PRE HATCHING DEVELOPMENTAL HISTOLOGICAL CHANGES OF THE TESTISIN MALLARD DUCK (*ANAS PLATYRHYNCHOS*)

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ABSTRACT

The current study was aimed to investigate the development of testis during pre-hatching periods to diverse the components of the testis in duck embryo from embryonic day 5 to 19 consequently; fifteen males' of mallard ducks embryos have been used. The results showed that development of testis has divided into two periods: the first one was the period of undifferentiated gonad (genital ridge) while the second period was the development into a testis which showed various stages. The undifferentiated stage has revealed of development a small protruded thickening of germinal epithelium and primordial germ cells on the ventromedial surface of the mesonephros and dorsal mesentery.The development periods has showed three stages: development of rete cords, sexual cords,primary somniferous tubules and Sertoli progenitor cells at the 8-10th day embryos. During 14th day old embryo was revealed of development immature somniferous tubule, tunica albuginea and testicular capsule. While the period of 19th day embryo showed that development of interstitial cells and thin septa from tunica albuginea, .in addition to marked division of spermatogonia cells. Size of left testis was larger than the right one also the epithelial layer of left gonad is thicker than that covering the right gonad.

INTRODUCTION

The mallard duck *Anas platyrhynchos*is one species of Anseri formes which interest in the Middle East by the Sumerians around 2000 BCE and then in Europe during the middle Ages (1). It gives rise to a large number of different breeds in different world countries (2&3). 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 and the mallard duck in one of these birds(4). (5) recorded about 10 sites in Iraq which represents as international importance source for ducks and geese, (Haur Al Hammar and Haur Al Hweaza) that supported more than 1% of the populations of six species of ducks in 1979, including nearly 60,000 Common Teal *Anascrecca*, thesesix species are dependent as a main source of protein for the population of Iraqi southern governorates' such as Missan, Dhi Qar and Basra governorate. In all poultry species, the testes are located in the coelomic cavity, in close to the kidneys, at the sexual maturity they increased tenfold in weight from around 3g to 30g, at this time the sperms are produced over a14 day period in the somniferous tubules.The testes have an endocrine function. Interstitial cells produce testosterone (6).Embryologically, during a period up to 27–28 days of hatching the gonad has develop as bipotential nature to make ita testis or an ovary for this reason the study of organogenesis of gonad development provides the developmental pathways and the processes by which cells that established the development. The development of the testes and ovary from a single primordial affords opportunities for comparative that the point of divergence. Many studies on the chick sexual organs development from hatching to sexual maturation have done by (7), (8), also there works on domestic fowl (*Gallus domesticus*) testis albunigeous tunic (9), on testicular net (10), and interstitial tissue (11).Several studies on testicular development have done on most mammals and some birds, so the current work was intended to investigate the development of the testis, in Mallard duck during pre-hatching period to determine the sequence of the histological changes in diverse components of the testis in duck embryo from embryonic day 5 to 19.

MATERIALS AND METHODS

Fifteen males' embryos of mallard ducks have obtained by hatching their eggs manually in the incubator for periods (5^{th}, 8^{th}, 10^{th}, $14^{th}$and 19^{th} days) in department of anatomy, college of veterinary medicine, university of Baghdad. The embryos have been fixed in 8% formalin saline for 3 days then conserved in solution contained (70% alcohol and three drops glycerin). The embryos' have been dehydrated by passing them for two

hours through a series of ascending alcohol (40%, 50%, 60%, 70%, 80%, 90% and 100%), then cleared in xylen for one hours after that embedded in paraffin wax for one hours and sectioned serially at 5µm thickness and stain with Hematoxylin and Eosin stain (12).

