Prenatal developmental study of the ovaries of the Egyptian Baladi rabbits (*Oryctolagus cuniculus*)

ABSTRACT

This study was carried out to find out the histological changes of the ovaries of the Baladi rabbits at different prenatal periods. A hundred Baladi rabbit embryos and fetuses were used in this study. The ethically approved pregnant rabbits were euthanized, dissected and, subsequently, the embryos and fetuses were collected. The samples were fixed with modified Davidson's fixative and 10% neutral buffered formalin for 24 to 48 hours and then subjected to routine paraffin embedding technique. They sections were stained by hematoxylin-eosin, Crossman's trichrome and Gomori's reticulin stains. Stained sections were examined by light microscope. The study revealed that the ovarian differentiation with the appearance of its cortex and medulla occurred at the 19th day postconception. The oogonial nests were observed within the ovarian cortex at the 24th day of fetal rabbit life. The primitive tunica albuginea ovarii could be recognized at the 28th day postconception. No primordial follicle occurred throughout the fetal rabbit life.

Keywords: Prenatal, ovaries, fetus, rabbit.

INTRODUCTION

The rabbit is one of the laboratory animals used for research due to its high breeding rate and giving high number of offsprings monthly. Since copulation is necessary to initiate ovulation, therefore, it easier to estimate the pregnancy timing and the ages of the embryos and fetuses. The gonadal primordium in mammals appears as a thickening of the coelomic epithelium medial to the mesonephros (Hyttel et al., 2012). The ovarian structure is important for sex determination and oocyte formation (Nicholas et al., 2010; Kaufmann et al., 2011). Ovaries of rabbit have been described as small and elongated organs (Fraser and Girling, 2009). Rabbit is a species which shows teratogenic defects on birth. It is commonly recommended that drugs should be examined for teratogenic activity in two species of animal such rat and rabbit (Cook and Fair-weather, 1986). The present study aimed to determine the time of female sexual differentiation and describe the main stages of the normal structural changes of the ovaries of the rabbit during the embryonic and fetal periods which will be useful for drug research to avoid ovary malformations in the rabbit along with human.

MATERIAL METHOD

The present study was done on 100 rabbit embryos and fetuses ranging from 9 day to full term. The abdominal cavities of the ethically approved adult, healthy, pregnant female rabbits were opened after euthanasia and the uteri were exposed and separate incisions were made within them.

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Research Article

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The whole embryos (9th to 13th day), the caudal halves of the fetuses (14th to 18th day) and the gonads of the fetuses (19 day up to full term) were collected after injection into the abdominal cavity with suitable amount of modified Davidson's fixative (Lihui et al, 2011) and left for few minutes. The samples were transformed into 10% neutral buffered formalin for 24 to 48 hours for more fixation. Then the specimens were processed by routine paraffin embedding technique and were sectioned at 4-6 µm thick and stained by different stains (Bancroft and Gamble, 2008) including hematoxylin-eosin, Crossman's trichrome, and Gomori's reticulin stains. Histological examination was performed using a light microscope (Leica model with camera attachment giving digital images). Images were digitally recorded.

RESULTS

The fetal ovaries could be identified at the 19th day postconception by the absence of the primitive tunica albuginea and slight differentiation of the primitive parenchyma into an outer cortex and an inner medulla. The ovaries were covered by a single layer of slender shaped columnar cells without basal lamina (Figure 1A). The ovarian epithelium changed to elongated columnar cells with intraepithelial oogonia at the 26th day postconception (Figure 1B). The ovarian surface epithelium consisted of a single layer of short columnar to cuboidal cells lacking basal lamina at the end of the gestation period (Figure 1C).

