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Pre-Hatching Development of the Alexandria Chicken Ovaries

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ABSTRACT

Background: The aim of this study was to explore the ultrastructure changes of the left and right ovaries at the pre-hatching period of Alexandria strain of chicken. **Methods:** One hundred fertilized egg of Alexandria breed of chicken used. The whole embryo has undergone the light microscopic examination at HH20 (E.3), HH21 (E.3.5), HH23 (E.4), HH29 (E.6) and HH34 (E.8). The ovary has undergone the light microscopic examination at HH38 (E.12) and HH42 (E.16), SEM at HH26 (E.5), HH29 (E.6), HH36 (E.10), HH38 (E.12), HH39 (E.13) and HH42 (E.16), TEM at HH38 (E.12) and HH42 (E.16). The genital ridge appeared by a thickening of the coelomic epithelium medio-ventral surface of the developing mesonephroi at HH20 (E.3). The boundaries of the undifferentiating gonads defined clearly separated from the mesonephroi. The undifferentiated gonads bulged as a distinct organ in the coelomic cavity at HH23 (E.4). **Results:** At the initial stages of the gonadogenesis, the germinal epithelium was stratified squamous epithelium. The PGCs appeared at the genital ridge at HH21 (E.3.5). The PGCs viewed at the dorsal mesentery with few microvilli and showed positive PAS reaction because of the glycogen content in their cytoplasm. The left-right gonadal asymmetry firstly detected by the number of PGCs migrating toward the left gonadal ridge more than the right at HH20 (E.3) and the macroscopic examination of gonadal asymmetry began at HH34 (E.8). **Conclusion:** The left ovary appeared a smooth rod-shape, its stroma showed lipid droplets and its parenchyma showed an extensive arrangement of interstitial cords at HH42 (E.16).

Keywords: *Ovary, Alexandria chicken, light microscopy, SEM, TEM.*

INTRODUCTION

Alexandria strain of chicken is a tetra hybrid cross mating of local Egyptian breed between Fayoumi and Barred Plymouth Rock then with Rhode Island Red and with White Leg Horn.[1,2] The fertility of Alexandria chicken reaches 88.17% and the hatchability is 76.82%. It reaches the sexual maturity at 172 days post-hatching and it characterized by high egg yield reaches about 47.4 and highest egg mass about 1987 gm.[2] Gonadogenesis is the process of embryonic gonads generation that divided into three phases: genital ridge formation, gonadal differentiation and gonadal function.[3] The gonadogenesis begins with the primordial germ cell migration.[4,5] The primordial germ cells (PGCs) are germ line stem cells that give rise into gametes in the vertebrates and they originate outside the embryo at very early stage of the development and migrate by a well-defined route into the genital ridge.[6] The PGCs are founder cells of the germ line and their descendants will form the functional gametes of the adult animal.[7] The two basic roles of the ovary are to deliver the ova and to function as an endocrine organ by the production of ovarian steroids.[8] Although vertebrates including the chicken display a superficial bilateral symmetry, most of the internal organs including the ovaries develop and locate with a consistent Left: Right (L: R) gonadal asymmetry as only the left gonad and left oviduct fully developed.[9]

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Considering the economic importance of the ovary in the egg production and the high fertility and early

maturity of Alexandria chicken attracted us to investigate the embryonic development of the ovary at the pre-hatching developmental period using the transmission and scanning electron microscopy supplemented with light microscopy.

MATERIALS ANDMETHODS

Egg incubation

One hundred fertilized egg of Alexandria chicken obtained from Abees farm that is belong to the Faculty of Agriculture, Alexandria University, Egypt. These eggs incubated horizontally at 37.5ºc ± 2ºc with 52% relative humidity until embryos reached the appropriate stages using the chick embryos staging guide.[10] The embryos harvested at various stages of development during embryonic period.

Samples

The whole embryo undergone the light microscopic examination at HH20 (E.3), HH21 (E.3.5), HH23 (E.4), HH29 (E.6) and HH34 (E.8). The ovary undergone the light microscopic examination at HH38 (E.12) and HH42 (E.16), SEM at HH26 (E.5), HH29 (E.6), HH36 (E.10), HH38 (E.12), HH39 (E.13) and HH42 (E.16), and TEM at HH38 (E.12), HH42 (E.16).

Collection of embryo

Immediately before procedures carried out, eggs sprayed with 70% ethyl alcohol. The cracked and their content transferred to sterile, clean and dry petri dish. The embryo and overlying vitelline membrane very gently peeled away from the yolk and the older embryos obtained after cutting the main umbilical blood vessels. The embryos transferred to a petri dish containing sterile phosphate buffer saline (PBS) then the embryos washed and removed by small spatula and transferred to 2 ml sterile Eppendorf tube. The smaller embryos from HH20 (E.3) until HH36 (E.10) wholly collected but after these stage from HH38 (E.12) until HH42 (E.16), the mid-ventral incision made and the abdominal organs removed then the female gonads dissected and separated from the embryos, washed in PBS and transferred into 2 ml Eppendorf tube.

