%) and 8-week-old (~0.16 ± 0.08%) chicks.

3.2. Histological findings

3.2.1. Ovarian cortex

The cortex of MVC ovaries is lined by a thin, single-layer cuboidal cell epithelium. In general, the cortex is composed of dense connective tissue and contains follicles at various developmental stages. The cortex and the medulla area were clearly defined as the chicks aged. The cortex of older MVC chicks contained follicles of different stages (Figure 3). The cortex of the MVC ovaries demonstrated a bell-shaped growth curve (Figure 4a). The 1-week-old MVC ovaries had a narrower cortex than the medulla. Over time, the cortex-to-medulla ratio increased, coinciding with the presence of developing follicles. The thickness of the ovarian cortex significantly increased ($p < 0.05$, $p = 0.001$) in the first 4 weeks of life, from 144.96 ± 43.9 µm to 256.54 ± 18.94 µm in 1- and 4-week-old ovaries, respectively. However, the cortical thickness was significantly reduced in 8-week-old ovaries (~171.80 ± 24.90 µm) compared to 4-week-old ovaries.

3.2.2. Ovarian medulla

The medulla is a highly vascularized connective tissue occupying the central part of the ovaries. This structure is also composed of lengthy and unevenly oriented bands of smooth muscle, areas of collagenous connective tissue, and a large number of fibroblasts. Unlike the cortex, the area occupied by the medulla decreased over time in the developing MVC chicks' ovaries (Figure 3). The thickness of the ovarian medulla was significantly reduced ($p < 0.05$,

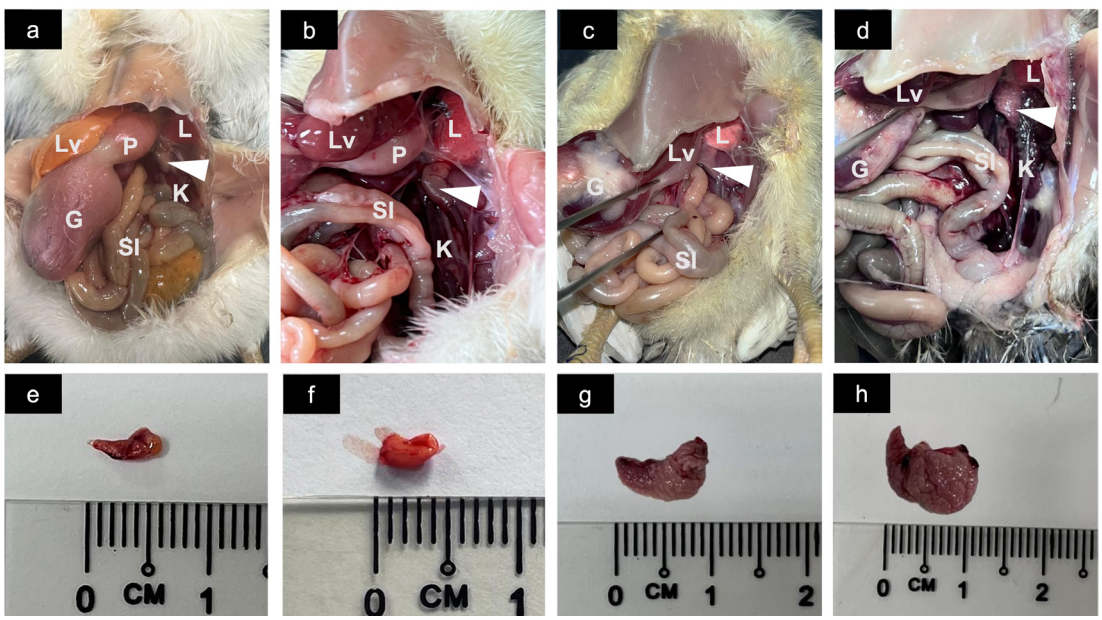

Figure 1. Representative photomicrographs showing in situ (a–d) and ex situ (e–h) examinations of the 1-week-old (a, e), 2-week-old (b, f), 4-week-old (c, g), and 8-week-old (d, h) MVC ovaries. Note the anatomical positioning of the ovaries (arrowhead) in association with other visceral organs. G: Gizzard; K: kidney; L: left lung; Lv: liver; P: proventriculus; SI: small intestine during in situ examination. Remarkable changes in the morphological appearance, including size, shape, and color, were observed in the sampled ovaries.