RESULTS AND DISCUSSION

At the 5th day old embryos

In the early fifth days of duck embryos the results was showing the developed of indifferent gonads (genital ridge). The ridge was developed from proliferating coelomic lining epithelium and other cells from the mesonephros.Both left and right gonads have appeared as small prominent thickening on the ventromedial surface of the anterior mesonephros at base of the dorsal mesentery and protrude into the coelomic cavity compressing of pseudo-stratified columnar epithelium (germinal epithelium) and primordium germ cells which appeared large cells with clear cytoplasm (Fig.1,2), this result have mentioned by (13) and (14). The thickening of genital ridge has formed mainly by the peritoneal epithelium that lined the coelomic cavity which formed the growing genital ridges, also cells from the mesonephros to the epithelial population of ridge have augmented the cell population in the male gonadal primordium (Fig.2) this result was agree with results of (15) and (16) who mentioned that " the primitive testis is resulted by series of cell proliferation, migration, and differentiation which are driven by the sequential activation of specific genes.Histologically in chick the Gonadogenesis is begins at around 72 hours of incubation period (17), but these genital ridges were not macroscopically evident until day 4.5 of incubation period(18) and(19), while the present result revealed that in male Mallard duck the gonadogenesis was at the 5th day of incubation period (Fig.2). The present result has suggested that the gonads were beginning in both male and female from similar structure(genital ridge) that composed of same cells components. The constitutional cells of mesonephros that plays a pivotal role for testis development has also mentioned in mammals embryos by (20).

At the 8-10thday old embryos

As the development was proceeding from the 8th to the 10th day of incubation periods the appearance of gonads was showing the male phenotype. Both testes have appeared asparenchymatous organat the anterior mesonephros and the left testis measured 200±3µm in diameter while the right one was 187±5µm which (Fig.3&4).The parenchyma of testis was composing of proliferating and migrating germinal epithelium cells (rete cords)that branched and migrated toward the core of testis and some of these migrating germinal epithelial cells with primordial germ cells to form the sexual cord (Fig.4,5), within testicle core the groups of migrating germinal epithelial cells were surrounding primordial germ cells to form the Sertoli progenitor cells or (nurse cells or sustentacular cells)which formed the small cylindrical cords that were the precursors of the primary somniferous tubules (Fig.5)this result has supported by results of (21) in ostrich and (13) they mentioned that first signs of testicular differentiation was formation of cords which are precursors of the adult spermatogenic tubules.The primary seminiferous tubule has composed of germ cells that enclosed by a layer of germinal epithelial cells which represented the Sertoli cells, this result has mentioned by(14) who refers that the cells of the male genital ridge was a mix of primitive Sertoli cells, primordial germ cells in addition to interstitial cells. The present result suggested that at this period both testes were having the same organization but, the epithelial layer of germinal epithelium of the left gonad were thicker than that of the right gonad which composed of stratified cells, this result was similar to those recorded by (19) in chick and (18).Also the present study revealed that the Sertoli cells were the first cells that differentiated within the gonad and determined that the gonad has passed from the undifferentiated stage into differentiated testis(22).The fact of cells migration and testis-cords formation was specific event in testis development and the cord formation was due to migration of mesonephric cells into the male gonad (23 &24), also (22) showed that the differentiation of cells from pre-Sertoli cells into Sertoli cells was marked by aggregation of epithelial cells that assemble into testis cords.

At the 14thday old embryos

At this period the results were showing that the size of both testes was increased due toproliferation of stromal cells, while the proliferation of germinal epithelium have been ceased at this period to form single layer that covered the testis (Fig.6) (24 &25) who referred that the processes of cells proliferating and migrating have occurred only in males after the onset of Sry gene expression. The present results showed that the proliferating cells beneath germinal epithelium lead to develop the primary tunica albuginea (Fig.6). The parenchyma of testis has characterized by marked division and anastomosing of sexual cords which lead to development of immature seminiferous tubules which have narrow lumen and measured 25.2±0.4 µm in diameter(Fig.6 and7). Within each seminiferous tubule a single layer of dividing spermatogonia cells and Sertoli cells (Fig.7).Also this period was showing marked develop of thick layer interstitial tissue with darkly stained interstitial cells outside these tubules (Fig.7) (13&24) mentioned that the mesenchymal cell population migrates from the mesonephros into the developing gonad to become peritubular myoid cells, pericytes and endothelial cells.