Light microscopic examination

The samples were fixed in 10% buffered neutral formalin for 48 hrs., and then dehydrated in ascending grades of ethyl alcohol.[11] They were cleared in xylene and embedded in three changes of paraffin. The paraffin blocks were cut at 4 µm thickness and stained by Harris hematoxylin and eosin for general studies, Periodic acid Schiff technique (PAS) for the demonstration of PGCs and Crossmon trichrome stain for demonstration of connective tissue and muscles.[12,13]

Scanning electron microscopic examination (SEM)

The specimens were immersed in a fixative (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2) at 4°C. Once fixed, the samples were washed in 0.1 M sodium cacodylate containing 5% sucrose, processed through tannic acid, and finally dehydrated in graded ethanol series for 15 minutes in each (50%, 70%, 80%, 90%, 95% and 100%). The samples were critical point dried in carbon dioxide, attached to stubs with colloidal carbon and coated with gold palladium in a sputtering device. Specimens examined and photographed with JEOL 5300 JSM scanning electron microscope operating at 25 K.V. at the Faculty of Science, Alexandria University.

Transmission electron microscopic examination (TEM)

Small cubes (1 mm3) from 12 days, 16 days old chicken embryonic left ovary were fixed in 6% solution of phosphate-buffered glutaraldehyde, pH 7.4, at 4°C for 6 hrs.[14] After initial fixation, tissues washed in several changes of cold (4°C) 0.1 M phosphate buffer every 15 min for 2 hrs. Samples were then rapidly dehydrated through increasing concentrations of ethanol, transferred to propylene oxide and placed over-night in a 1:1 mixture of propylene oxide and epoxy araldite. Semi-thin sections (1 mm) were first cut, stained with toluidine blue and viewed with light microscopy to specify areas suitable for transmission electron microscopy. Then ultrathin sections (60-100 nm) were then cut by a glass knife with an L.K.B. microtome and stained with uranyl acetate followed by lead citrate. The ultrathin sections were examined with JEOL transmission electron microscope operating at 100 Kv. at the Faculty of Science, Alexandria University.

RESULTS

Genital ridge formation

HH20 (E.3) was the beginning of the gonad development that started with the gonadal ridge formation that firstly appeared as rod-shape structures of tiny bilateral thickening of coelomic epithelium at the thoraco-lumbar region at the level of the urogenital ridge that divided into the lateral urinary ridge where the developing mesonephroi located and the medial genital ridge [Figure 1]. Nevertheless, by HH21 (E.3.5) the gonadal ridge became more thick and obvious. The gonadal ridge located at the medio-ventral surface of the developing mesonephroi and near the base of the dorsal mesentery on its both sides. The undifferentiated gonads appeared firstly as paired rod-shape structures with the left gonad slightly larger than the right one. The boundaries of the undifferentiating gonads were well defined and separated from the developing mesonephroi

[Figure 2]. At HH23 (E.4), the undifferentiating gonads protruded into the coelomic cavity as a distinct organ with rounded boundaries [Figure 5]. The primary sex cord of the undifferentiating gonads developed from the migrating PGCs together with the coelomic epithelial cells that are two of the cellular components of the early developing gonads.

The cellular components of the gonadal ridge and early developing gonads

The coelomic epithelial cells at the basal lamina of the gonadal ridge form the germinal epithelium. They are simple columnar epithelium at the left gonadal ridge and stratified squamous epithelium at the right gonadal ridge [Figure 3]. The mesenchymal cells are stellate shape cells with thin cytoplasmic processes, basophilic cytoplasm and centrally located nucleus. These cells have wide intercellular spaces between each other [Figure 3]. One or two cells of RBCs found in the area of the gonadal ridge coming from the adjacent blood vessels [Figure 9]. These RBCs had the characteristic morphology of the avian RBCs, which are elongated cells with clear eosinophilic cytoplasm. The nucleus is spherical in shape and centrally located with clear mitotic figures [Figure 3].

Primordial germ cells (PGCs)

By light microscopic examination

PGCs firstly observed at the gonadal ridge at HH21 (E.3.5). By H&E staining technique, PGCs appeared as densely staining large spherical cells as solitary cells or as a mass composed of several cells. These cells had a large spherical eccentric nucleus and a large amount of glycogen that partially masking the dense granular cytoplasm [Figure 3]. The glycogen content of PGCs gave a positive reaction with PAS staining technique. Therefore, these cells stained red except the nucleus. However, the surrounding coelomic epithelial cells were smaller give negative PAS reaction, and did not stain red [Figure 4]. The majority of PGCs presented at the gonadal ridge and few cells observed at the dorsal mesentery at HH21 (E.3.5) [Figure 4]. However, more cells appeared in both gonadal ridge and dorsal mesentery at HH23 (E.4) [Figure 7].