The ovarian stroma: The supportive tissue of fetal ovaries from 19th up to 22nd day postconception was represented by undifferentiated mesenchymal tissue and fibroblasts. They were distributed in the medullary region but were scant in the cortex (Figure 1D). The primitive tunica albuginea ovarii firstly appeared at 28th day postconception and the ovarian cortex was supported by few collagen fibers between the oogonial nests, while the medulla contained extensive network of collagen fibers housing blood vessels (Figure 2E). The ovaries of full term rabbit fetus were supported by a well-developed fibrous framework of collagen and reticular fibers, which was few around the cortical elements but form extensive network in the ovarian medulla around the oogonial nests (Figure 2F; 2G).

The ovarian cortex: The ovarian cortex was formed of stroma cells, which were derived from the down growth from the ovarian surface epithelium, mesenchymal cells and proliferating cells derived from the mesonephros housing few oogonia. The oogonia appeared as large rounded cells with rounded euchromatic nuclei with one to three nucleoli and have a clear, finely granular and lightly stained cytoplasm (Figure 2H). From the 24th to 28th day postconception, the ovarian cortex became more organized with marked increase in the numbers of the oogonia which form the oogonial nests which were surrounded by basal laminae (Figure3I). On reaching the full term rabbit fetus, the oogonia increased in number in which many oogonia entered the leptotene of prophase of the first meiotic division while other oogonia suffered from apoptotic changes. However, the ovaries remained containing proliferating oogonia with no primordial follicles formation throughout the fetal life (Figure 3J).

Ovarian medulla: The medullary portion was formed of loose undifferentiated mesenchymal cells and few fibroblasts permeated by blood vessels. From the 22nd to the 24th day postconception, the ovarian medulla became more differentiated; formed mainly of stroma cells and fibroblasts and invaded by few oogonia with mitotic activity. The vascularization of the ovarian medulla became highly pronounced. The medullary cords were firstly recognized and they appeared as solid cords of stroma cells of

about 2 to 4 cell-thick. (Figure 3K). In full term rabbit fetus, the ovarian medulla appeared highly fibrous housing blood vessels. The oogonia within the ovarian medulla were disintegrated. The ovaries of the rabbit contained no interstitial cells during the fetal life and the medullary cords remained without lumen (Figure 3L).