At the 19thday old embryos

At this period both testes have been increased in sizes, but the left testis was larger than the right one and the right testis was reducing its attachment with mesonephros (Fig.8).In both testes the seminiferous tubules displayed relatively wide lumen that filled with more than a layer of dividing spermatogonia cells and Sertoli cells which rested at the basement membrane (Fig.9). In both testes the seminiferous tubule was measured 37.1±0.7µm in diameterand this diameter was significantly than that of previous ages, this result was agree with (27).Spermatogonia cells have showed pale stained nucli and mitotic figures. Sertoli cells appeared tall pyramidal cells which rested at the basement membrane of seminiferous tubules and showed darkly stained nucli with eosinophlic cytoplasm. (Fig.10),the present result were showing that interstitial cells which represented the precursors of Leydig cells were gathering with other flattened peritubular myoid cells which formed layer of flattened cells(Fig.10), the present result suggested that, peritubular myoid cells have originated and migrate from the mesonephros cells into

The male gonad and play an important role in future movement of sperm within mature seminiferous tubules (26), also such observation has recorded by (25). At this period a thin layer of connective tissue which formed the tunica albuginea has composed mainly of fibroblast that was immersing into the interstitial tissue(Fig.10)(13&14) referred that the component of interstitial tissue has contains the interstitial cells in addition to cords cells of tunica albuginea, on other hand other cell types were recorded by (24) those included endothelial cells, fibroblasts, macrophages, lymphocytes, and mast cells which. migrating from mesonephros

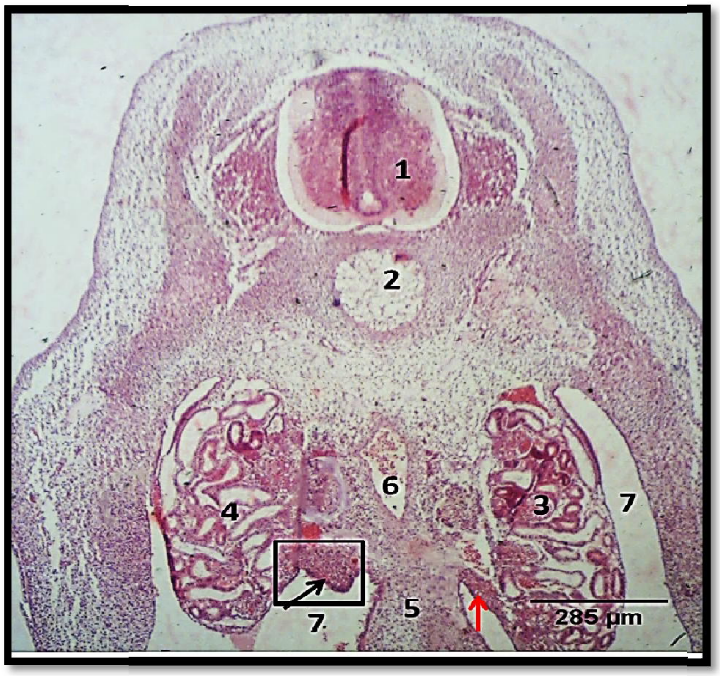


Fig.1: Transverse section through the anterior mesonephros (5th day old embryo) shows: spinal cord (1), notochord (2), right mesonephros (3), left mesonephros (4), dorsal mesentery (5), aorta (6) and coelom (7). H&E stain. 40 X.