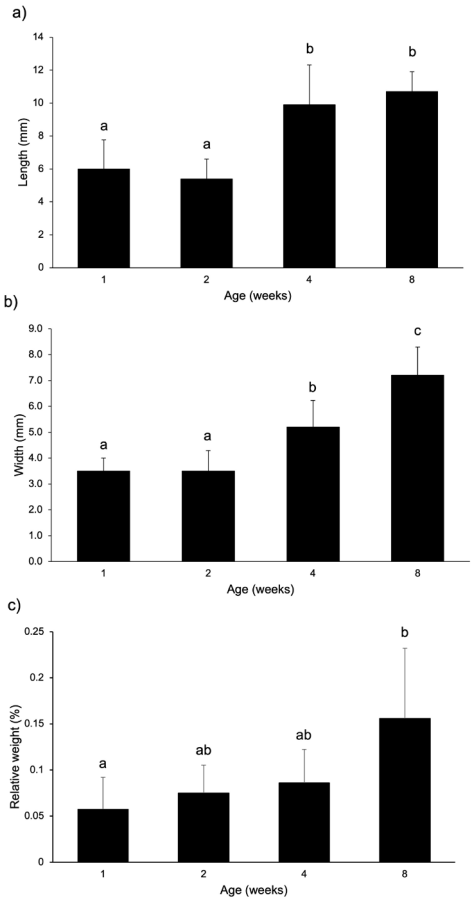

Figure 2. The (a) ovarian length (mm), (b) ovarian width (mm), and (c) relative ovarian weight (%) of MVC ovaries at 1, 2, 4, and 8 weeks of age (n = 5 per age group). Relative ovarian weight (%) is the relative weight of the ovary according to the chick's body weight. Data are presented as mean ± standard deviation. Bars marked with different letters (a, b) indicate significant differences according to age ($p < 0.05$).

p = 0.003) with age (Figure 4b). The medullary thickness decreased from 341.95 ± 155.67 µm to 102.66 ± 17.62 µm in 1-week and 8-week samples, respectively.

3.2.3. Follicle numbers

Ovarian follicles of varying sizes and developmental stages were observed in MVC chicks of different ages (Figure 5). In general, each follicle consisted of a developing oocyte with a round nucleus known as a germinal vesicle, surrounded by yolk-laden cytoplasm. In terms of prevalence, primordial follicles were the only type present in 1-week-old ovaries (Figures 6 and 7a). These follicles were distributed throughout the ovarian cortex and interspersed with stromal connective tissue, making individual follicles difficult to distinguish. Each follicle consisted of a small oocyte with a clearly visible nucleus and lightly stained cytoplasm, surrounded by a single layer of squamous pregranulosa cells. Primary follicles began to appear in 2-week-old ovaries, with a prevalence of ~ 8% (Figures 6 and 7b). They were slightly larger than primordial follicles and morphologically distinct, marked by oocyte enlargement and the presence of a single layer of cuboidal granulosa cells surrounding the oocyte. These follicles were concentrated near the cortical margin, close to the surface epithelium. Growing primary follicles were observed in 4-week and 8-week ovaries. At 4 weeks, follicles were distributed throughout most of the cortex and they displayed more structural features of early zona pellucida formation, with a prevalence of ~ 9% (Figures 6 and 7c), whereas at 8 weeks, they were interspersed across the entire cortical region (Figure 8), with a prevalence of about ~12% (Figure 6).