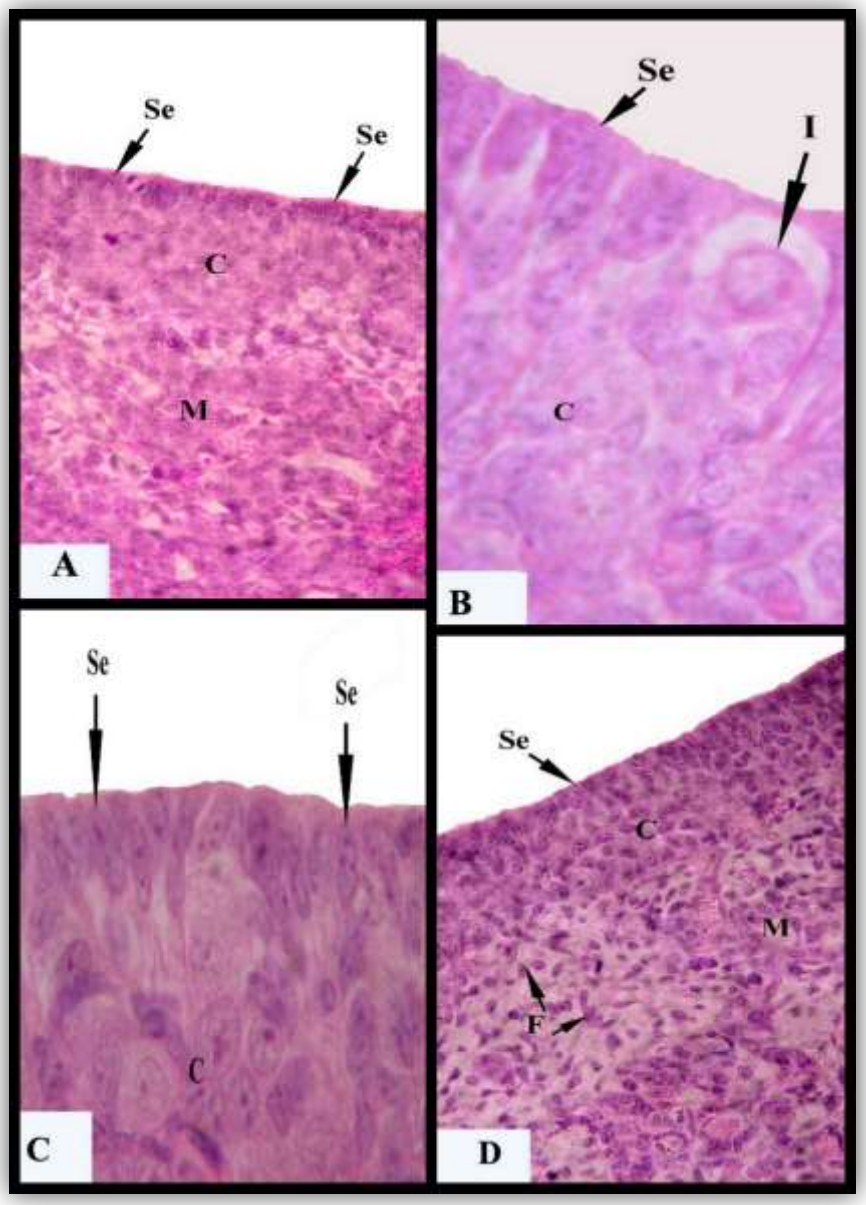

Figure 1: A photomicrographs of the developing ovary of 19th day old rabbit fetus showing ovarian surface epithelium, ovarian cortex and ovarian medulla (A- H&E stain, X400). A high magnification of the ovary of 26th day old rabbit fetus showing intraepithelial oogonial (B - H&E stain, X1000). A full term rabbit fetus showing short columnar to cuboidal cells ovarian surface epithelium without basal lamina (C- H&E stain, X1000). The ovary of 22nd day old rabbit fetus showing the supportive tissue of the ovary represented by mesenchymal tissue with few fibroblasts widely distributed in the medulla but very scant in the cortex (D- H&E stain, X200). C- Ovarian cortex; I– Intraepithelial oogonia; M – Ovarian medulla; Se – Ovarian surface epithelium.