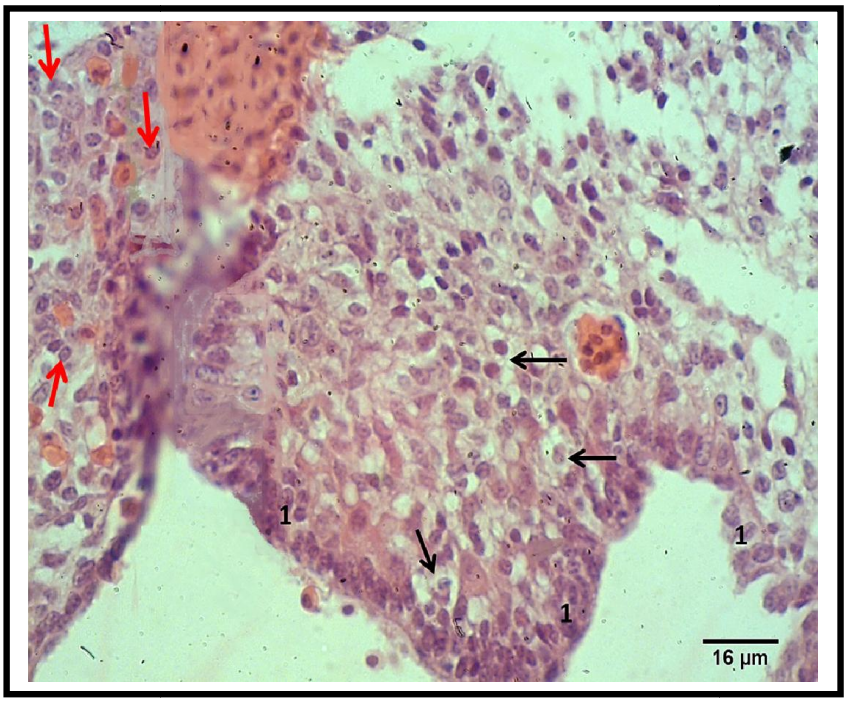


Fig.2: The Magnified black box in figure (1) shows:Germinal epithelium (1),Nephrogenic tissue (2)Primordial germ cells (black arrows). H&E stain.400 X.

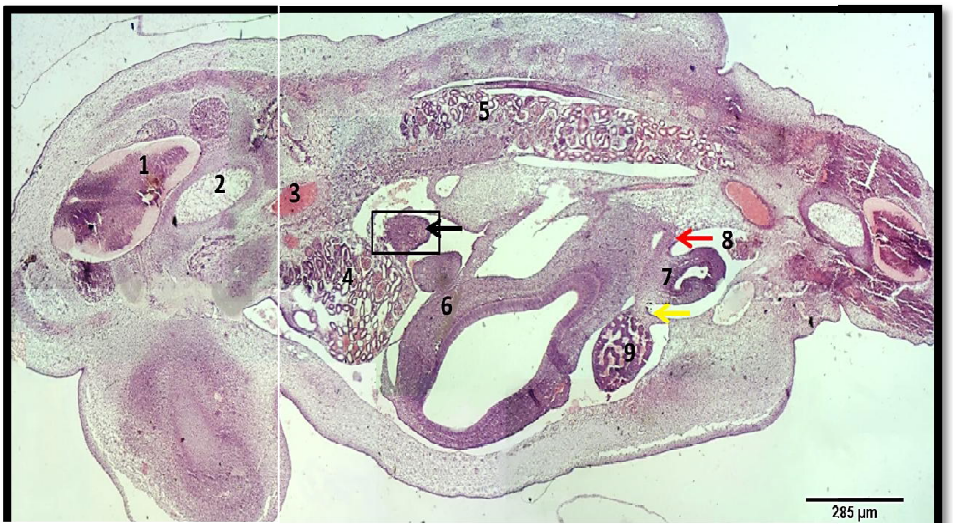


Fig. 3: Longitudinal section at the left side of body (8th day old mallard duck) shows: Spinal cord (1), notochord (2), aorta (3), left anterior mesonephros (4), posterior mesonephros (5), gizzard (6), intestine (7), metanephros (8), liver (9), left testis (Black arrow), dorsal mesentery (Red arrow) and ventral mesentery (Yellow arrow). H&E stain. 40 X.

