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RESEARCH ARTICLE

Histomorphological study of the pre hatching development of the female genital system in Indigenous Mallard Duck (Anas platyrhynchos)

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Manuscript Info Abstract	
Manuscript History:	The current study is endeavored and intended to obtain principal data on the
Received: 12 August 2014 Final Accepted: 25 September 2014 Published Online: October 2014	histomorphological developmental changes in the female genital system of Mallard ducks in Iraq. Twenty females of Mallard ducks are used to monitor the embryonic changes may occurs during the following pre-hatching periods: 7-8 th , 14 th and 19 th day.
Key words:	Results revealed that the intermediate cell mass of mesoderm is the primordia of the reproductive organs and their ducts. Gonads are developed
Mallard, duck, ovary ontogeny, oviduct ontogeny, development	at the median face of the mesonephros. Paramesonephric duct of mesonephros undergoes morphological changes ended by oviduct formation
*Corresponding Author	which derived from the peritoneal covering of mesonephros subsequently converted into urogenital ridge. In 7-8 th day old embryo, the left and right ovaries revealed undistinguished cortex and medulla, whereas in 14 th day old
F. J. Al-Saffar	embryo, the left ovary is significantly enlarged, whereas in 14 day old embryo, the left ovary is significantly enlarged, whereas, the right is small and rudimentary. At the anterior region of urogenital ridge both oviducts are observed, but the left is completely fused tube and invaded the tissue of the left mesonephros, while right tubal ridge remain not fused yet. In middle region of the ridge the left and right tubal ridges are fused. In caudal region of the ridge, the left and right tubal ridges are still not fused and they attached to the lateral border of the left and right metanephros. In 19 th day old embryo, the left ovary is extensively enlarged with obvious tunica albuginea separating cortex from the medulla. In this period an important developmental changes are recognized in the regions of the oviduct.

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Introduction

Ducks are well known for their attractive egg's size and their highest resistance against different avian diseases (Jalaludeen *et al.*, 2004; Patki and Lucy, 2012). Ducks and other species such as geese and swans are members of the aquatic bird family (*Anatidae*) which is classified as one of the three families of the order *Anseriformes* (waterfowl) (Bruce *et al.*, 1992; Johnsgard, 2010). Approximately 50 species of ducks are well identified in the genus *Ansa* which is one of the important genera of the family *Anatidae*. These species are characterized by their compact bodies with thick subcutaneous fat coat which enable them for better swimming and movement in the water environment. The latter trait plays a role in preserving body temperature during bird diving in the water (Phillips, 1915).

Geographically, Mallard ducks are distributed mainly in the wetlands and marshlands throughout the various parts of world. For breeding and during winter season most of Mallard ducks are migrated toward the warm parts of the world (Peters *et al.*, 2004).

The Mesopotamian marshlands in Iraq are one of the most important sites of several migratory birds. If restored, they will become once again a vital strategic stop-over site for millions of water birds migrating between breeding areas in northern Russia and Africa (UNEP, 2005). Scott and Rose (1969) recorded about 10 sites in Iraq for their international importance for ducks and geese. Haur Al Hammar and Haur Al Hweaza are the most familiar once of these sites. According to survey conducted in 1979, these sites support more than 1% of the populations of six species of ducks, including nearly 60,000 Common Teal (*Anas crecca*) The latter species are dependent as a main source of protein for the population in southern parts of Iraqi such as Misan, Thi-quar and Al-Basraha governorates.

Embryologically, during a period up to 27–28 days of hatching, the ducks oviduct is derived from the left Mullerian duct. The female gonads are paired, accordingly the bird have two Mullerian ducts and during embryonic development only the left oviduct undergoes continuous growth to form the laying oviduct, whereas the right one is entirely regressed (Johnson, 1986).