By the SEM examination

At HH38 (E.12) PGCs were large and surrounded by smaller germinal epithelial cells. The cortical PGCs were large spherical cells (8-14 micrometer in diameter) with few short atypical microvilli [Figure 30]. However, the medullary PGCs were smaller, irregular spherical in shape and surrounded by medullary lacunae [Figure 31].

By the TEM examination

At HH38-42 (E.12-16), PGCs were large spherical cells with a large spherical eccentric nucleus that showed chromatin condensation [Figure 32&38].

At HH38 (E.12), more than one mitochondria investigated at the cytoplasm of the PGCs as well as few number of small size smooth and rough endoplasmic reticulum. Some cells contained one centriole and the medullary germ cells not contained any centrioles. No Golgi apparatus observed at this stage of development [Figure 32]. Tight junctions observed between the PGCs and each other. However, tight junctions and desmosomes observed between the PGCS and the adjacent somatic cells [Figure 32].

At HH42 (E.16), the number of the mitochondria increased more than at the previous age. PGCs at this stage of development contained large-size smooth and rough endoplasmic reticulum. Unlike the previous age, the cytoplasm of the PGCs contained Golgi apparatus as well as one pair of centrioles [Figure 38].

Gonadal Asymmetry

The gonadal asymmetry between the left and right ovary firstly appeared at HH20 (E.3).

By light microscopic examination

The gonadal asymmetry firstly appeared at HH20 (E.3) when the thickening of the coelomic epithelium toke a wide range at the left side than the right side of the gonadal ridge [Figure 1].

At HH21 (E.3.5), the proliferation of the coelomic epithelium at the gonadal ridge, which will be the germinal epithelium in further developmental stages, was somewhat more in the left gonadal ridge than the right one [Figure 2]. At the same stage of development (E.3.5) when PGCS began to migrate to the gonadal ridge, the number of the migrating cells to the left gonadal ridge was more than to the right one [Figure 4&7].

At HH23 (E.4), with the increase of the migration of PGCs to the gonadal ridge, the unequal arrival of PGCs to the left and right indifferent gonad was more obvious. At the same stage of development (E.4) the difference in the size between left and right indifferent gonad was more prominent with increasing the left indifferent gonad than the right one [Figure 8] The increasing in size of the indifferent gonad was back to the increase of the proliferation of the germinal epithelium that became stratified columnar epithelial cells in the left indifferent gonad. However, the germinal epithelium at the right indifferent gonad remained stratified squamous epithelium [Figure 6].

By SEM examination

The scanning electron microscopy observations revealed the presence of the gonad by this technique began at HH29 (E.6) and not observed at HH26 (E.5) [Figure 9].

At HH29 (E.6), the left and right ovaries appeared on the ventromedial surface of the left and right mesonephroi with left ovary cranial than the right one that appeared medial. The abdominal aorta separated between the both left and right ovaries. The left ovary

was taller (6.4m length) and wider (0.88m width) than the right ovary that estimated 4.4m length and 0.6m width [Figure 10]. The epithelia covering the right ovary appeared as hexagonal shaped cells with fewer rounded pilli [Figure 11]. However, the epithelia covering the left ovary were rounded cells with the presence of rounded pilli [Figure 12]. At HH36 (E.10), the left ovary was taller (6.5m length) and wider (1m width) than the right ovary that estimated 4.6m length and 0.78m width [Figure 13]. The epithelia covering the right ovary was more globular with many rounded pilli [Figure 14]. However, the epithelia covering the left ovary was more flat cells with few pilli [Figure 15].

At HH38 (E.12), the left ovary viewed taller (6.8m length) and wider (1.1m width) than the right ovary that estimated 5m length and 0.58m width. The right ovary was medial to the right mesonephros while the left ovary was dorsolateral to the left mesonephros [Figure 16]. The pilli was many and small oval elevations over the surface of the right ovary [Figure 17]. However, the epithelia covering the lateral surface of the left ovary contained few number of large ovoid pilli [Figure 18].

At HH39 (E.13), the L-R ovarian asymmetry in the size became more obvious and clear with the very small size right ovary (5m length and 0.58m width) and large size left ovary (10m length and 2.5m width) [Figure 19]. The epithelia covering the right ovary partially lost its globular shape and became flattened cells with few number dome shape pilli [Figure 20]. However, the epithelia covering the left ovary were more globular with many small size sandy shape pilli [Figure 21].

