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Developmental Asymmetry of Ovary in Rhode Island Red Chicken Embryos: A Histological, Histochemical and Immunohistochemical Study

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Abstract

THE present study aimed to investigate the developmental asymmetry of the left ovary of Rhode Island Red (RIR) chicken embryos using histological, histochemical, and immunohistochemical techniques. Embryos at the following stages were examined at days: 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 19 & 21, with three embryos per stage. Serial 3-5 µm sections of whole embryos and ovaries were prepared and stained using Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Masson's Trichrome stain, along with immunohistochemical staining. The results revealed significant bilateral asymmetry, with the left ovary exhibiting a more developed cortex and medulla at all stages. The primitive left ovarian cortex was composed of germinal epithelium, germ cells, and numerous somatic cells. As development progressed, the left ovary enlarged, with an increase in the size of secondary sex cords. The medulla of the left ovary contained isolated germ cells, numerous lacunar channels, interstitial cells, blood vessels, and nerves. Immunohistochemical analysis at 14 and 18 days of embryonic development revealed estrogen receptor-positive cells in both the ovarian surface epithelium and the germ cells. Progesterone receptor-positive cells, however, were only identified in the germ cells and ovarian surface epithelium at day 18**.**

Keywords: Embryonic development, Germ cells, Immunohistochemistry, Ovarian asymmetry, Rhode Island Red chicken.

Introduction

The Rhode Island Red (RIR) chicken breed was primarily developed in the United States, specifically in Rhode Island, through crossbreeding various Asian breeds (e.g., Asian Malays) with European breeds (e.g., Italian Brown Leghorns). This breed is recognized for its dual-purpose use in both meat and egg production [1]. RIR chickens are known for their high egg production potential, with mature hens beginning to lay eggs at approximately 4 to 5 months of age and producing around 200 to 300 eggs annually [2, 3], and the incubation period for RIR eggs typically lasts about 21 days [4].

In birds, the female reproductive system is characterized by the development of only the left genital (gonadal) primordium into a functional organ, while the paired anlagen remain present but non-functional, except in birds of prey [5, 6]. Gonadogenesis, the process by which embryonic gonads are formed, involves three stages: first, the formation of the gonadal ridge; second, gonadal differentiation; and third, the onset of gonadal function [7]. Gonadal differentiation typically begins between the 5th and 7th days of the pre-hatching period, with complete differentiation into either testes or ovaries occurring between the 8^{th} and 10^{th} days [8]. During this process, the development of the right ovary occurs more slowly than the left one, results in gonadal asymmetry (Mizia et al., 2023). Estrogens are crucial for female sexual differentiation, reproduction, vitellogenesis, the development of secondary sexual characteristics in females, and the sexual differentiation of the nervous system [9]. The physiological effects of estrogens are mediated by specific nuclear receptor proteins called estrogen receptors, which bind estrogen and function as transcription factors [10]. Chicken tissues contain

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two distinct forms of estrogen receptor-α (ER-α): cER-α form I and cER-α form II [11].

Although the development of chicken gonads had been previously described (Andrews et al., 1997; González-Morán, 2011; Mfoundou et al., 2021), limited detailed histomorphological data exists regarding the developmental asymmetry of the left ovary in RIR chicken embryos. Therefore, the present study aimed to provide a detailed histological, histochemical, and immunohistochemical analysis of the developmental asymmetry of the left ovary in RIR chickens during the pre-hatching period.

Material and Methods

Egg incubation and sample collection

Thirty-nine fertilized Rhode Island Red chicken eggs were procured from Al-Azzab Farm, Fayoum Governorate, Egypt. The eggs were incubated horizontally at 37.5°C ± 2°C with a relative humidity of 52% in an incubator located in the Hatchery Unit, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, following the breed guidelines [12].

The eggs were turned manually once every 6 hrs. Embryos were dissected from the collected eggs, staged in accordance to the Hamburger-Hamilton stages (Hamburger and Hamilton, 1992), and harvested from the following embryonic period: Hamburger–Hamilton (HH) stage 23 (Embryonic day 4 "E4"), HH26 (E.5), HH29 (E.6), HH31 (E.7), HH34 (E.8), HH35 (E.9), HH36 (E.10), HH38 (E.12), HH40 (E.14), HH42 (E.16), HH44 (E.18), HH45 (E.19), and HH46 (E.21). Three embryos were collected from each stage of incubation.

During the early developmental stages, from HH23 (E.4) to HH38 (E.12), the entire embryos were collected. For embryos from HH40 (E.14) to HH46 (E.21), a mid-ventral incision was made to expose and remove the abdominal organs, after which the gonads were carefully harvested. The whole embryos (up to the 12^{th} day of incubation) and ovarian specimens, approximately 0.5 cm in thickness (from the 14^{th} day of incubation to the day of hatching), were immediately immersed in Bouin's solution for fixation overnight.

Histological procedures and staining

After dehydration in a graded alcohol series (50% to 100%), the fixed embryos and specimens were cleared in xylene for one hour, embedded in paraffin wax, and sectioned serially at a thickness of 3 µm using a rotary microtome. The sections were mounted on clean, dry glass slides and stained with Hematoxylin and Eosin (H&E) to visualize the general histological structure, Periodic Acid-Schiff (PAS) to identify neutral glycosaminoglycans, and Masson's trichrome to detect collagen and smooth muscle fibers.

The aforementioned fixatives and staining methods were applied according to [13].

Immunohistochemical staining

Immunohistochemical detection of progesterone receptor (PR) isoforms and estrogen receptor (ER) was performed using the Universal DakoL-SAB+ peroxidase kit (Dako Corporation, CA, USA). After washing and rehydrating, 4 µm thick slides were subjected to two cycles of microwave treatment at 750 W for 10 minutes each, with an interval of 10 minutes between cycles, using 10 mM citric acid (pH 6.0). Following microwave treatment, the slides were washed twice with 0.05 M sodium phosphate buffer (PBS, pH 7.4).