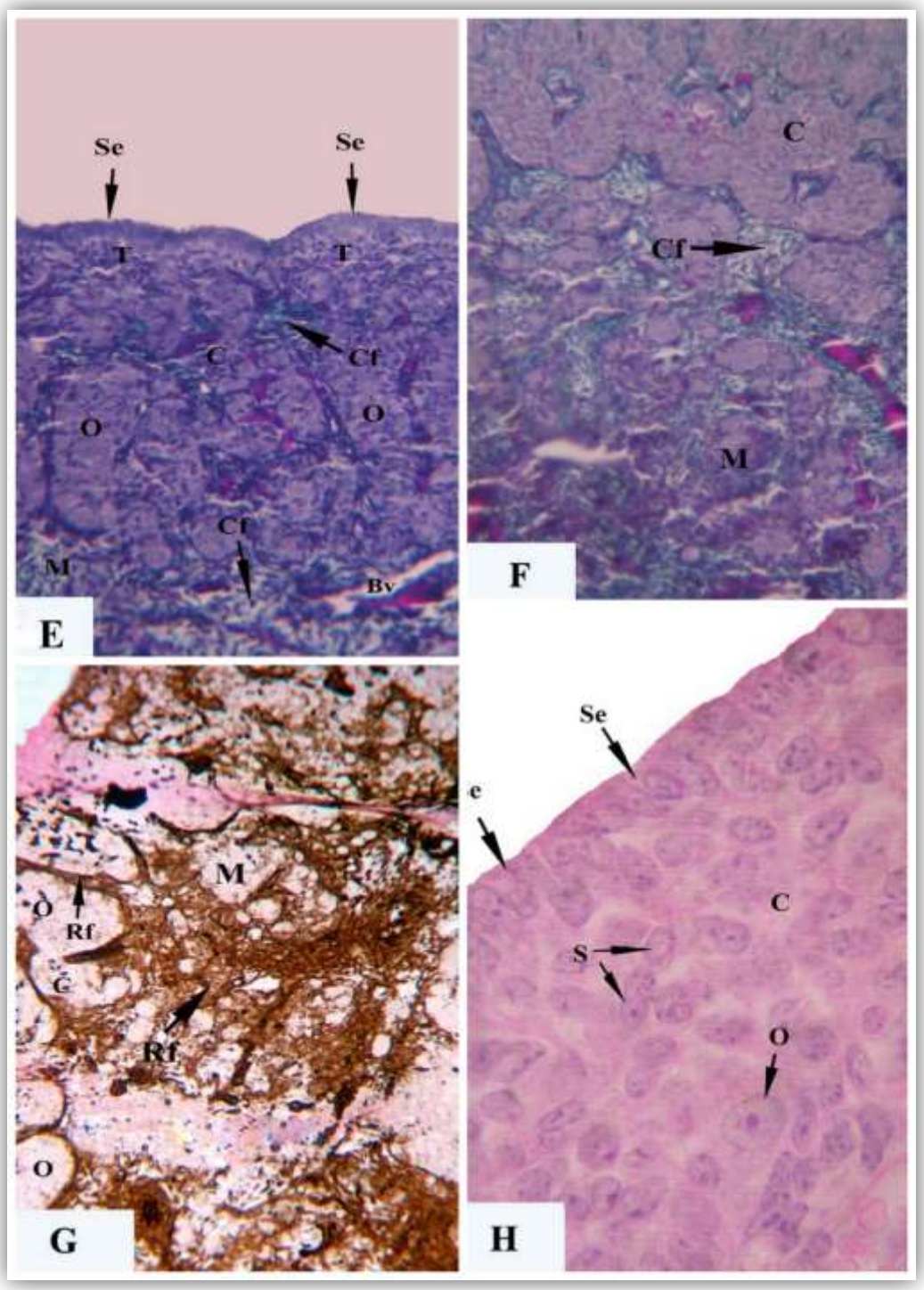

Figure 2: A photomicrographs of 28th day old fetal rabbit ovary showing primitive tunica albuginea ovarii formed mainly of fibroblasts. Note, the ovarian cortex supported by few collagen fibers while the medulla contained extensive network of collagen fibers (E-Crossman's trichrome stain, X200). A section through the ovary of full term rabbit fetus showing well developed fibrous framework especially in the medulla (F-Crossman's trichrome stain, X200). A section through the ovary of full term rabbit fetus showing well established reticular framework in the ovarian medulla and few fibers in the cortex (G-Gomori reticulin stain, X200). A 19th day old rabbit fetus showing ovarian cortex formed of stromal cells irregularly distributed housing few oogonial (H-H&E stain, X1000). C – Ovarian cortex; Bv – Blood vessel; Cf– Collagen fibers; M– Ovarian medulla; O- Oogonial nests; Rf – Reticular fibers; S – Stroma cells; Se – Ovarian surface epithelium; T – Tunica albuginea ovarii.