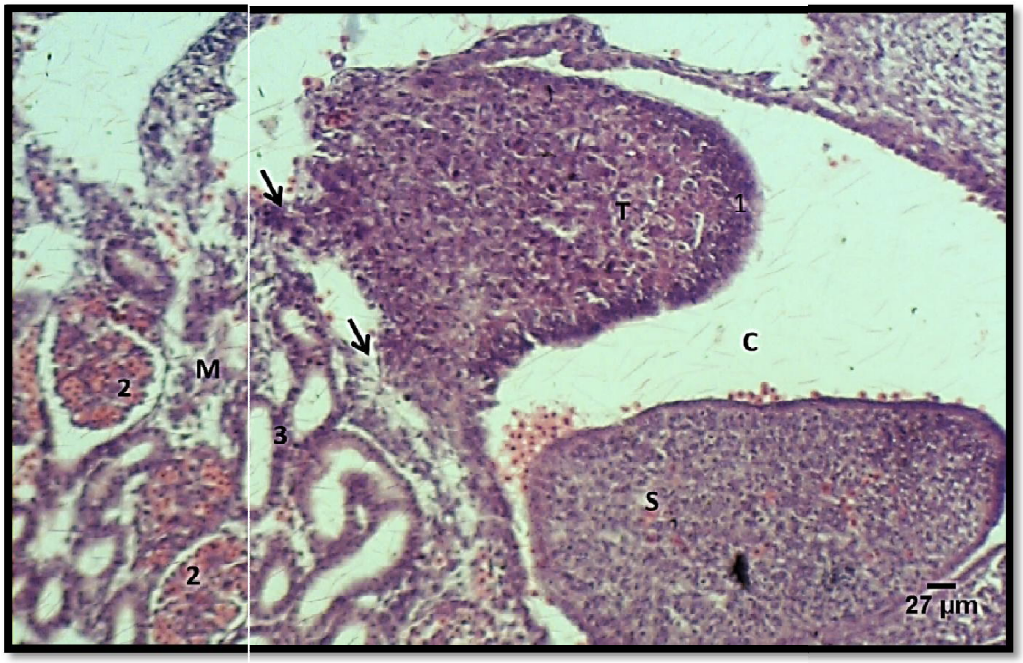


Fig.4: Magnified of black box area in figure (3) shows: proliferating germinal epithelium (1), glomeruli (2), mesonephric tubules (3), mesonephros (M), Testis (T), spleen (S) , coelomic cavity (C) and proliferating nephrogenic tissue (Black arrows).. H&E stain.100 X.

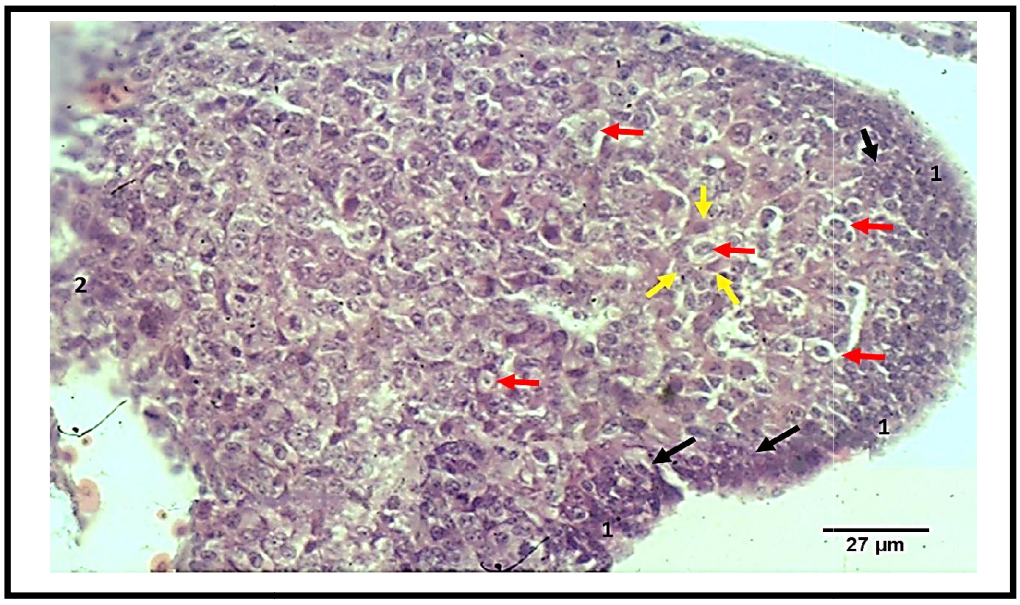


Fig. 5: Magnified of the testis section in figure (4) shows: proliferating germinal epithelium (1), migrating epithelial cells (Black arrows), primordial germ cells (Red arrows) that surrounded by Sertoli-progenitor cells (yellow arrows). H&E stain. 400