The sections were then incubated in 3% hydrogen peroxide for 10 minutes, followed by 0.5% Triton X-100 in PBS for 20 minutes, and 1% normal swine serum in PBS for 20 minutes. For antibody incubation, the slides were placed in a humid chamber at 4°C for 72 hours for PR and 24 hours for ER with monoclonal antibodies PgR Ab-8 or Ab-6 (for PR) or alpha-ER (ER Ab-10) (4 µg/mL) in PBS containing 0.3% Triton X-100 and 0.1% gelatin. After primary antibody incubation (rabbit antibody), sections were incubated with biotinylated secondary antibody for 30 minutes at room temperature, followed by streptavidin-peroxidase conjugate for an additional 30 minutes. After each incubation period, slides were washed twice with PBS. Peroxidase activity was visualized using the 3,3-diaminobenzidine (DAB) chromogen solution with hydrogen peroxide. Finally, the slides were counterstained with Hematoxylin, dehydrated, and mounted for observation [14].

The morphometrical measurements

The diameter of the right and left gonads, the diameter of the left cortex and medulla, the diameter and number of lacunar channels, and the number of both somatic and germ cells of the right and left ovaries at all examined ages were measured with the aid of the image J analysis software program. Microsoft Company using a LEICA (DFC290 HD system digital camera) connected to the light microscope using a 10× objective lens.

Statistical analysis

All data using SPSS Statistics for Windows (ibmspss 22; IBM) were submitted to one-way ANOVA.

Results

The current study demonstrated that the gonadal primordia (referred to as the gonadal ridge or gonadal anlage) in RIR chickens first appeared on the HH23 (E4) as a bilateral thickening of the coelomic epithelium on either side of the mesonephros (Fig. 1A), with the left side slightly thicker than the right (Fig. 1B). The boundaries of

the gonadal ridges were clearly defined from the developing mesonephros. The left gonadal ridge exhibited a stratified coelomic epithelium, and the gonadal core was composed of a few germ cells and numerous mesenchymal cells (Fig. 1C). The basement membrane between the coelomic epithelium and mesenchymal core displayed a moderate fuchsinophilic reaction, which was also observed in the germ cells (Fig. 1D).

By the HH26 (E.5) of incubation, the gonadal primordia had increased in size and appeared to protrude into the coelomic cavity with rounded boundaries (Fig. 1E). At this stage, the left indifferent gonads were covered by a layer of columnar coelomic epithelium surrounding a core of numerous mesenchymal cells and germ cells, which formed the primary sex cords (Fig. 1F). The mesenchymal cells observed were stellate-shaped, with numerous thin cytoplasmic processes, centrally located nuclei, and basophilic cytoplasm, with the cells widely separated. In contrast, the germ cells were larger, spherical in shape, with large, eccentrically located nuclei, and were arranged either sporadically or in groups composed of multiple cells.

As development progressed, by the HH29 (E.6), a notable increase in the size of the indifferent gonads was observed, with the left gonad protruding more into the coelomic cavity than the right (Fig. 2A). The left indifferent gonad was covered by a single layer of columnar coelomic epithelium, with patches of stratified epithelium in certain regions. The central core consisted of numerous undifferentiated mesenchymal cells interspersed with germ cells (Fig. 2B).

On the HH31 (E.7), the left indifferent gonads showed a significant increase in size compared to the right gonads (Fig. 2C). The left gonads were externally covered by a single layer of cuboidal coelomic epithelium, enclosing a gonadal core composed of undifferentiated mesenchymal cells and clusters of germ cells (Fig. 2D). A strong fuchsinophilic reaction was observed in the basement membrane of the gonadal coelomic epithelium, with a moderate reaction noted in the gonadal core (Fig. 2E).

By the HH34 (E.8) and HH35 (E.9), the left indifferent gonad continued to increase in size and protrude further into the coelomic cavity. At this stage, the indifferent gonads were covered by a single layer of cuboidal epithelium, and the gonadal core consisted of undifferentiated mesenchymal cells and clusters of germ cells (Fig. 3A, 3B). A strong fuchsinophilic reaction was observed in the basement membrane of the gonadal surface epithelium, with a moderate reaction in the gonadal core (Fig. 3C). Additionally, the indifferent gonadal cells were supported by fine collagen fibers (Fig. 3D).

The right indifferent gonads at HH23 (E4) to HH35 (E.9) revealed a single layer of cuboidal germinal epithelium from HH23 (E4) to HH34 (E.8) and squamous at HH35 (E.9), whereas the gonadal core was composed of few germ cells and mesenchymal cells (Fig. 4A-E). The number of germ cells was slightly increased by advanced development. The size of the right indifferent gonad was increased as the development progressed but was smaller than that of the left side at HH23 (E4) to HH34 "E.8" (Table 1).

At HH36 (E.10), a marked gonadal asymmetry between the left and right ovaries was observed, with the left ovary showing more pronounced development in the form of further increase in size and more protruding into the coelomic cavity (Fig. 5A). The left ovary was covered by a simple cuboidal ovarian surface epithelium and had a thick cortex and a thin medulla, which contained numerous lacunar spaces (Fig. 5B). The ovarian cortex featured numerous secondary sex cords, composed of clusters of germ cells and somatic cells (Fig. 5C).

At HH38 (E.12), the left ovary exhibited significant growth, primarily due to an increased number of both the somatic and the germ cells (Table 1). The cortex and medulla had become thicker. The left ovary and left mesonephros were connected by a hilum, visible within the left ovarian medulla. The number and size of the medullary lacunae had increased compared to earlier stages (Fig. 6A). The left ovarian surface epithelium was low cuboidal, and the cortex contained numerous secondary sex cords composed of germ cells and somatic cells (Fig. 6B). Fine collagen fibers were observed beneath the ovarian surface epithelium and between the ovarian cortical and medullary cells (Fig. 6C).