At HH42 (E.16), the L-R ovarian asymmetry appeared obvious than at HH39 (E.13). The right ovary estimated 5.7m length and 1.3m width and occupied the cranial part of the right mesonephros. while, the left ovary estimated 12m length and 3.6m width and extended from the cranial pole of the left mesonephros to near its caudal pole along its dorsolateral surface [Figure 22]. The characters of the epithelia covered the both ovaries were the same as HH39 (E.13) except the pilli covering the right ovarian surface was few, small and rounded pilli [Figure 23]. However, the pilli at the left ovarian surface increased in the size than the pilli on the surface of the left ovary of the previous age [Figure 24]. The pilli present on the ovarian surface of both ovaries were many on the lateral ovarian surface than the medial surface.

Sexual differentiating stage

The undifferentiating gonads enter a stage of sexual differentiation between male and female gonads firstly at HH29 (E.6).

The gonadal function stage

At HH29 (E.6), the left ovary showed a thick ovarian cortex and medulla that faintly separated from each other. The left ovarian cortex covered by the germinal epithelium and contained the cells of the secondary sex cord [Figure 25]. The germinal epithelium was a thick layer of cuboidal epithelial cells that enclosed many PGCs. The secondary sex cord composed of two types of the cells, somatic cells as well as PGCs that enclosed between the somatic cells. The identification between the two cell types occurred through the large size and spherical shape of the PGCs than the somatic cells. The PGCs had the same characters of the same cells at the undifferentiating gonads except for the PGCs of the definitive ovary had a clear cytoplasm and lost its positive PAS reaction. The PGCs of the undifferentiating gonads had granular cytoplasm and positive PAS reaction because of the presence of glycogen granules in the cytoplasm. The cortical PGCs increased in number by the advanced development and appeared mainly in clusters. The parenchyma of the left ovary contained interstitial cells with dense connective tissue and few numbers of small size lipid droplets as well as small blood vessels containing avian RBCs. The medulla of the left ovary showed lacunar spaces (unfilled space) and some of these lacunae were spherical. Some of the medullary lacunae contained PGCs, which were involved in the formation of the primary sex cord, and these PGCs had the same profile of the cortical PGCs. The left ovarian medulla showed a hilum that was a stalk connected the left ovary with the left mesonephros. Some small lacunae resemble the medullary lacunae observed beneath the cortex [Figure 26].

At HH38 (E.12), the left ovary showed a thicker cortex than the left ovary of HH29 (E.6) with a higher density of germ cells that viewed as single cells or in cells clusters. The density of the cells of the secondary sex cord increased because of increase both somatic and germ cells in both size and number. The left ovarian medulla showed more lacunae as well as lacunar channels. The parenchyma of the left ovary showed mesenchymal cells that viewed as single cells or clusters of mesenchymal cells [Figure 27,28]. The parenchyma also showed interstitial cells that arranged in interstitial cords and involved lipid droplets that increased in size and number than the previous age [Figure 29].

At HH42 (E.16), the left ovary showed a well-defined thick cortex and medulla. The germinal epithelium at the cortex composed of one layer of cuboidal epithelial cells enclosed many germ cells in the cortex. The interstitial cells arranged as interstitial cords at the left ovarian parenchyma. Also, the ovarian parenchyma showed small blood vessels [Figure 35,36]. A thin layer of primitive tunica albuginea of loose vascularized connective tissue noted between the cortex and the medulla and projected into the medulla. The medullary lacunae of HH42 (E.16) left ovary increased in the size than the previous age with germ cells at the wall of this lacunae [Figure 37].

Gross anatomy of the embryonic ovary

The embryonic ovary appeared as a whitish creamy rod-shape with a smooth surface. The left ovary estimated 6 mm length and 2 mm width. While, the right ovary estimated 3 mm length and 1 mm width and this marked the regression of the right ovary. The left and right ovaries located at the thoraco-lumbar region hanged at the dorsal body wall. They bounded dorsally by the mesonephric kidney and separated from each other by the last two thoracic vertebrae and first three lumber vertebrae that represented the medial boundaries of left and right ovary. Laterally, left and right ovaries bounded by the last two ribs and abdominal air sacs. The left ovary related; cranio-ventrally by the caudal end of the esophagus, ventrally by fusiform proventriculus and gizzard that located caudo-ventral to the left ovary. The right ovary related ventrally with the right lobe of the liver and caudo-ventrally by the small intestine. At the pre-hatching period, there was no relation between the embryonic ovaries and the lung [Figure 33-34].