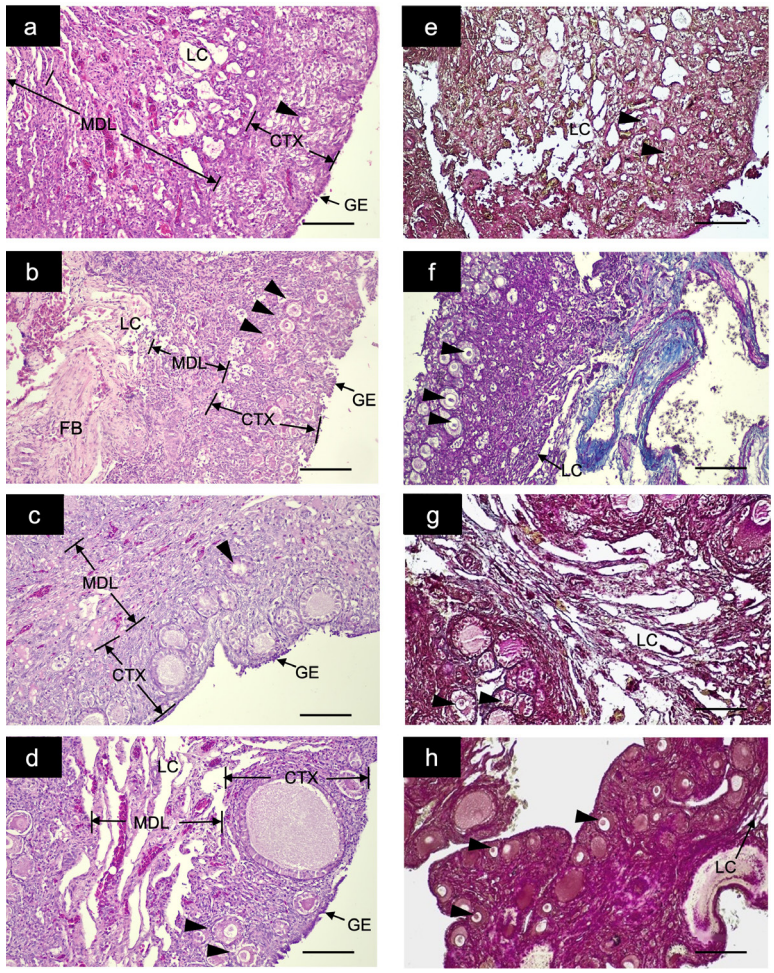

Figure 3. Representative photomicrographs of MVC ovaries at different ages: (a, e) 1 week, (b, f) 2 weeks, (c, g) 4 weeks, and (d, h) 8 weeks. Cortical thickness (CTX) was measured from the germinal epithelium (GE) and the cortex–medulla border. Fibroblasts (FB) were observed within the interstitial connective tissue. Follicles at various developmental stages are indicated in the cortex (arrowhead). Lacunae (LC) were prominent in the medullary (MDL) region of the ovaries (H&E (a–d) and Masson's trichrome staining (e–h); scale bar: 100 µm).

3.2.4. Follicle diameters

The changes in follicle diameter over time are presented in the Table. In 1-week-old ovaries, only follicles of ≤25 µm were present. The prevalence of follicles of ≤25 µm significantly decreased ($p < 0.05$) from 100% in 1-week-old ovaries to approximately 88% in 2-week-old ovaries. Follicles between >25 and ≤50 µm in size first appeared in 2-week-old ovaries, accounting for approximately 13% of all follicles. The prevalence of follicles between >25 and ≤50 µm in size continued to increase over time, reaching 16% in 8-week-old ovaries. Follicles of >75 µm were observed at 4 and 8 weeks of age, each representing less than 1%. Concurrently, the development of these larger follicles caused cortical swelling and reduced the medullary space.

4. Discussion

The development of ovaries in most birds is described as asymmetrical, with only the left ovary continuing to grow and remain functional in female chicks after hatching [24]. In this study, only the left ovary was observed in 1-week-old MVC chicks, contrasting with Hy-line brown layers, where the right ovary could still be traced at the same age [21]. Initially, both ovaries develop in female embryos [23], but the right ovary regresses over time, leaving only the left ovary in most birds [25]. This regression is associated with the absence of an estrogen receptor gene in the right ovary [26] and is considered an evolutionary adaptation in birds that optimizes flight efficiency and egg production.

The morphology of the developing ovary varies with age and across breeds. In 1-week-old Hy-line and White