By HH40 (E.14), the left ovary had grown significantly larger (Table 1). An invagination of the ovarian surface epithelium was noted, and the medulla exhibited an increase in the number and size of lacunar channels. The cortex was thicker than at previous stages and contained a greater number of secondary sex cords and germ cell clusters (Fig. 7A and Table 1). The interstitial spaces between the medullary lacunar channels were occupied by germ cells and numerous somatic cells (Fig. 7B). An extensive network of collagen fibers supported the ovarian cells (Fig. 7C). Strong immunostaining for estrogen receptors was observed in the ovarian surface epithelial cells and germ cells (Fig. 7D), while weak immunostaining for progesterone receptors was detected in the germ cells and ovarian surface epithelium (Fig. 7E).

At HH42 (E.16) and HH44 (E.18), the left ovary was larger than at previous stages (Table 1). The left ovarian surface epithelium consisted of cuboidal to squamous cells, and the cortex contained numerous secondary sex cords. The medulla was composed of numerous lacunar channels and interstitial cells (Fig. 8A). Strong fuchsinophilic reactions were observed in the basement membrane of the ovarian surface epithelium, cortical secondary sex cords, and medullary lacunar channels at 18 days (Fig. 8B).

Positive immunostaining reactions for estrogen receptors (Fig. 8C) and progesterone receptors (Fig. 8D) were detected in the cortical germ cells.

At the HH45 (E.19) to the HH46 (E.21), the cortex of the left ovary showed the appearance of primordial follicles (Fig. 9A), and the medulla contained numerous lacunar channels associated with interstitial cells (Fig. 9B). Strong PAS reactions were detected in the basement membranes of the ovarian epithelium, secondary sex cords, and lacunar channels (Fig. 9C). The left ovary was supported by a network of collagen fibers located beneath the ovarian surface epithelium and between the sex cords and lacunar channels (Fig. 9D).

The right ovary at HH38 (E.12) to HH46 (E.21) showed a single layer of flattened ovarian surface epithelium. The ovary had only ovarian medulla with no cortex. The medulla was composed of numerous lacunar channels and interstitial cells composed of germ cells and somatic cells (Fig. 10A-E). The number and diameter of lacunar channels increased with the advanced development (Table 1).

Discussion

The gonad's development (testes and ovaries) is an invaluable model for researching developmental pathways because despite coming from the same primordia, they grow in two distinct ways [15]. Chicken embryos are a great model for researching cell biology and the development of embryonic organs [16, 17].

Although the gonadal development of chickens has been extensively studied, little is known about the developmental asymmetry of RIR chicken's gonad.

During the embryonic stage, the first signs of gonadal development are marked by the appearance of two thickenings of the coelomic epithelium on the ventromedial surface of the mesonephros, referred to as the gonad anlagen or genital (gonadal) ridge [18]. This gonadal ridge firstly appeared on the 4th day in chicken embryos as recorded in the current study. Gonadal ridges in chickens typically appear between the 3.5^{th} and 4.5^{th} days [19]. Similarly, it appeared at the same time point in quails [20] and chickens [21]. Other studies reported their appearance on the third day of embryonic development in chickens [22, 23], on the 3.5th day in Japanese quail [24], and in Muscovy ducks [25]. Later points of gonadal ridge appearance were also reported on the fifth day in Mallard ducks [26] and the 6th day in Rock doves [27].

During the early stages of gonadogenesis, the gonads cannot be morphologically distinguished as either testes or ovaries and are therefore referred to as "indifferent gonads" [7]. In the current study, the indifferent gonads of RIR chickens were observed to protrude into the coelomic cavity at HH26 (E.5), forming a distinct organ. Similar findings were reported [20]. However, previous studies have identified the indifferent gonads as early as HH23 (E.4) [28] and HH24 (E.4.5) [29]. Consistent with our observations in RIR chickens, the studies by [29] in chickens and [24] in quail found that the indifferent gonads consisted of an outer germinal epithelium, a thickened coelomic epithelium, and somatic cells arranged in primitive sex cords, with mesenchymal cells interspersed between them.

The current investigation demonstrated that on the HH23 (E4), the thickened coelomic epithelial cells on the left side were elongated and arranged in multiple layers. In contrast, the right side showed only a slight thickening of the coelomic epithelium, consisting of a single layer. The thickening of the coelomic epithelium served as the first indication of gonadal development (gonadal anlage), with the difference in thickness marking the initial sign of gonadal asymmetry. Similar asymmetry was observed by [28] and [30] at HH20 (E.3) and HH23-24 (E.4-4.5), respectively. In contrast, [31] reported that the development of the chicken embryonic gonads does not exhibit discernible left-right morphological asymmetry, while [32] found no left-right gonadal asymmetry during the indifferent stage (prior to sexual differentiation), with such asymmetry becoming apparent only after sexual differentiation.

The asymmetric development of the right and left ovaries has been attributed to several factors, including the asymmetric expression of the PITX2 gene and estrogen receptor alpha (ESR1). PITX2 and ESR1 are thought to inhibit cell proliferation and induce a lack of responsiveness to estrogen in the right ovary [33, 34] while promoting increased cell number, meiotic divisions, and cell proliferation in the left gonad [35]. Additionally, several genes, including BMP7, OVEX1, FET-1, and R-Spondin1, have been identified as side-biased genes, showing side-dependent expression in the developing chicken gonads [36].