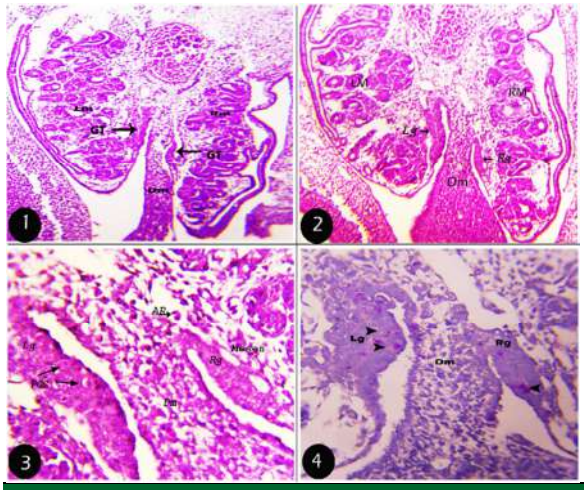

Figure 1: Photomicrograph showing the bilateral thickening of the coelomic epithelium to form the gonadal ridge (GT) at 3 days old chicken embryo. Dorsal mesentery (Dm), Left mesonephros (Lm), Right mesonephros (Rm). [H&E, Mag. X10]. (2): Photomicrograph showing the left gonadal ridge (Lg) and the right gonadal ridge (Rg) at the medio-ventral surface of the mesonephroi (LM &RM) at 3.5 days old chicken embryo. Dorsal mesentery (Dm). [H&E, Mag. X10]. (3): Photomicrograph showing the cellular component at the left genital ridge (Lg) and the right genital ridge (Rg) with its stratified squamous epithelium (black arrow) of 3.5 days old chicken embryo. Primordial germ cell (PGC), Mesenchymal cells (Mesen), Avian RBCs (AR), Dorsal mesentery (Dm). [H&E, Mag. X100]. (4): Photomicrograph showing the migrating PGCs (arrowhead) into the left gonadal ridge (Lg) and the right gonadal ridge (Rg) at 3.5 days old chicken embryo. Dorsal mesentery (Dm). [PAS, Mag. X10]

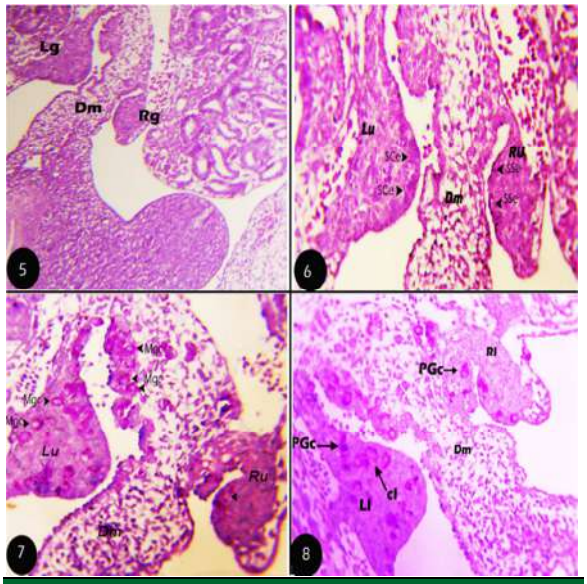

Figure 5: Photomicrograph showing the bulging of the left genital ridge (Lg) and the right genital ridge (Rg) into the coelomic cavity of 4-day-old chicken embryo with more rounded boundaries. Dorsal mesentery (Dm). [H&E, Mag. X10]. (6): photomicrograph showing the L: R gonadal asymmetry at the level of germinal epithelium as it clear that it was stratified columnar epithelium (SCe) at the left undifferentiating gonads (LU) and still squamous epithelium(SSe) at the right undifferentiating gonad (RU) of 4 days old chicken embryo.[PAS, Mag. X40]. (7): Photomicrograph showing the increase the migratory germ cells (arrowhead) through the dorsal mesentry (Dm). Left undifferentiated gonad (Lu), right undifferentiated gonad (Ru). [PAS, Mag. X40]. (8): photomicrograph showing the L: R gonadal asymmetry at the level of migrating (PGC) at the left indifferent gonad (LI) more than those migrating to the right indifferent gonad (RI) in 4 days chicken embryo. Cluster of PGCs (CL), dorsal mesentery (Dm). [PAS, Mag. X40]

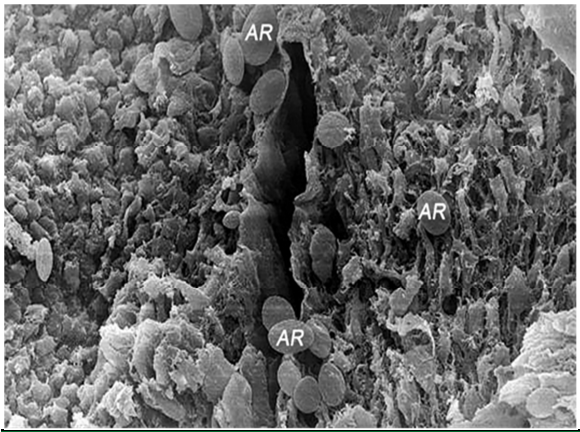

Figure 9: scanning electron micrograph (SEM) at the gonadal region of 5 days old chicken embryo. Avian RBCs (AR). [Mag. X2000]