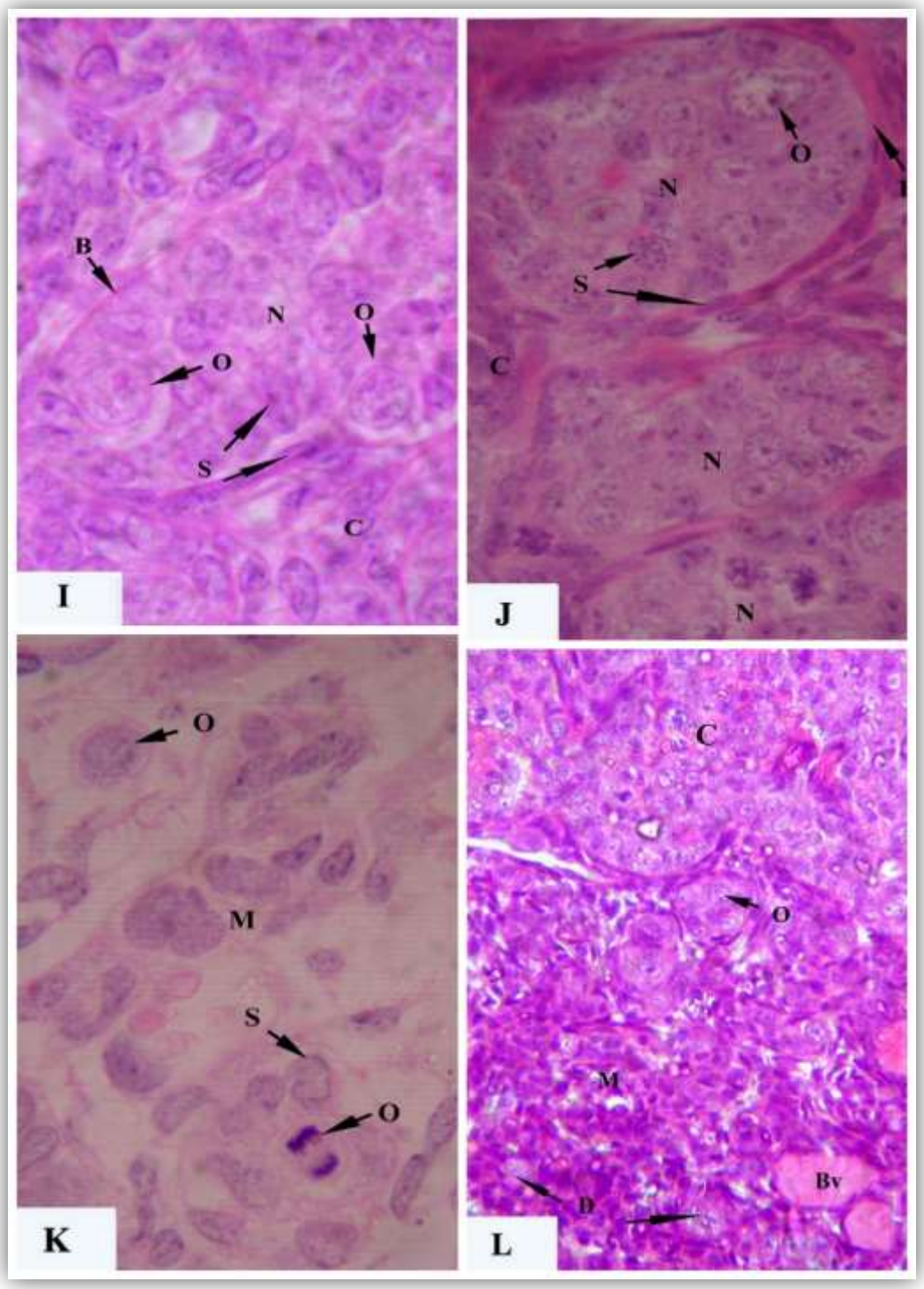

Figure 3: A high magnification of the ovarian cortex of 26th day old rabbit fetus showing oogonial nest containing many oogonia and stroma cells. The nest was surrounded by interrupted basal lamina and stroma cells (I- H&E stain, X1000). A high magnification of the ovarian cortex of full term rabbit fetus showing oogonial nests filled with numerous oogonia and stroma cells. The nests were surrounded by thick basal lamina and stromal cells (J- H&E stain, X1000). A 22nd day old rabbit fetus showing ovarian medulla contains oogonia with mitotic activity and stroma cells (K- H&E stain, X1000). The ovary of full term rabbit fetus showing disintegration of medullary oogonia. Note: the medulla appeared highly fibrous housing blood vessels (L-H&E stain, X400). B–Basal lamina; Bv – Blood vessels; C– Ovarian cortex; D – Disintegrated oogonia; M – Ovarian medulla; N – Oogonial nests; O – Oogonia; S – Stroma cells.