Along the embryonic periods, the epithelium lining the indifferent gonads and ovaries of both sides of RIR chicken embryos differed as follows: the right side's epithelium started out as a single layer of cuboidal cells in the early stages of embryonic life and evolved into a simple squamous layer in the later stages. Conversely, the left side's epithelium started out as patches of stratified epithelium with simple columnar epithelium, followed by a single layer of columnar, cuboidal, and, in the latter stages of embryonic life, squamous cells. The current study's findings concerning the epithelium lining the gonadal ridge were different from those of Alsafy et al. (2017) in the Alexandria Chicken, who found that the right gonadal ridge was covered by stratified epithelium, while the left had a single layer of columnar cells. The timing of sex differentiation in indifferent gonads varies among bird species. For example, in quail, differentiation occurs at day 6.5 [37], while in chickens it happens at day 7 [20], at day 8 in mallard ducks [26], at day 9 in Muscovy ducks [25], at day 11.5 in emus [19], and as late as day 20 in ostriches [38]. These variations in the

timing of sexual differentiation are likely associated with differences in incubation periods; species with shorter incubation periods tend to exhibit earlier gonadal differentiation, while those with longer incubation periods show a delayed onset of gonadal differentiation.

Sexual differentiation, as observed in the current investigation, was marked by the arrangement of the left ovary into two distinct regions: the outer primitive cortex, composed of extended inward cords of gonadal epithelium known as cortical cords, and the inner medulla, which consists of medullary cords and expanded inner spaces referred to as medullary lacunae. The present study found that the left ovarian germinal epithelial cells exhibited high levels of estrogen receptor-alpha (ER-α) expression at the studied ages (14- and 18-day-old embryos). These findings are consistent with previous research by [39] and [14], who reported similar ER-α expression during early gonadal development. [40] suggested that estrogens may directly influence germinal epithelial cells through ER-α interaction and regulate the developmental growth of the left ovarian cortical tissue, which increases with age. Additionally, [41] noted that the absence of ER-α expression in the right ovary's germinal epithelial cells contributes significantly to unilateral ovarian development.

The results obtained in this study are consistent with those of [42], who detected estrogen receptor mRNA expression in the gonads of presumed male and female chick embryos at 4.5, 5.5, and 6.5 days of age. [43] also reported the presence of estrogen receptors in differentiated ovaries from 7.5-day-old embryos through to hatching. Similarly, [14] observed ER-α expression in the nuclei of the ovarian germinal epithelium, cortical germ cells of the left ovary, as well as in the medullary interstitial cells and epithelial cells of the lacunar channels in chicken embryos from 13 days of age until the end of the incubation period.

Civinini et al. (2010) reported that estrogenic activity influences various cell types in the chick ovary during the 3.5 days preceding the end of incubation. These cell types include the ovarian surface epithelial cells, cortical germ cells, parafollicular cells, as well as medullary germ cells, somatic cells, and interstitial cells. The effects of estrogen involve not only nuclear transcription but also other mechanisms that require the cytoplasmic action of the hormone.

The current results, along with previous research by [14, 44], demonstrate that both ER-α (estrogen receptor alpha) and PR (progesterone receptor) are expressed in all cortical germ cells of the left ovary. These findings suggest the involvement of estrogens and progesterone in the proliferation and development of cortical germ cells. [45] reported the production of progesterone (P4) in the chick ovary throughout the embryonic stage. Based on this secretory activity, [44] proposed that progesterone interacts with progesterone receptors (PR) to directly affect ovarian cells, with the effects being mediated by the PR-B isoform.

The appearance of primordial follicles in the developing ovary varies among bird species. In quails, primordial follicles first appear at 13 days of age [46], in chicken embryos at 14 days [23], in mallard ducks at 19 days [47], and at 21 days in chicken embryos, as observed in the RIR breed (current investigation). However, other studies report the initial development of primordial follicles occurring post-hatching, including in Alexandria chickens [48], Japanese quail [49], geese [50], Uttara chickens [51], and turkeys [52] at one day, four days, one week, and nine days of age, respectively. According to [53], during the late embryonic and early post-hatching stages, the germ cell cysts disintegrate to form individual oocytes, which are then surrounded by granulosa cells to create primordial follicles.

Conclusion

The developmental pathways of chicken gonads, particularly in the RIR breed, offer important insights into the broader mechanisms of gonadogenesis across species. The study confirmed that gonadal development begins with the formation of the gonadal ridges around day 4, leading to the differentiation of the gonads into "indifferent gonads". Developmental asymmetry between the left and right ovaries was observed. This asymmetry, along with the delayed onset of sexual differentiation, contributes to the distinct development of the left ovary. The ovarian surface epithelium and the germ cells had estrogen receptor-positive cells at HH40 (E.14) and HH44 (E.18). However, progesterone receptor-positive cells were detected in the ovarian surface epithelium and germ cells only at HH44 (E.18). These findings contribute to a deeper understanding of how gonadal development asymmetry and hormonal regulation shape reproductive organ development.

Acknowledgments

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Funding statement

This study didn't receive any funding support.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The current study was approved by the Beni-Suef Institutional Animal Care and Use Committee (BSU-IACUC) at the Faculty of Veterinary Medicine, Beni-Suef University, Egypt (BSU/FVM/2022/021-216).