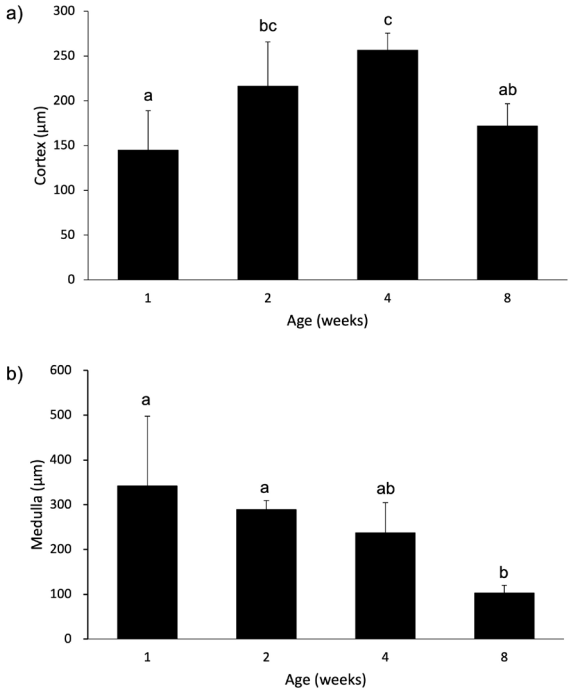

Figure 4. The (a) cortical thickness (µm) and (b) medullary thickness of MVC ovaries at 1, 2, 4, and 8 weeks (n = 5 per age group). Data are presented as mean ± standard deviation, analyzed using one-way ANOVA. Bars marked with different letters (a, b, c) indicate significant differences according to age ($p < 0.05$).

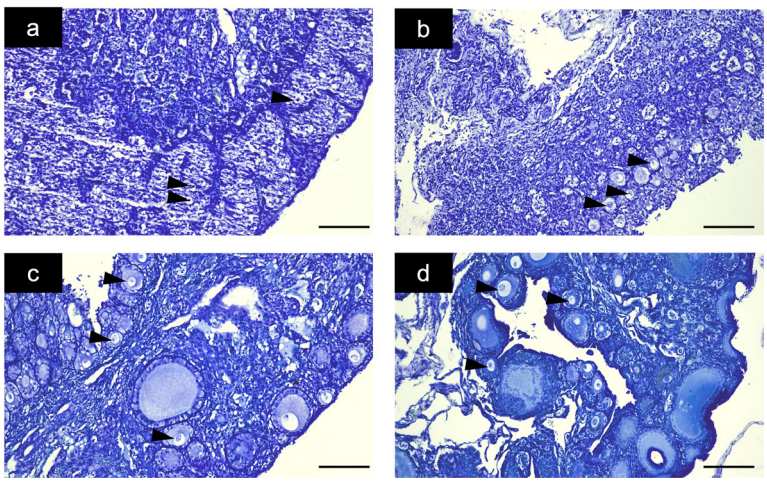

Figure 5. Photomicrographs showing ovarian follicles in MVC ovaries at (a) 1 week, (b) 2 weeks, (c) 4 weeks, and (d) 8 weeks. Oocytes are shown with the nucleus (germinal vesicle) indicated by arrowheads (toluidine blue staining; scale bar: 100 µm).

Leghorn chicks, the ovary is irregular and elongated [21,23], whereas in Alexandria chicks it is fan-shaped [22]. In contrast, the 1-week-old MVC ovary exhibited a rounded or spherical shape. As MVC chicks matured, the ovary gradually acquired a fan-shaped morphology, demonstrating that developmental timing and shape transitions may differ between indigenous and commercial breeds. The progressive changes in the ventral surface of

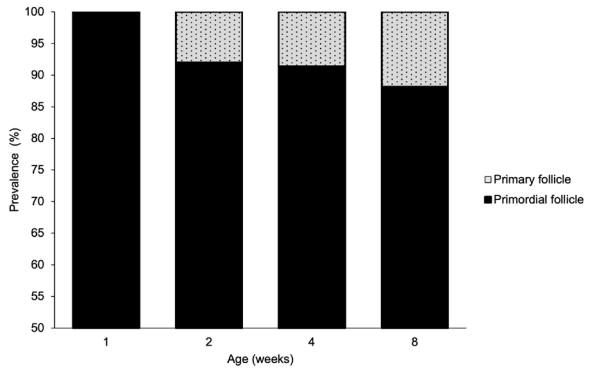

Figure 6. Prevalence of primordial and primary follicles (%) in 1-, 2-, 4-, and 8-week-old MVC ovaries.