Anatomically, the avian oviduct is long convoluted organ in completely developed and mature birds and it is divided into five apparent regions, that are infundibulum which forms a strong perivitelline membrane around the egg yolk, the magnum which is responsible for the synthesis and secretion of the albumin, the isthmus which forms a fibrous membrane around the egg white, the uterus which forms the egg shell, and finally the vagina which is last part of the oviduct that helps in pushing the egg out (Draper *et al.*, 1968; Nickel *et al.*, 1977 and Yaniz *et al.*, 2006). The maturation of oviduct plays an important role in the production of eggs (Kelany *et al.*, 1993) and histologically it composed of a collection of exocrine glands which synthesizes and secretes albumen, carbohydrates and ions that form an egg mass (Draper and Davidson, 1970). It is characterized by an internal fertilization feature which is facilitating storage of spermatozoa in specialized region that is located at the junction of the vagina and uterus (Froman, 2003).

To our knowledge, there is no obtainable embryological study to date on the histomorphological as well as histochemical development changes in the female genital system of the Mallard ducks in Iraq. Accordingly, the current research was endeavored and intended to investigate the development of the female reproductive system in the Mallard duck during pre-hatching (embryonic) period with special attention to the features that distinguished early duck ontogeny.

Materials & Methods

Bird's preparation and study design

Twenty females of Mallard ducks are obtained by hatching their eggs in the incubator for three various periods in order to gain the required pre-hatched embryos that are 7-8th day (10n), 14^{th} days (5n) and 19^{th} day (5n) (Fig. 1).

Technique of embryos preparation and preservation

The egg shell was incised transversely using fine pointed scissor and most of remaining albumen was eliminated and the required embryo was obtained. By using the scissor, the embryo was incised by making a circle on its surrounding membranous tissue, leaving it at the center. Using blunt forceps the embryo was pulled out and the adherent extra-embryonic membranes were removed. After that embryos were well rinsed by using tap water. Following through washing, the embryo transferred and kept in a clean container containing 8% formalin for three days. Then the formalin solution poured off and the specimen rinsed again in the tap water. Finally, specimen was placed into 70% alcohol as a final preservative agent containing 3 to 4 drops of glycerin (glycerin keeps the specimen softer and the skin translucent in ages of pre-hatching period).

Histological procedures of embryos preparation

After well fixation the embryos' were dehydrated by passing them through a series of ascending alcohol each for two hours (30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) and then specimens were cleared in xylene for one hours after that embedded in paraffin wax for one hours and then sectioned serially at 5μ m thickness and stain with Hematoxylin and Eosin stain (Luna, 1968).

Results and discussion

Microscopic examination revealed that the intermediate cell mass of mesoderm is the primordial for development of the longitudinally oriented thickened urogenital ridge. The ridge is responsible for the development of the pronephros, mesonephros, metanephros as well as the reproductive organs and their ducts. The gonads are situated on the median face of the mesonephros from which they subsequently raised. The paramesonephric duct (Mullerian duct) of the mesonephros underwent morphological changes ended by the formation of the oviduct. The oviduct is derived as in-folding of the coelomic peritoneal epithelium of the urogenital ridge covering the mesonephros.

Current microscopic findings showed that the early female gonads (ovaries) are developed from the median surface of mesonephros as a ventromedial thickening of urogenital ridge. Whilst their oviducts are developed as a dorso-lateral thickening of the mesonephros. The urogenital ridge is running longitudinal structure initiated from the pronephros and ended at the metanephros in the pelvic region, thus according to the changes that is observed, the developed oviduct divided into three regions. The first or anterior region which is located at the levels of the first half of mesonephros that possessed the ovary on the median surface, whereas, the oviducts and mesonephric duct are found on the lateral surface near the dorsal angle of the celom as well as proventriculus and gizzard. The second or middle region which is located at the second half of the mesonephros and extended into the first half of the metanephros and possessing the ends of the gizzard and liver. The third or caudal region which is located at the level of end metanephros toward the pelvic region and possessing the ends of liver and intestine.