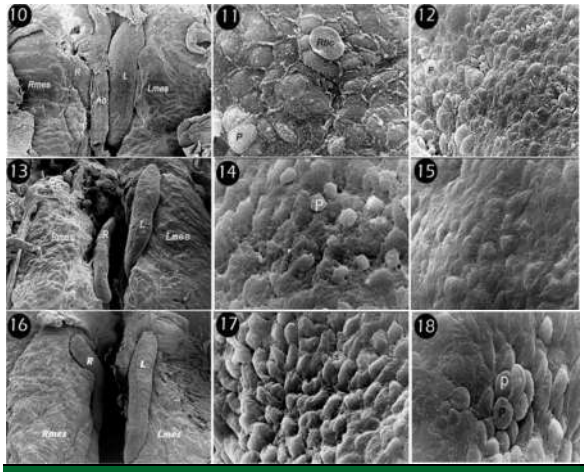

Figure 10: scanning electron micrograph (SEM) showing the L:R gonadal asymmetry between the left ovary (L) and the right ovary (R) of 6 days old chicken embryo. Right mesonephros (Rmes), left mesonephros (Lmes). [Mag. X100]. (11): scanning electron micrograph (SEM) showing the covering epithelium of the right ovarian surface of 6 days old chicken embryo containing Pilli (P). Red blood cell (Rbc). [Mag. X5000]. (12): scanning electron micrograph (SEM) showing the covering epithelium of the left ovarian surface of 6 days old chicken embryo. Pilli (P). [Mag. X5000]. (13): scanning electron micrograph (SEM) showing the L: R gonadal asymmetry between the left ovary (L) and the right ovary (R) of 10 days old chicken embryo. Right mesonephros (Rmes), left mesonephros (Lmes). [Mag. X100]. (14): scanning electron micrograph (SEM) showing the covering epithelium of the right ovarian surface of 10 days old chicken embryo containing rounded Pill (arrowhead). [Mag. X5000] (15): scanning electron micrograph (SEM) showing the covering epithelium of the left ovarian surface of 10 days old chicken embryo. [Mag. X5000]. (16): scanning electron micrograph (SEM) showing the L: R gonadal asymmetry between the left ovary (L) and the right ovary (R) of 12 days old chicken embryo. Right mesonephros (Rmes), left mesonephros (Lmes). [Mag. X100]. (17): scanning electron micrograph (SEM) showing the covering epithelium of the right ovarian surface of 12 days old chicken embryo containing numerous Pilli. [Mag. X5000]. (18): scanning electron micrograph (SEM) showing the covering epithelium of the left ovarian surface of 12 days old chicken embryo containing ovoid Pilli (P). [Mag. X5000]

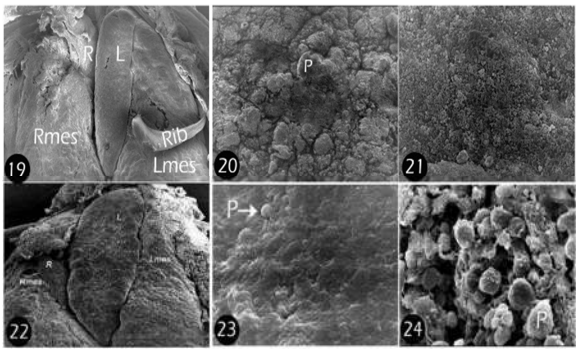

Figure 19: scanning electron micrograph (SEM) showing the L: R gonadal asymmetry between the left ovary (L) and the right ovary (R) of 13 days old chicken embryo. Right mesonephros (Rmes), left mesonephros (Lmes). [Mag. X100]. (20): scanning electron micrograph (SEM) showing the less globular right ovarian surface with few dome shape pilli (P) in 13 days old chicken embryo. [Mag. X5000]. (21): scanning electron micrograph (SEM) showing the more globular left ovarian surface with numerous small size and shape Pilli in 13 days old chicken embryo. [Mag. X5000]. (22): scanning electron micrograph (SEM) showing the L: R gonadal asymmetry between the left ovary (L) and the right ovary (R) of 16 days old chicken embryo. Right mesonephros (Rmes), left mesonephros (Lmes). [Mag. X100]. (23): scanning electron micrograph (SEM) showing the more flattened right ovarian surface of 16 days old chicken embryo with few little size rounded pilli (P). [Mag. X5000]. (24): scanning electron micrograph (SEM) showing the numerous large size mammalian Rbcs like pilli at the left ovarian surface of 16 days old chicken embryo. [Mag. X5000]