DISCUSSION

In this study, the ovarian differentiation of rabbit fetuses could be distinguished at 19th day postconception while some investigators reported that it occurs at 16th, 18th days of fetal life in rabbit (Byskov et al., 1985; Duke, 1941; Mario et al., 2018). Moreover, the fetal ovaries of rabbit could be identified by absence of the primitive tunica albuginea and slight demarcation of the primitive parenchyma into an outer cortex and inner medulla. Meanwhile the ovaries of the rabbit are distinguished from the testes by the absence of seminiferous cord formation and tunica albuginea (Gondos et al., 1983).

In the opinion of most embryologists, the presence of intraepithelial oogonia is mainly due to the misdirection of these oogonia during their intragonadal migration (Rodrigues et al., 2009). Some authors postulated that the surface epithelium plays a distinct role in production of oogonia in rabbit (Duke, 1947). In our opinion, the second theory could not be accepted due to the absolute microscopic difference between oogonia and surface epithelial cells in addition to the presence of extra gonadal germ cells on their way towards the developing ovary.

Our findings reported that ovarian surface epithelium had no basal demarcation during the prenatal rabbit life while the basement membrane of the germinal epithelium of rabbit ovary is formed from reticular fibers at 17th day postcoitum (Duke, 1947).

Our observations stated that the separation between the ovarian surface epithelium and ovarian parenchyma occurred via mesenchymal tissue and fibroblasts, while the primitive tunica albuginea ovarii appeared as an interrupted layer of fibroblasts and few fibers under the surface epithelium at 28th day postconception. The germinal epithelium in rabbit is separated from ovarian parenchyma by mesenchymal tissue at 17th day postcoitum, while the complete separation occurred after birth via tunica albuginea (Duke, 1941).

The present study observed that the ovarian cortex of recently differentiated ovary at the19th day old rabbit fetus was formed of stroma cells irregularly distributed all over the cortex intermingled with few oogonia. While, the ovarian cortex consists of diffuse sheets of germ cells and granulosa cells at the16th day old rabbit fetus (Gondos et al., 1983).

The obtained results declared that the ovarian cortex was formed from stroma cells derived from the down growth from the ovarian surface epithelium, mesenchymal cells of the ovarian cortex and proliferating cells derived from the mesonephros while the precursors of the follicular cells of the ovaries of the rabbits were derived from the coelomic mesothelium lining the surface of the ovary (Duke, 1941).

The ovaries remained containing proliferating oogonia with no primordial follicles formation throughout the fetal life while the primordial follicles could be seen in the epithelium of the ovary at the 18th days old rabbit fetus (Mario et al., 2018).

In the work under discussion, the oogonia within the ovarian cortex entered mitotic division during the prenatal life. While all germ cells are oogonia and many mitoses are found among them in the ovaries on the day of birth of rabbit (Anderson et al., 1970). On the other hand, the ovaries on 22nd, 26th and 28th day of gestation are in the proliferative phase and most of oogonia are in mitotic phase and few are in resting stage but at 30th day of gestation most of the oogonia are in resting stage and a few are in mitotic stage (Sasabe et al., 1990).

The results obtained from this study that the ovarian medulla of the rabbit fetus contained medullary solid cords at 24th day postconception and they remained without lumen until birth while the medulla of the

ovary of the rabbit contained well-defined twisted epithelial cords (Duke, 1947). Moreover, the medullary solid cords arose from ovarian stromal cells while the epithelial cords arise from the growth of connective tissue in the ovarian medulla of the rabbit (Duke, 1941).

CONCLUSION

The current study detected the time of the sexual differenation of the ovary of the rabbit and recorded the normal microscopic structural changes that occurred in the ovary at different prenatal periods. Knowledge gained through this research will give some limitations for drug research to avoid teratogenesis and ovary malformation in laboratory animals along with human.

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The author declare that there are no conflicts of interest.

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