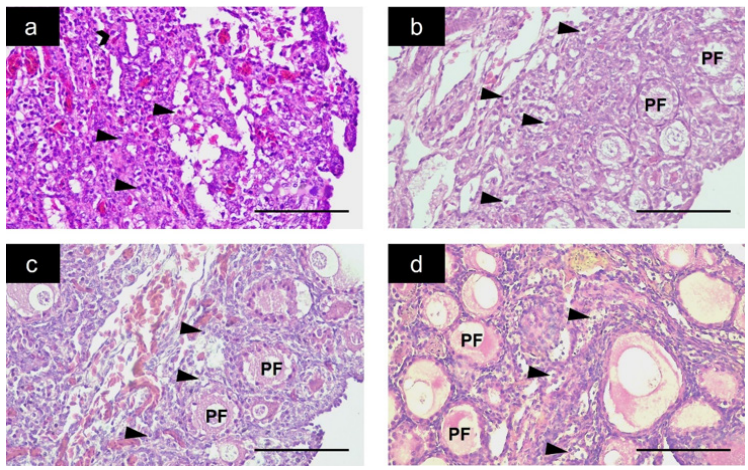

Figure 7. Representative photomicrographs showing primordial follicles (arrowheads) and primary follicles (PF) in the ovaries of MVC chicks at different ages: (a) 1 week, (b) 2 weeks, (c) 4 weeks, and (d) 8 weeks (H&E staining; scale bar: 100 µm).

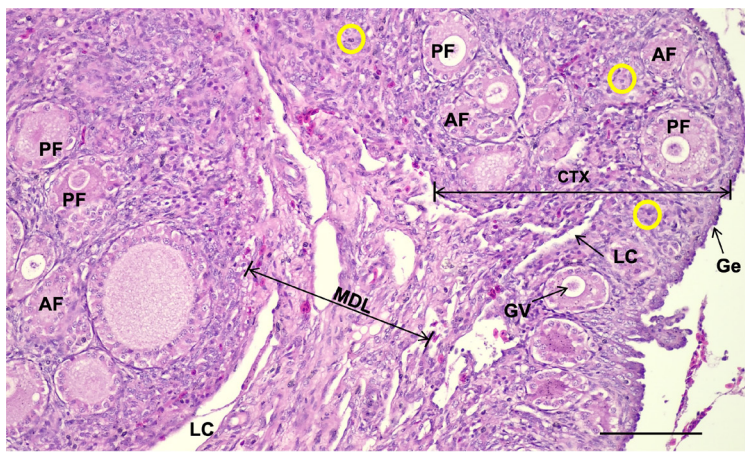

Figure 8. Higher magnification (400×) photomicrograph showing the histology of MVC chick ovary at 8 weeks of age. It consists of germinal epithelium (Ge), cortex (CTX), medulla (MDL), lacunar channel (LC), atretic follicle (AF), primordial follicle (yellow circle), primary follicles (PF), and germinal vesicle (GV) (H&E staining; scale bar: 100 µm).

Table. Diameters of ovarian follicles according to age.

Diameter (μm)	Age			
	1 week	2 weeks	4 weeks	8 weeks
≤25	200 (100%) [a]	175 (87.5%) [b, w]	178 (89%) [b, w]	167 (83.5%) [b, w]
>25 to ≤50	-	24 (12%) [x]	15 (7.5%) [x]	24 (12%) [x]
>50 to ≤75	-	1 (0.5%) [a, y]	5 (2.5%) [a, b, y]	8 (4%) [b, y]
>75 to ≤100	-	-	1 (0.5%) [y]	1 (0.5%) [z]
>100	-	-	1 (0.5%) [y]	-

Data are the mean prevalence of ovarian follicle presence in the ovarian cortex of MVC chicks with age; 200 follicles were measured randomly per replicate (n = 5 per age group).
Data were compared using the chi-square test. Data with different superscripted letters a, b, and c differ significantly between groups ($p < 0.05$), while data with different superscripted letters w, x, y, and z differ significantly within groups ($p < 0.05$).

the MVC ovary, from smooth to lobulated, correlate with growing follicles in the cortex [21]. Compared to Hy-line chicks, in which a lobulated surface appears by 4 weeks [21], this feature in MVC ovaries became prominent only at 8 weeks. Increased vascularization in the 8-week-old MVC ovary is likely essential to support folliculogenesis [27].

The MVC is a slow-growing breed, which is reflected by morphometric ovarian measurements. The weight, length, and width of MVC ovaries increased with age in this study; however, the growth rate was slower than that of commercial breeds [21,22]. For example, the ovary weight in MVC chicks increased by 252% from 1 to 4 weeks and 85% from 4 to 8 weeks, whereas in Hy-line chicks, the weight increased by 500% and 92% over the same periods [21]. Additionally, the 1-week-old MVC ovary was approximately 40% smaller in length than that of the Alexandria breed. These differences likely reflect the higher genetic potential for egg production in commercial breeds.