Evolutionary ontogeny at 7-8th day old embryos Ovary ontogeny at 7-8th day old embryos

At this age, the left and right ovaries appeared as prominent thickening and laying between the base of the dorsal mesentery and the first half of the mesonephros. These embryonic ovaries revealed undistinguished cortex and medulla (Fig. 2). The established thickening developed from the peritoneal epithelium that could be called germinal epithelium. The thickened mass of epithelial cells invades the median surface of the mesonephros and lead to the projection of this mass into the celom. The parenchyma of the embryonic ovaries is structured from two types of cells that are the peritoneal and primordial germ cells. The latter germ cells are significantly larger than those of the peritoneal cells and characterized by wide clear cytoplasm forming the primary sexual cords (Fig. 3).

The current findings revealed early development of both left and right ovaries in the Mallard ducks before the 7th day of incubation and such observation was approximately similar to those observed previously by Wolf (1979) in 6 to 7 days-old embryos of quails and ducks, whereas, the ovaries differentiated and developed earlier (before 4-5 days of incubation) in the embryos of Gallus domesticus (Gonzalez -Moran, 2011) and in chick embryos too (Dennis, 1936). In another aspect, ovaries of Mallard ducks is developed as a thickening at the base of the dorsal mesentery and mesonephros which was similar also to their development in the above mentioned species. Actually, at this period the epithelial and parenchymal structures of the left ovary of Mallard was as those described by Lillie (1952) and Dennis (1936) at 5 to 5.4 days of incubation, whereas the left ovary of the 8th day old chick embryos showed well distinguished and separated cortex from the medulla (Gonzalez-Moran, 2011).

Oviduct ontogeny at 7-8th day old embryos

In the embryos of eight days old, the left and right oviducts appeared short and they did not seen at the level of anterior end of mesonephros, mid-part of the gizzard and end of proventriculus. While at the level of middle region of urogenital ridge, the oviducts appeared as incomplete tubes of thickened cellular structure called tubal ridges attached to latero-ventral border of the left and right mesonephros adjacent to the lateral body wall (Fig. 4). At the level of

posterior region of urogenital ridge, they were seen as a crescent thickening of peritoneal epithelial cells which lined the dorsal body wall (Fig. 5). This outcome revealed that the origin of the left and right oviducts was started in an anterior-posterior direction.

The present findings revealed that, the primordial ridge of the oviduct in Mallard duck appeared as a thickening of the dorsal body wall's lining peritoneum which observed at mid part of future oviduct. This finding was in parallel with those observed by Gonzalez –Moran (2011) at 4-5 days old and Lillie, (1952) at 4 days old chick embryos. It suggests that initiated thickening appeared first at the mid part of the embryonic oviduct then their subsequent growth toward the anterior direction and then to the posterior direction of urogenital ridge.

Morphological ontogeny at 14th day old embryos Ovary ontogeny at 14th day old embryos

At this developmental period, the left and right ovaries appeared larger than those observed at 8th day aged embryos, but the left ovary was larger than the right one. The left ovary showed an initial cortex which characterized by an increase in the thickened germinal epithelial layer that composed of two cellular layers; the outermost cells layer was vertically arranged formed of growing cells of the peritoneal epithelium, the inner layer was a layer of germ cells which intermingling germinal epithelial and formed primary sex cords, the sex cords appeared migrated from the inner surface of the germinal epithelium toward sub epithelial tissue to form a primitive tunica albuginea which separated the initial cortex from parenchyma of ovary. The parenchyma of the left ovary showed a series of sexual cords which resulted from extended the primary sex cords, thus, the primary sex cords become centrally located and formed the initial medulla which showed increased of primordial germ cells population. The right ovary appeared smaller in size and the germinal epithelium layer was rudimentary or lacked and the parenchymal tissue showed few primordial germ and peritoneal cells (Fig. 6).

The present developmental differentiation of the left ovary displayed at this period an initial cortex and medulla in addition to the appearance of the primitive tunica albuginea which was similar to those observed at the 9^{th} day of incubation in chick embryos (Gonzalez -Moran, 2011) and at 9^{th} -10th days old white Pekin ducks (Lewis,1946). But at 13^{th} days of incubation, the left ovary of chick embryos displayed lacunars medulla (Gonzalez -Moran, 2011).