TABLE 1. The morphometrical measurements of the right and left indifferent gonads and ovarian structures in Rhode Island Red Chicken Embryos

	The embryonic day	Right						left					
		Diameter/ µm	No. of germ cells/ field	No. of somatic cells/field	No. of Lacunar spaces	Size of Lacunar spaces/µm	Diameter/ µm	No. of germ cells /field	No. of somatic cells /field	No. of Lacunar spaces	Size of Lacunar spaces/ µm	Cortex/ µm	Medulla/ µm
The indifferent gonad	4	84±.0.5	2±0.002	18±0.01	-	-	85±1.405	2±0.010	21±0.0	-	-	-	-
	5	86±0.96	3±0.001	22±0.02	-	-	134±0.28	4±0.011	28±0.03	-	-	-	-
	6	147±0.7	4±0.010	27±0.01	-	-	192±1.31	7±0.021	36±0.02	-	-	-	-
	7	229±0.2	5±0.012	31±0.01	-	-	342±.2.3	10±0.02	41±0.03	-	-	-	-
	9	414±0.5	7±0.008	35±0.01	-	-	515±2.331	13±0.02	49±0.03	-	-	-	-
The Ovary	10	535±2.4	8±0.003	41±0.41	4±0.01	12±0.01	751±4.83	16±0.02	55±1.01	10±0.11	27±0.26	579±1.0	216±0.6
	12	695±2.4	11±0.00	66±0.44	8±0.02	17±0.03	1412±4.4	41±0.03	103±2.1	18±0.44	47±0.41	1076±2.	386±0.9
	14	653±2.7	14±0.00	80±0.56	11±0.03	23±0.01	1780±6.6	58±0.13	217±3.3	24±0.51	61±0.52	1105±3.	412±0.5
	16	556±2.5	15±0.00	112±1.	14±0.030	34±0.12	2130±6.9	74±0.33	335±3.8	36±0.62	78±0.63	1518±4.	566±1.0
	18	507±2.6	17±0.00	188±2.2	16±0.410	41±0.22	2528±4.5	112±0.5	487±4.7	42±0.63	91±0.86	1925±1.	612±1.3
	19	456±1.8	19±0.00	244±3.1	17±0.040	49±0.34	2628±7.2	144±0.6	618±4.9	52±0.66	102±0.6	2043±2.	649±1.7
	21	402±1.3	20±0.00	280±4.1	19±0.030	51±0.62	3014±9.1	156±0.8	988±6.8	62±0.82	123±1.0	2489±3.	731±1.9
P value		0.0105*	0.0161*	0.1126	0.0109*	0.0088*	0.0105*	0.0161*	0.1126	0.0109*	0.0088*	0.0019*	0.0019*

-Value <0.05 considered significance

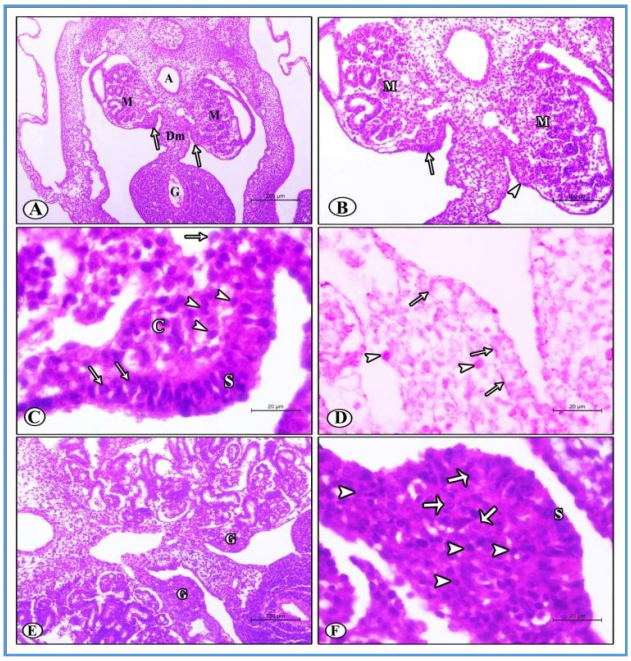

Fig. 1. Photomicrographs of 4- and 5-day-old RIR chicken embryos show: A) Gonadal anlagen (arrows) on the medial sides of the mesonephros (M), with the aorta (A), dorsal mesentery (DM), and gut (G) of the 4-day-old embryo. Stain: H&E, ×100; B) The left gonadal ridge (arrow) of the 4-day-old embryo is slightly larger than the right one (arrowhead), beside the mesonephros (M). Stain: H&E, X200; C) Higher magnification of the left gonadal ridge of the 4-day-old embryo, showing stratified epithelium (S) over a core (C) of germ cells (arrows) and mesenchymal cells (arrowheads). Stain: H&E, ×1000; D) Fuchsinophilic reaction in the basement membranes of the coelomic epithelium (arrows) and germ cells (arrowheads) of the 4-day-old embryo. Stain: PAS, ×1000; E) The indifferent gonad (G) of the 5-day-old embryo, protruding into the coelomic cavity as a distinct organ. Stain: H&E, X200; F) Germ cells (arrows), mesenchymal cells (arrowheads), and primary sex cords (stars) of the 5-day-old embryo, covered by the columnar coelomic epithelium (S). Stain: H&E, ×1000.