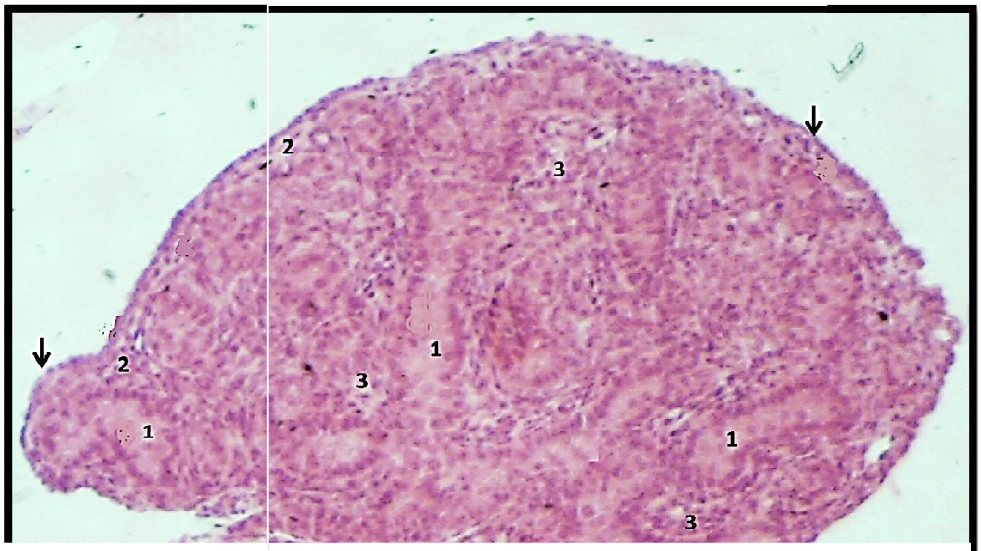


Fig.6: Section of the testicle (14th day embryo) shows immature seminiferous tubules (1), primary tunica albuginea (2), interstitial tissue (3) and proliferating germinal epithelium (Black arrows). H&E stain, 100 X.

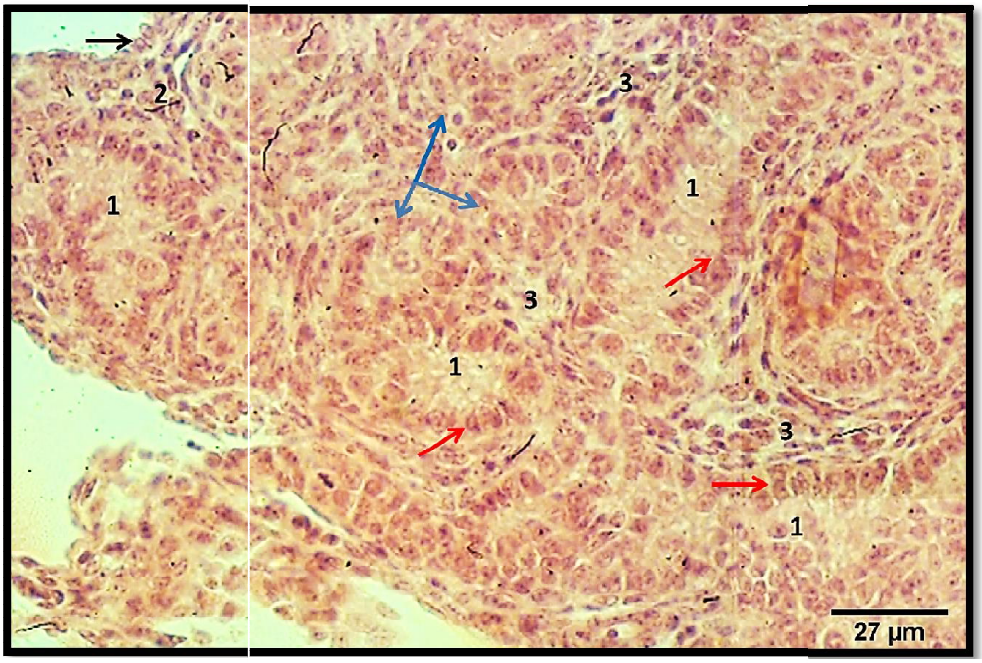


Fig.7: magnified section of the testicle (14th day embryo) shows immature seminiferous tubules (1), tunica albuginea (2), interstitial cells (3), dividing spermatogonia & Sertoli cells (Red arrows), dividing and anastomosing cords (blue arrows and germinal epithelium (Black arrow). H&E stain, 400X.