Oviduct ontogeny at 14th day old embryos

Both oviducts appeared at the level of anterior region of urogenital ridge. The lips of the left tubal ridge (primordial of the left oviduct) fused and invaded the tissue at the lateral borders of the left mesonephros whereas; the right tubal ridge showed more invaginated and not fused yet. At the level of middle region of the urogenital ridge, the left and right tubal ridges of oviduct appeared fused and invaded the tissue at the lateral borders of the left and right metanephros. At the level of posterior region of the urogenital ridge and within the pelvic region the left and right tubal ridges were not fused and attached to the lateral border of the left and right metanephros (Fig.7). The present result revealed that, this stage most lips of tubal ridge were fused in the early formed region of the oviduct and started to fused during subsequent developmental stages.

Morphological ontogeny at 19th day old embryos Ovary ontogeny at 19th day old embryos

At this stage of development, the left ovary characterized by the enlarged size and showed well-differentiated cortex which was well separated from the medulla by the tunica albuginea. The cortex was packed with the clusters of primordial follicles which surrounded by secondary sex cords and few of lacunae. The primordial follicles formed of large Oocytes which possessed large nucleus surrounded by single layer of cuboidal granulosa cells. The granulosa cells were enclosed by flattened pre-granulosa cells of spindle-shaped nucleus and scanty cytoplasm. The other cortical cells were consisted of interstitial sex cords that filled the cortical stroma. The germinal epithelium that covered the cortex was reduced into single layer of superficial cuboidal epithelial cells (Fig. 8). The underlying tunica albuginea which separated the cortex from medulla was a layer of well vascularized loose connective tissue with solitary clusters of primordial germ cells. The medulla appeared well-vascularized with enlarged lacunae. The germ cells were scattered

as solitary cells at the region of tunica albuginea which was projected into the medulla (Fig. 9). The right ovary appeared smaller in size showed ceased developed cortex and reduce sized medulla (Fig. 8). The left ovary of Mallard ducks at 19th day old embryos showed definite cortex with its developed follicles and ideal medullary region, whereas previous findings of Haffen (1975) in 14-15 days old chick embryos, described the cortex content as an epithelial and germ cells cords only. The development of medulla region in Mallard was similar to those recorded by Gonzalez - Moran, (2011) in 8th day old chicken embryos. This observation indicated that the development in chicken embryos was clearly earlier compared to those found in the Mallard.

Oviduct ontogeny at 19th day old embryos

Microscopic examination of 19th day's old embryos revealed morphological developmental changes occurred in the anterior, middle and caudal regions of the oviduct. At the anterior region of urogenital ridge throughout the level of mesonephros and proventriculus ends, only the left oviduct was observed at this stage of development. It was appeared as tiny tube of mesenchymal cells situated on the left side of the coelomic cavity next to the left dorsal body wall in relationship with both left mesonephros and proventriculus, whereas the microscopic examination revealed absence of the right oviduct at this level (Fig. 9). At the level of the distal end of the proventriculus, the part of left oviduct that corresponded its cranial end appeared completely surrounded by the peritoneal membrane nearby to the newly differentiating cranial end of the oviduct which developed from of peritoneum. This newly differentiated cranial end showed low epithelial folds surrounded by sub epithelial mesenchymal tissue and thin layer of smooth muscle fibers, all were enclosed by peritoneal membrane (Fig. 9). At the level of mid gizzard, the left oviduct appeared thick tube with narrow elliptical lumen and measured $97.8 \pm 0.02 \mu m$ in diameter. The mucosal lining of the left oviduct was stratified epithelium characterized by darkly stained nuclei and eosinophilic cytoplasm. The sub epithelial tissue composed of densely packed mesenchymal cells with few capillaries (Fig.10). The dorsal and ventral oviductal ligaments were morphologically differentiated and appeared as extensions of the sub epithelial mesenchymal tissue. The tube of the oviduct was enclosed with cells similar to those lined the body cavity, characterized by large and darkly stained flattened epithelial cells (Fig. 10). At the level of middle region of urogenital ridge (distal end of gizzard) the right oviduct appeared as regressed tube of mesenchymal cells positioned between the right mesonephros and the cranial ends of pelvic bones (Fig.11), this gave rise the thought that the regression of right oviduct started interiorly and continued toward the posterior direction. The middle and caudal regions of oviduct revealed the oviduct was extended from the first half of metanephros toward the cloaca within pelvic region and both oviducts appeared as bilateral tubes of mesenchymal tissue. They were located on the left and right sides of coelomic cavity next to the left and right lateral body walls and the oviduct measured about $140\pm0.03\mu$ m in diameter (Fig. 12). The current findings revealed incomplete regression of the right oviduct at this stage of development.