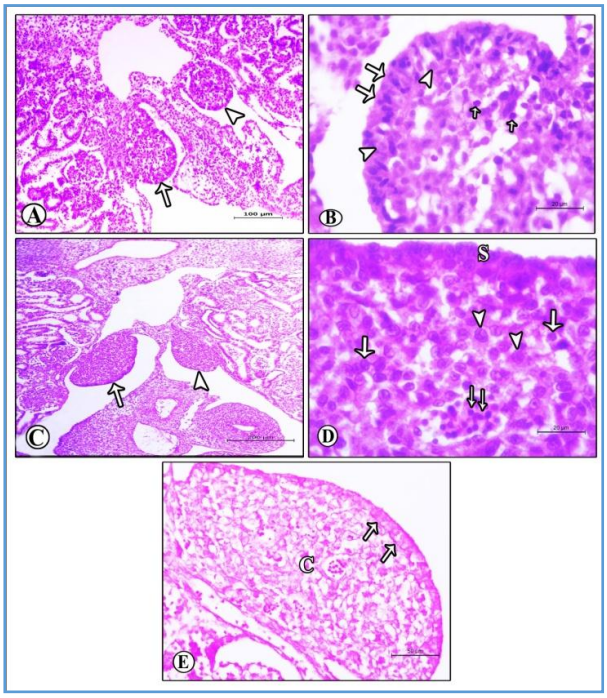

Fig. 2. Photomicrographs of 6- and 7-day-old RIR chicken embryos showing: A) A marked increase in the size of the indifferent gonad of the 6-day-old embryo, with the left side (arrow) larger than the right one (arrowhead). Stain: H&E, X200; B) The left indifferent gonad of the 6-day-old embryo is covered by a single layer of columnar coelomic epithelium, with patches of stratified epithelium in certain areas (double arrows). Numerous undifferentiated mesenchymal cells (arrows) are interspersed with germ cells (arrowheads) forming the central core. Stain: H&E, ×1000; C) A significant increase in the size of the left (arrow) indifferent gonad of the 7-day-old embryo, compared to the right (arrowhead). Stain: H&E, ×100; D) The left indifferent gonads of the 7-day-old embryo are covered externally by a single layer of cuboidal epithelium (S) enclosing a core of numerous undifferentiated mesenchymal cells (arrows) and germ cells (arrowheads). Stain: H&E, ×1000; E) A strong fuchsinophilic reaction in the basement membrane of the gonadal surface epithelium (arrows) and a moderate reaction in the gonadal core (C) of the 7-day-old embryo. Stain: PAS, ×400.

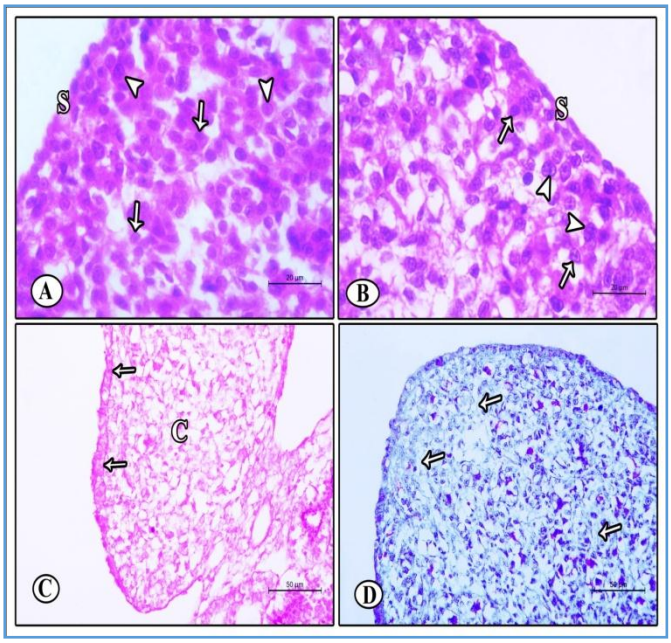

Fig. 3. Photomicrographs of 8- and 9-day-old RIR chicken embryos showing: A) The left indifferent gonads of the 8-day-old embryo, covered by a single layer of cuboidal epithelium (S), enclosing a gonadal core with clusters of germ cells (arrowheads) and undifferentiated mesenchymal cells (arrows). Stain: H&E, ×1000; B) The left indifferent gonads of the 9-day-old embryo, covered by cuboidal epithelium, with undifferentiated mesenchymal cells (arrows) and numerous germ cells (arrowheads) in the gonadal core. Stain: H&E, ×1000; C) A strong fuchsinophilic reaction in the basement membrane of the gonadal surface epithelium (arrow) and a moderate reaction in the gonadal core (C). Stain: PAS, ×400; D) Fine collagen fibers (arrows) supporting the indifferent gonadal cells in the 9-day-old embryo. Stain: Masson trichrome stain, ×400.

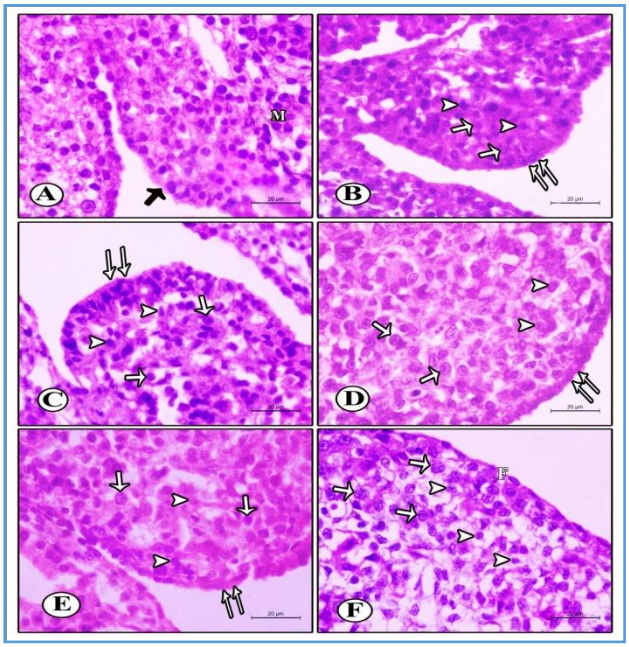

Fig. 4. Photomicrographs of the right indifferent gonads at: A) 4 days, B) 5 days, C) 6 days, D) 7 days, E) 8 days, and F) 9 days old RIR chicken embryos show: The right gonadal primordia (black arrow), mesonephros (M), cuboidal coelomic epithelium (double arrows), mesenchymal cells (arrowheads), germ cells (arrows), somatic cells (arrowheads), and a single layer of flattened coelomic epithelium (F). Stain: H&E; X1000.