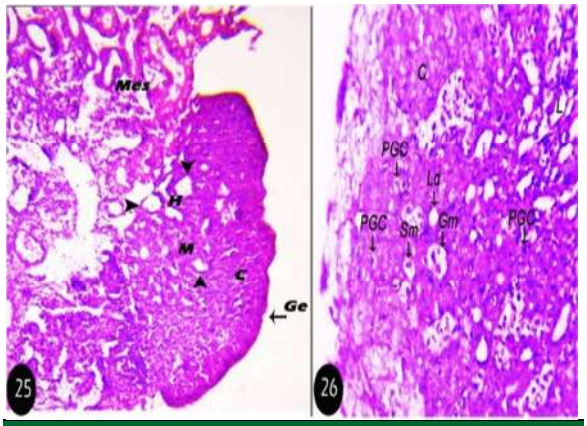

Figure 25: photomicrograph showing the differentiated left ovary of 6 days old chicken embryo. Cortex (C), Germinal epithelium (Ge), Medulla (M), Hailum (H), Lacunae (arrowhead). Mesonephros (Mes). [H&E, Mag. X10]. (26): photomicrograph showing the left ovarian cortex of 6 days old chicken embryo. PGCs (black arrow), somatic cells (arrowhead), lipid droplets (Ld), Avian RBCs (AR), interstitial cords (IC), lacunae (L). [PAS, Mag. X40]

DISCUSSION & CONCLUSION

The genital ridge began to appear by a thickening of the coelomic epithelium at HH20 (E.3).[7,15,16] In contrary Fargeix, Didier noted the genital ridge appeared at HH13 (E.2),[17] Sekido and Lovell-Badge marked that it appeared at HH23 (E.4) and Al-Saffar and Ab.Abood claimed the genital ridge appeared at 7-8 days old duck embryo.[9,18] The genital ridge located at the medio-ventral surface of the developing mesonephroi near the base of the dorsal mesentery.[16,18]

The current study revealed the boundaries of the undifferentiating gonads were well defined and separated from the mesonephroi. While Chang, Chen found the undifferentiated gonads shared the same parenchyma with the kidney tube suggesting that,[19] some kidney cells shared in the gonadal formation. The undifferentiated gonads bulged into the coelomic cavity at HH23 (E.4) as a distinct organ. The same results approved by Fargeix, Didier in chicken,[17] Intarapat and Satayalai in quail.[16] While Guioli, Nandi observed that at HH24 (E.4.5),[8] Sekido and Lovell-Badge found that at HH26 (E.5).[9] At the initial stages of the gonadogenesis, the germinal epithelium was stratified squamous epithelium, while Carlon and Stahl explained that,[20] at the initial stages of the development, the epithelial ridge composed of columnar cells. The primary cord of the undifferentiated gonads composed of peritoneal epithelial cells as well as PGCs, similar findings by Hoshino, Koide in chicken and Al-Saffar and Ab.Abood in duck.[18,21]

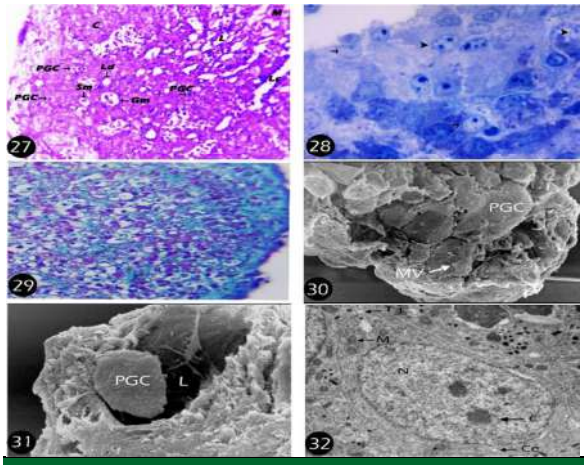

Figure 27: photomicrograph showing the left ovary of 12 days old chicken embryo. Cortex (C), lipid droplet (Ld), solitary mesenchymal cell (Sm), group of mesenchymal cells (Gm), medulla (M), lacunae (L), lacunar channel (Lc). [PAS, Mag. X40]. (28): photo micrograph showing the distribution of the PGCs in the left ovarian cortex of 12 days old chicken embryo as a solitary cells (black arrow) or in cell clusters (arrow head).[Toluidine blue, Mag. X10000]. (29): photomicrograph showing the density of the interstitial cords at the left ovarian cortex of 12 days old chicken embryo. [Trichrome, Mag. X40]. (30): scanning electron micrograph (SEM) showing the cortical PGC with its short atypical microvilli (Mv) at 12 days old chicken embryo. [Mag. X1500]. (31): scanning electron micrograph (SEM) showing the medullary PGC at the medullary lacunae by a cross section made in the left ovary of 12 days old chicken embryo. [Mag. X3500]. (32): transmission electron micrograph (TEM) showing the ultrastructure of the PGC at 12 days old chicken embryo. Nucleus (N), mitochondria (M), chromatin condensation (C), centriole (Ce), tight junction (TJ). [Mag. X4000]