In conclusion, the current findings revealed that post 7^{th} days of incubation is considered the onset time for development of the reproductive system and the mesodermal intermediate cells mass is the primordia of the reproductive organs and their ducts. At 14^{th} day old embryo, the primitive tunica albuginea is formed as well as initial medulla. At 19^{th} day old embryo, the left ovary was greatly enlarged and showing well separated cortex from medulla and the cortex is covered by simple cuboidal epithelium and composed of clusters of the primordial follicles surrounded by interstitial sex cords while the medulla is composed of enlarged lacunae channels.

Oviducts post 7th days of incubation appeared as incomplete thickened tube located at latero-ventral border of the left and right mesonephros. At 14th day old embryo both oviducts showed various stages of fused tubal ridge (primordium of oviduct) in different regions of urogenital ridge, but left tubal ridge is appeared as completed fused tube and invade the left mesonephros as early as. The processes of fusion are starting from the cranial ends of tubal ridge toward the caudal ends (from the mesonephros toward the metanephros). At 19th day old embryo, both oviducts were completed their development as tiny tubes of mesenchymal cells, but the right one underwent regression.



Fig.1: Gross appearance of embryos of Mallard ducks during different embryonic developmental periods that are post 7 days (A), 8 days (B), 14 days (C) and 19 days of incubation (D).



Fig. 2: Transverse section through the anterior mesonephros of 8th day old embryo. It showed the spinal cord (Sc), dorsal mesentery (Dm), aorta (Ao), left mesonephros (Lm), right mesonephros (Rm), left ovary (black arrow) and right ovary (red arrow), H&E.



Fig. 3. Left gonad of the 8th day-old embryo showed the glomerulus (G), germinal epithelium (Ge), parenchymal tissue (Pt), primordial cells (black arrows), primary cords (blue arrows) and peritoneal epithelial cells (red arrows), H&E.



Fig. 4.The 8th day-old embryo. Upper left panel: transverse section through anterior mesonephros and mid gizzard of the urogenital ridge. It showed the spinal cord (Sc), dorsal mesentery (Dm), aorta (Ao), gizzard (G), liver (Li), celom (C), right mesonephros (Rm) and left mesonephros (Lm). Upper right panel: Transverse section of the right and left tubal ridge through the end of the mesonephros showed spinal cord (Sc), dorsal mesentery (Dm), right mesonephros (Rm), left mesonephros (Lm), aorta (Ao), liver (Li), right tubal ridge (black arrow) and left tubal ridge (red arrow). Lower panel: Higher magnification the pointed right & left oviducts in the right panel showed tubal ridge of oviduct (black arrows), kidneys secreting tubules (st), kidneys collecting tubules (ct) and posterior cardinal vein (PCV), H&E.



Fig. 5. Transverse section of left tubal ridge through the posterior region of urogenital ridge of the 8th day old embryo. It showed the spinal cord (Sc), dorsal mesentery (Dm), aorta (Ao), right metanephros (black arrow), left metanephros (red arrow) and thickening of left tubal ridge (magnified area of the rectangle), H&E.



Fig. 6. Transverse section of left & right ovaries through the anterior mesonephros of the 14th day old embryo (upper panel). It showed dorsal mesentery (Dm). celom (C). left mesonephros (Lm) right mesonephros (Rm), left ovary (Black arrow) and right ovary (red arrow). Lower panels: Magnified right and left ovaries showed outer layer (1), inner layer (2), zoon of thickened germinal epithelium (Ge), primordial germ cells (black arrows), initial medulla (Im) and initial cortex (Ic), H&E.