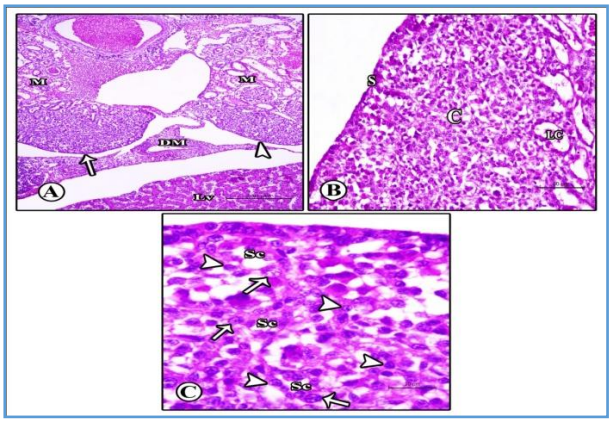

Fig. 5. Photomicrograph of a 10-day-old RIR chicken embryo showing: A) Marked gonadal asymmetry between the left (arrow) and right (arrowhead) ovaries, located on either side of the mesonephros (M), with the liver (Lv) and dorsal mesentery (DM) indicated. Stain: H&E, ×100; B) The left ovary covered by a simple cuboidal (S) ovarian surface epithelium with a thick cortex (C) and a thin medulla containing numerous lacunar spaces (LC). Stain: H&E, ×400; C) The ovarian cortex, containing numerous secondary sex cords (Sc) composed of germ cells (arrows) and somatic cells (arrowheads). Stain: H&E, ×1000.

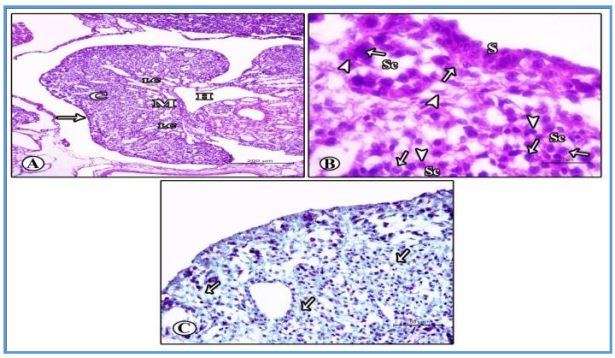

Fig. 6. Photomicrograph of a 12-day-old RIR chicken embryo showing: A) Left ovary with the ovarian surface epithelium (arrow), thick cortex (C), thin medulla (M), hilum (H), and medullary lacunae (LC). Stain: H&E, ×100; B) Ovarian surface epithelium (S), secondary sex cords (Sc) containing germ cells (arrows) and somatic cells (arrowheads). Stain: H&E, ×1000; C) Fine collagen fibers (arrows) located beneath the ovarian surface epithelium and between the ovarian cells. Stain: Masson trichrome stain, ×400.

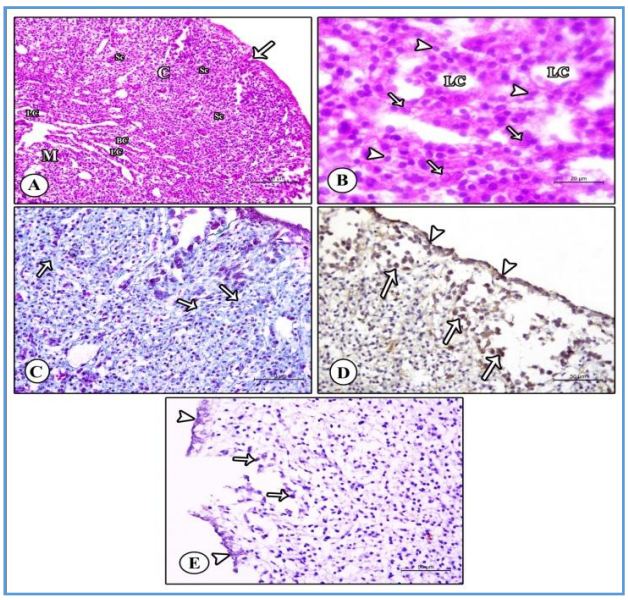

Fig. 7. Photomicrograph of the left ovary of a 14-day-old RIR chicken embryo showing: A) Invagination of the ovarian surface epithelium (arrow), thick ovarian cortex (C), secondary sex cords (Sc), ovarian medulla (M), lacunar channels (Lc), and blood capillary (Bc). Stain: H&E, X200; B) Higher magnification of panel (A) showing lacunar channels (Lc), germ cells (arrows), and somatic cells (arrowheads). Stain: H&E, ×1000; C) Extensive network of collagen fibers (arrows) supporting the ovarian cells. Stain: Masson trichrome stain, ×400; D) Strong estrogen receptor immunostaining in the ovarian surface epithelium (arrowheads) and germ cells (arrows). Immunohistochemical reaction, ×400; E) Weak progesterone receptors immunostaining reactions in the germ cells (arrows) and ovarian surface epithelium (arrowheads). Immunohistochemical reaction, ×400.

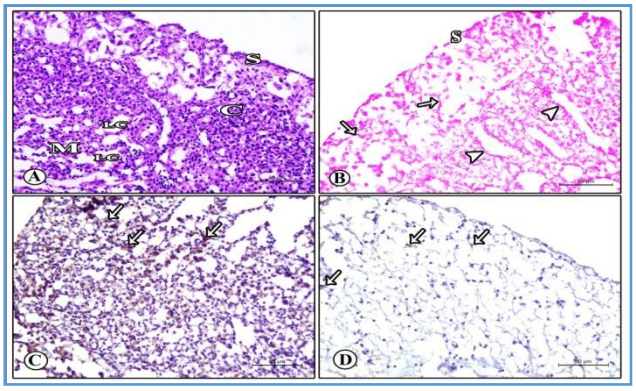

Fig. 8. Photomicrograph of the left ovary of 16- and 18-day-old RIR chicken embryos showing: A) Ovarian surface epithelium (S), cortex (C), medulla (M), and lacunar channels (Lc). Stain: H&E, ×400; B) Strong PAS reaction in the ovarian surface epithelium (S), cortex (arrows), and medullary lacunar channels (arrowheads) of 18-day-old embryos. Stain: PAS, ×400; C) Strong estrogen receptor immunostaining in the cortical germ cells (arrows) of 18-day-old embryos. Immunohistochemical reaction, ×400; D) Some cortical germ cells (arrows) showing positive progesterone receptor immunostaining in 18-day-old embryos. Immunohistochemical reaction, ×400.