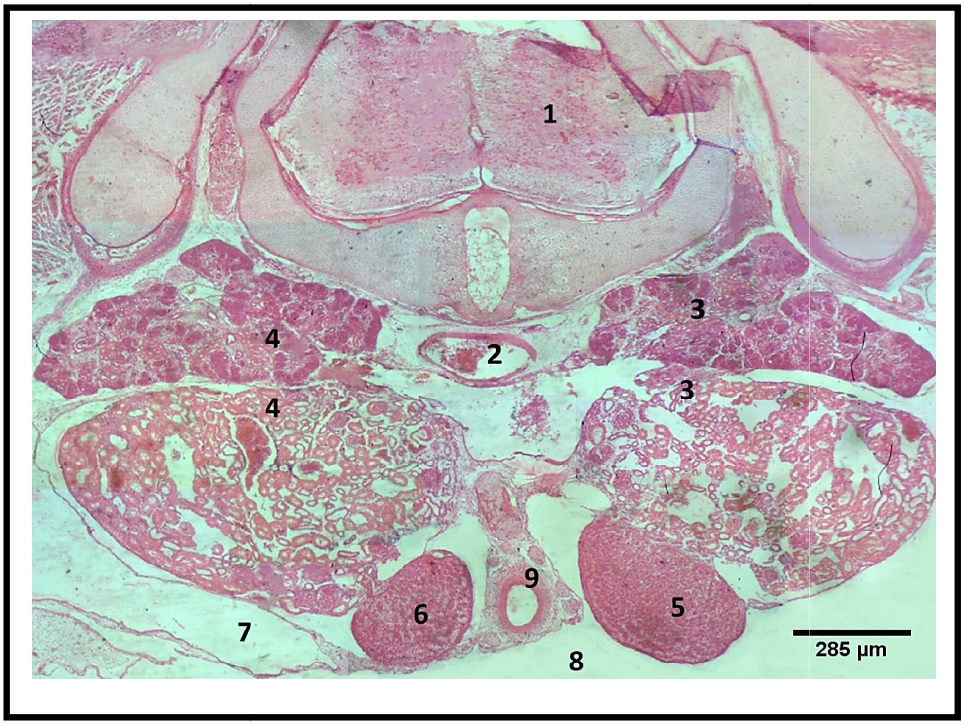


Fig. 8: Transverse section through the mesonephros of the 19th day embryo shows spinal cord (1), aorta (2), left mesonephros (3), right mesonephros (4), left testicle (5), right testicle (6), abdominal air sac (7) and coelomic cavity (8). H&E stain, 40X.