Fig.7. The 14th day old embryo. Upper left panel: Transverse section of the left oviduct through the ends mesonephros. It showed spinal cord, (Lm) left mesonephros (Sc), right mesonephros (Rm), left tubal ridge (Black arrow), right tubal ridge (red arrow), gizzard (G) and liver (Li). Upper right panel: Transverse section through the anterior metanephros. It showed kidneys secreting tubules (st), kidneys collecting tubule(ct), glomerulus (g), invaded left oviduct (black arrow) and invaded right oviduct (red arrow). Left lower panel: Transverse section of tubal ridge through end of metanephros. It showed right metanephros (Rm), left metanephros (Lm), left tubal ridge (black arrow), right tubal ridge (red arrow). kidneys secreting tubules (st), kidneys collecting tubules (ct), celom (C). aorta (Ao). glomerulus (g), H&E.



Fig. 8.The 19 days old embryo. Upper panel: Transverse section of the left & right ovaries. It showed left ovary (red arrows). right ovary (black arrow), cortex (C). and medulla (M), H&E. Lower left panel: Left ovary. It showed cortex (C). tunica albuginea (ta), medulla (M), primordial follicles (P). lacunae (L) and germinal epithelium (black arrow). Lower right panel: A primordial follicles in the cortex (rectangle). It showed nucleus (N). germinal epithelium (black arrows), granulosa cells (red arrows) and pregranulosa cells (blue arrows), H&E.



Fig. 9.The 19th days old embryo. A. Ovarian medulla revealed tunica albuginea (ta), blood vessels (v), lacunae (L) and primordial germ cells (black arrow). B. Transverse section through the end of proventriculus & mesonephros showed vertebra (V), right mesonephros (Rm), left mesonephros (Lm), aorta (Ao), proventriculus (Pr), body wall (Bw), regressed cranial end of left oviduct (red arrow) and newly developed cranial end (black arrow). C. magnified left oviduct red arrow in B which is the newly developed cranial end showed epithelial folds (E), smooth muscles (Sm), subepithelial tissue (Se), peritoneal tissue (P). D. Regressive end of oviduct showing epithelium (E), ligament (Dl), sub-epithelial mesenchymal tissue (Se), peritoneal tissue (red arrows) and incomplete lumen (blue arrow), H&E.



Fig.10: Transverse section of left oviduct through mid gizzard of 19 days old embryo (upper panel). It showed spinal cord (Sc), vertebra (V), right mesonephros (Rm), left mesonephros (Lm), aorta (Ao), gizzard (G). body wall (Bw), abdominal air sac (As), celom (C) and oviduct(arrow). Left panel: magnified of the left oviduct (rectangle) showed epithelium (E), subepithelial mesenchymal tissue (Sc), lumen (L), dorsal ligament (DL). ventral ligament (VL), capillaries (black arrows) and flattened cell of serosa (red arrows), H&E.



Fig. 11.The left and right oviducts through the end of gizzard of 19 days old embryo (upper panel). It showed spinal cord (Sc), vertebra (V), right mesonephros (Rm), left mesonephros (Lm), gizzard (G), abdominal air sac (As), celom (C), left oviduct (black arrow) and regressive right oviduct (red arrow). Both left & right oviducts are magnified in the lower panel where it showed oviduct (ov), left Mesonephros (Lm), right mesonephros (Rm), celom (C), dorsal ligament (black arrow) and ventral ligament (red arrow).



Fig. 12. The left & right oviducts through middle and caudal regions of urogenital ridge of the 19 days old embryo (upper panel). It showed spinal cord (Sc), vertebrae (V), right metanephros (Rm), left metanephros (Lm), rectum (R), abdominal air sac (As), left oviduct (black arrow) and right oviduct (red arrow). Both left & right oviducts are magnified in the lower panel where it showed epithelium (e), sub epithelial tissue (Se), dorsal ligament (Dl), ventral ligament (Vl) and blood vessels (black arrows), H&E.

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