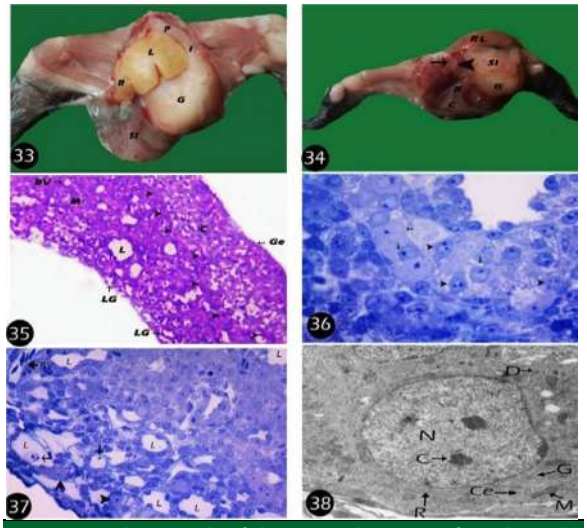

Figure 33: photograph of the visceral organs of 16 days old chicken embryo showing proventriculus (P), Ismuth (I), gizzard (G), small intestine (SI), and the left lobe of the liver (L) over the left ovary and the right lobe of the liver (R). (34): photograph showing the left ovary (arrowhead) and the right ovary (black arrow) of 16 days old chicken embryo after reflecting the viscera. Right lobe of liver (RL), small intestine (SI), gizzard (G), rectum (R), cloaca (C). (35): photomicrograph showing the left ovary of 16 days old chicken embryo. Cortex (C), Germinal epithelium (Ge), tunica albuginea (arrow head), blood vessel (BV), medulla (M), lacunae (L), medullary germ cells at the wall of the lacunae (LG).[PAS, Mag. X40]. (36): Photomicrograph showing the distribution of the PGCs in the left ovarian cortex of 16 days old chicken embryo as solitary cells (black arrow) or in cell clusters (arrowhead). [Toluidine blue, Mag. X10000]. (37): photomicrograph showing the left ovarian medulla of 16 days old chicken embryo. Blood vessel (BV), medullary lacunae (L), germ cells (arrowhead), PGCs inside the lacunae (black arrow). [Toluidine blue, Mag. X400]. (38): transmission electron micrograph (TEM) showing the ultrastructure of the PGC at 16 days old chicken embryo. Nucleus (N), mitochondria (M), chromatin condensation (C), centriole (Ce), desmosome (D), rough endoplasmic reticulum (R), Golgi apparatus (G). [Mag.X4000]

The PGCs appeared at the genital ridge at HH21 (E.3.5) in chicken.[22] In contrast to Hoshino, Koide observed the PGCs at the genital ridge of the chicken embryo at HH24 (E.4.5) and Méndez,[21] Carrasco observed it at HH26-28 (E.5-6) in chicken.[23] The PGCs observed at the dorsal mesentery and showed positive PAS reaction because of the glycogen content in their cytoplasm while Armengol,[24,25] Carretero recorded the quail PGCs were PAS negative because of absence of glycogen in their cytoplasm.[26] By SEM, the PGCs had few microvilli,[27] while, England and Matsumura stated the PGCs covered by many microvilli.[28] The PGCs and somatic cells had tight junction and desmosomes between them, these junctions important for nutrient,

gaseous and energy transmission from somatic cells to PGCs.[18]

The left and right gonadal asymmetry detected at HH20 (E.3),[17,29] different from Carlon and Stahl mentioned the L: R gonadal asymmetry appeared at HH23-24 (E.4-4.5).[20] The L: R gonadal asymmetry detected by the number of PGCs migrating toward the left gonadal ridge more than the right where the left gonadal ridge produce a chemotactic for PGCs more than that produced by the right one.[30] The macroscopic examination of gonadal asymmetry began at HH34 (E.8), in the same line with Rodríguez-León, Esteban in chicken, different from Al-Saffar and Ab.Abood in duck embryo this because of the different the incubation period between the chicken and duck.[18,31] The undifferentiated gonads enter the stage of sexual differentiation at HH29 (E.6),[32] while, Smith and Sinclair revealed the sexual differentiation in chicken embryo occurred at HH23 (E.4),[33] Sekido and Lovell-Badge at HH31 (E.7),[9] González□Morán at HH34 (E.8).[29] While Intarapat and Satayalai noted the sexual differentiation stage in the quail embryo at days 5-7 of the development.[16]

The differentiated left ovary composed of an outer cortex and inner medulla separated from each other by a thin layer of connective tissue that form the primary tunica albuginea.[18,29] The stroma of the left ovary showed lipid droplets and have a role of a steroid production.[29,34] The left ovarian parenchyma at HH42 (E.16) showed an extensive arrangement of interstitial cords, while González□Morán stated that at HH39 (E.13).[29] However, this extensive density of the interstitial cords were because of the formation of the theca layers around the oocyte at the stage of the folliculogenesis at the post-hatching period. The PGCs appeared at the medullary lacunae of the medulla of the left ovary and it might be apoptosis.[27] The left embryonic ovary was smooth and rod in shape, while Nickel, Schummer stated the embryonic left ovary was smooth and triangular in shape.[35]

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