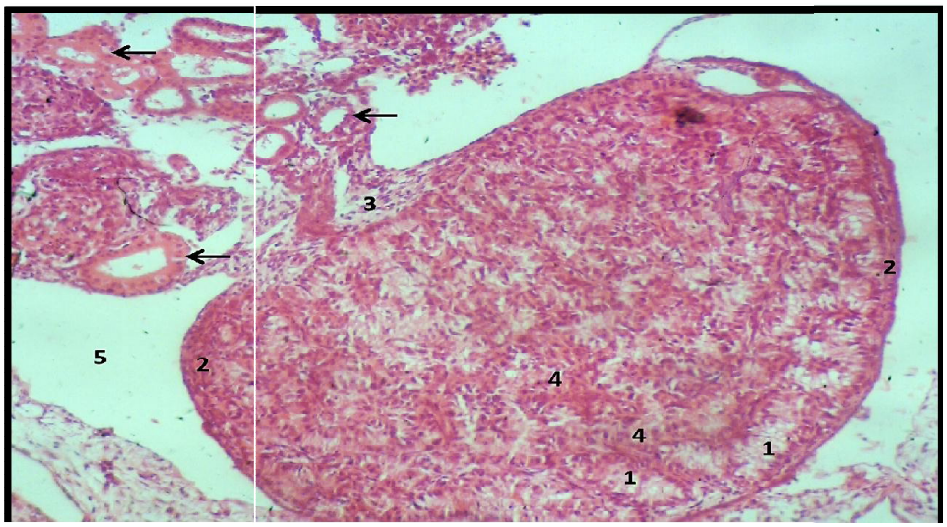
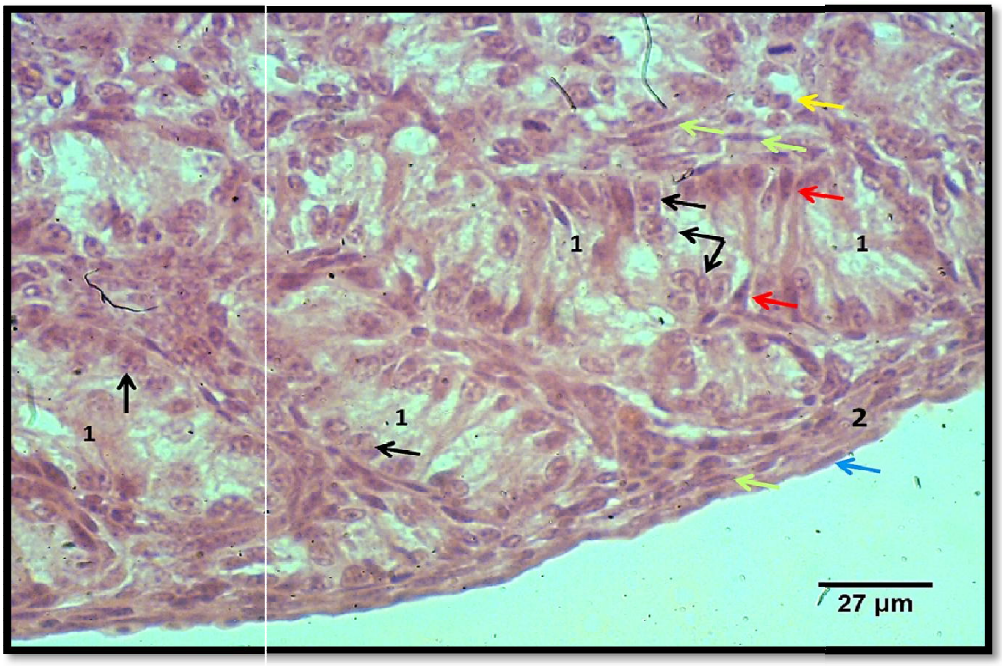


Fig.9: magnified section of the right testicle (19th day old embryo) shows seminiferous tubules (1), capsule (2), nephrogenic tissue (3), interstitial tissue (4), coelomic cavity (5) and mesonephric tubules (Black arrows). H&E stain, 100X.



tubules (1) that filled spermatogonia cells (Black arrows) & Sertoli cells (Red arrows), tunica albuginea (2), interstitial cells (Yellow arrow), flattened peritubular myoid cells (Green arrows), and germinal epithelium (blue arrows). H&E stain, 400X.

التطورالنسجي لفترات ماقبل الفقس لخصية البط المحلي
(*Anas platyrhynchos*)

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الخلاصة

اظهرت النتائج التطور الخصيتين يكون على مرحلتين: الاولى كانت مرحلة القند غير المتمايز والثانية كانت مراحل تطور الخصية. بينت مرحلة الاولى تطور القند بشكل تثخن صغير،مؤقت وبارز لظهارة الجوف العام بين السطح البطني الانسي للكلية الوسطى وقاعدة المساريق الظهري ويتكون من الخلايا الظهارية والخلايا بدئية الجرثومية. اما تطور الخصية كانت على ثلاثة مراحل: الاولى اظهرت تطور الحبال الجنسية، النبيبات الخصوية الاولية، وخلايا المولدة لخلية سيرتولي خلال اليوم 8 والى اليوم 10. اليوم 14 من عمر الجنين اظهرت تطور النبيبات المنوية والغلالة البيضاء وخلايا سيرتولي وخلايا بدئية الامشاج. بعمر 19 يوم تمايزالنسيج الخلالي للخصية مع تكون حويجزات نحيفة واحاطة الخصيتين بمحفظة. اظهرت نتائج مراحل التطور ان حجم الخصية اليسرى اكبر من نظيرتها في جهة اليمنى وكذلك الحال لطبقة الظهارة المولدة في الخصية اليسرى كانت اسمك من تللك في جهة اليمنى.

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