A significant increase ($p < 0.05$) in cortical thickness was observed in MVC ovaries during the first 4 weeks of life, likely due to progressive folliculogenesis and recruitment of primordial follicles into growing follicles. In 1-week-old MVC ovaries, 100% of follicles were ≤25 μm in diameter. By 4 weeks, ~12% of follicles were between >25 and ≤75 μm in size, and by 8 weeks, this proportion increased to ~17%. The growth of the nonstromal area in the cortex may plateau as the surface epithelium develops, as observed in bovine fetal ovaries [28]. We also suggest that atresia and apoptosis regulate the number of growing follicles, maintaining cortical thickness after a certain stage.

The ovarian medulla houses blood vessels, lymphatics, nerves, fibrous connective tissue, and smooth muscle [29]. Using Masson's trichrome staining, we observed that the cortex-to-medulla ratio decreased with age (1.7, 1.06, and 0.4 in 1-, 4-, and 8-week-old MVC ovaries), indicating a relatively thicker medulla in younger chicks. Histological changes in the medulla, including lacuna size and blood vessel distribution, likely support follicle growth and hormonal activity. Larger lacunae in more mature ovaries accommodate enlarging follicles and facilitate follicle stalk formation [29], while increased vascularization provides nutrients and oxygen to developing follicles [30].

Avian follicles are characterized by a single layer of granulosa cells [10], unlike the multilayered granulosa cells in mammals. This observation in MVCs may explain functional differences between avian and mammalian granulosa cells [31]. Primordial follicles begin recruitment immediately after hatching [32], and in 1-week-old MVC ovaries, primary follicles (≤25 μm) were the only type present in the cortex.

Histological evaluation revealed that follicle development in MVC proceeded slowly from 1 to 4 weeks, although the number of larger follicles (>25 μm) increased with age. The follicle population shifted significantly at 4 and 8 weeks, with follicles measuring >25 to ≤50 μm and >50 to ≤75 μm becoming more prominent, consistent with findings from Hy-line birds, where follicles undergo slow growth initially followed by a faster phase from 4 weeks onward [21]. Despite slow early growth, primary follicles were detected as early as 2 weeks. This onset is earlier than in other breeds, where primary follicles typically appear around 4 weeks [21,33], although some indigenous breeds, such as Uttara fowl, also show early primary follicle development (~3 weeks) [34]. This suggests that early follicular activation may occur in MVCs despite their slow growth and delayed sexual maturity at ~24 weeks [35]. Taken together, these findings suggest that early follicular activation may be an adaptive trait shared among certain indigenous or noncommercial breeds, even when overall growth and sexual maturity are delayed. However, this interpretation should be made cautiously, as interstudy differences may reflect variations in breed genetics, husbandry, or methodology. Further comparative studies are required to determine whether early folliculogenesis is consistent among indigenous breeds or specific to MVC.

Each female bird hatches with a finite ovarian reserve, reflecting the ovary's capacity to provide fertilizable eggs [36,37]. In sexually mature hens, the oocyte pool is estimated at approximately 12,000 [17,18]. Oocyte numbers are regulated via atresia, and only a few hundred are recruited into mature follicles and ovulated [38]. In this study, atretic follicles were observed in MVC ovaries of 2 weeks and older, while the consistent proportion of primordial follicles (≤25 μm; approximately 88%–89%) at 2, 4, and 8 weeks indicated stable maintenance of the ovarian reserve. Characterizing these follicular patterns in MVC improves our understanding of ovarian development in this breed and provides a foundation for further comparative studies among indigenous chickens.

5. Conclusion

The ovarian tissues of MVC chicks exhibited clear age-related morphological and histological changes, including cortical thickening, medullary remodeling, and the onset of folliculogenesis at 2 weeks after hatching. This study thus provides detailed insight into early follicular and stromal dynamics in MVCs and establishes essential baseline data to support future comparative and developmental research on indigenous avian species.

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Ethical approval

All necessary ethical protocols were diligently followed throughout the course of this study.

Author contributions

NALAR performed the experiments, collected and analyzed the data, and wrote the first draft of the manuscript. MM and ISAR contributed to data validation and manuscript revision. RMZ contributed to revising the manuscript. AHAJ conceived and designed the study, contributed to data validation, and was involved in writing and revising the manuscript. All authors read and approved the final version of the manuscript.

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