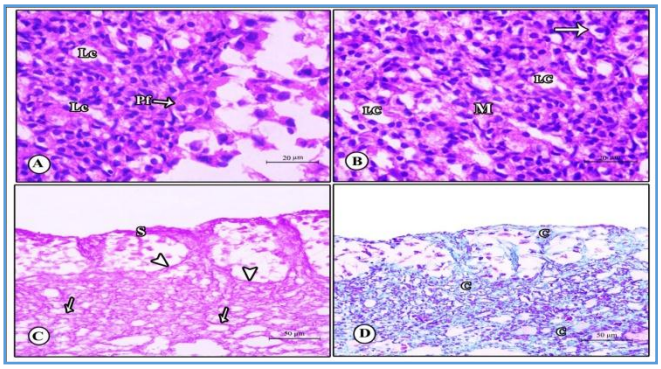

Fig. 9. Photomicrograph of the left ovary of 21-day-old RIR chicken embrYos showing: A) primordial follicle (Pf) and lacunar channels (Ls). Stain: H&E, ×1000; B) Ovarian medulla (M), lacunar channels (Lc), and primordial follicle (arrow). Stain: H&E, ×1000; C) Strong PAS reaction in the ovarian surface epithelium (S), sex cords (arrowheads), and lacunar channels (arrows). Stain: PAS, ×400; D) Extensive collagen fiber network (C) beneath the surface epithelium, around the sex cords, and between lacunar channels. Stain: Masson trichrome stain, ×400.

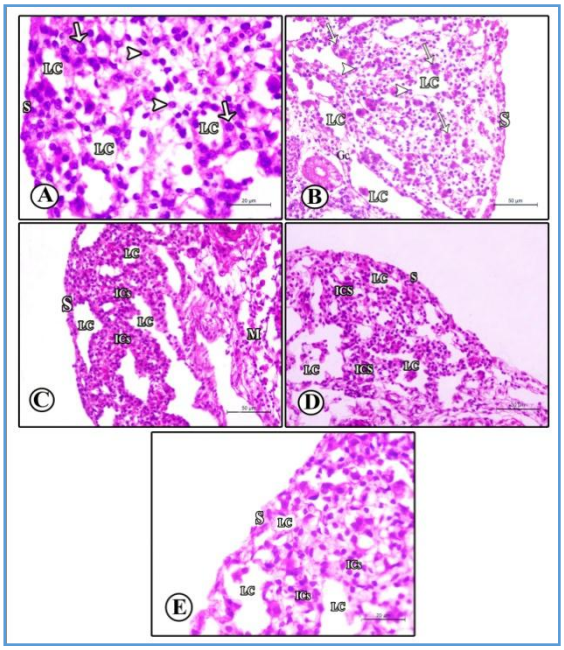

Fig. 10. Photomicrograph of the right ovary of A) 12 days, B) 14 days, C) 16 days, D) 19 days, and E) 21 days old RIR chicken embryos show: ovarian surface epithelium (S), interstitial cells (ICS), mesonephros (M), lacunar channel (Lc), germ cells (arrows), and somatic cells (arrowheads). Stain: H&E; X1000, X400, X400, X400, and X1000, respectively.

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عدم التماثل التطوري للمبيض الأيسر في أجنة دجاج رود آيلاند الأحمر: دراسة نسيجية وكيميائية نسيجية ومناعية نسيجية

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الملخص

هدفت الدراسة الحالية إلى التحقيق في عدم التماثل التطوري للمبيض الأيسر لأجنة دجاج رود آيلاند الأحمر باستخدام تقنيات الأنسجة، الكيمياء النسيجية، والكيمياء النسيجية المناعية. تم فحص الأجنة في الايام التالية :4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 19,21 فترة ما قبل الفقس وتم اخذ ثلاثة أجنة لكل مرحلة. تم تحضير مقاطع متسلسلة بسمك 3-5 ميكرومتر من الأجنة والمبايض الكاملة وصبغها باستخدام الهيماتوكسيلين والإيوسين وصبغة شيف الحمضية الدورية وصبغة ماسون الثلاثية، بالإضافة إلى الصبغ المناعي الكيميائي. كشفت النتائج عن عدم تماثل ثنائي الجانب بشكل كبير، حيث أظهر المبيض الأيسر قشرة ونخاع أكثر تطوراً في جميع المراحل. كانت القشرة المبيضية اليسرى البدائية تتكون من الظهارة الجرثومية، والخلايا الجرثومية، والعديد من الخلايا الجسدية. مع تقدم التطور، زاد حجم المبيض الأيسر، مع زيادة في حجم الحبال الجنسية الثانوية. أحتوى نخاع المبيض الأيسر على خلايا جرثومية معزولة، قنوات فجوية عديدة، خلايا بينية، أوعية دموية، وأعصاب. أظهر التحليل المناعي النسيجي في اليومين 14 و18 من التطور الجنيني خلايا إيجابية لمستقبلات الإستروجين في كل من الظهارة السطحية للمبيض والخلايا الجرثومية. على الجانب الأخر، تم تحديد خلايا مستقبلات البروجستيرون الإيجابية فقط في الخلايا الجرثومية والظهارة السطحية للمبيض في اليوم الثامن عشر.

الكلمات الدالة: التطور الجنيني، الخلايا الجرثومة، الكيمياء النسيجية المناعية، عدم التماثل المبيضي، دجاج رود آيلاند الأحمر.