**Original Paper****Histo-morphological comparative investigations on the ovary of turkey (*Meleagris gallopavo*) in immature and mature age.**Sozan A. Ali¹, Hassan Emam², Maha S. Kilany¹¹ Histology and Cytology Department, Faculty of Veterinary Medicine, Zag. University, Egypt² Anatomy and Embryology Department, Faculty of Veterinary Medicine, Zag. University, Egypt**ARTICLE INFO****Keywords**

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ABSTRACT

The current investigation was performed on 30 healthy slaughtered female turkey, the samples were processed for the histo-morphological examination. Whereas ovaries were dissected after caught and slaughtered birds directly. The morphological findings showed that, the ovaries of immature (6 months) and mature (one year) ages of turkey were located in the Coelomic cavity with pale to dark yellow color and irregular shapes resemble a bunch of grapes due to different sized follicles on its surface., at six months of age, the ovary was composed of an outer cortex and inner vascular medulla, there was a clear separation between them. The cortex enclosed ovarian follicles of different sizes. In addition, there were some large vacuolated cells known as thecal gland. Some small secondary follicles had vacuoles that resemble pre-antral follicles in primates and other birds. The ovarian medulla represented the inner vascular part of the ovary. At one year of age, the ovarian follicles showed an advanced stage of growth. The large growing follicles migrated to the ovarian surface and the covering ovarian epithelium became thinner. Postovulatory regressed follicle that resembles corpus luteum was observed. At six months: highly connective tissue fibers presented as collagen, elastic, reticular. Whereas connective tissue decreased at one year of age, so the age of laying and maturation in turkey is nearly at one year of age. This study showed the ovary of female turkey at one year of age contained ovarian follicles at advanced stage of growth and Postovulatory regressed follicle that resembles corpus luteum. So, the age of laying and maturation in turkey is nearly at one year of age.

1. INTRODUCTION

Turkey is a large type of poultry species originated in America and domesticated by Indian tribes (Becker, 2006). The domesticated turkey is a large poultry spp. in *Meleagris* genus (Aslam, 2012). Turkey birds have the goal to be a competitive meat production to other birds (Nixey, 1986). Turkey meat is considered one of the best points for alternative protein source in Bangladesh. Turkey production is a major agricultural branch with growing demand for its products (Yakubu et al. 2013). Reproduction is an important part of producing new offspring and protecting the species from extinction. If there is no reproduction, the population will not increase (Murphy, 2012). The reproductive system of livestock will develop the reproductive process when the animal has entered the sexual maturity stage. Reproductive organs play an important role in the reproductive activity of an animal species (Wahyuni et al. 2014). The ovary had irregular surface in the abdominal cavity, the left ovary is functional in most avian species. Islam (2021) recorded that the ovary of turkey composed of cortex and medulla, whereas ovarian follicles of different sizes present in the cortex and a germinal layer covering the cortex from the reflection of the abdominal cavity on it.

It is necessary in diagnosis of various diseases, whereas the information of such bird is scanty in academic records. So,

this research has been designed to understand normal histo-morphology of the ovary in female turkey.

2. MATERIAL AND METHODS*2.1. Birds and Tissue Specimen's*

The present study was carried on 30 healthy female turkeys species at six months and one year of age, Ovary was obtained immediately after slaughter, where birds were purchased from the turkey breeders in El Sharkia Governorate Egypt, Birds (6 months, one year) were apparently healthy with (2.5–3.5 Kg) average body weights.

This work has been reviewed and approved by the ZU-IACUC committee with approval number ZUIACUC/2/F/86/2023.

2. Anatomical evaluation

The present study was carried at Department of Anatomy and Embryology, Zagazig Univ., Egypt, 2 groups were used. The first group (n=15) of female immature turkeys (6 months age) while, the second group (n=15) of female mature turkeys (12 months age). All turkey birds were feed and added libitum water before they slaughtered. The whole ovaries of slaughtered turkeys were dissected and photographed. The color of dissected ovaries was observed by visual inspection. The weight, length, width, and

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thickness of ovaries were measured. The weight was measured by electronic balance. The length, width and thickness were measured by using Calipers.

3. Histological and histochemical preparations

The specimens were preserved and fixed in 10% buffered neutral formalin for 72 hrs at room temperature, dehydrated, then cleared in Xylol. All specimens were embedded in paraffin. Paraffin sections were stained with Harris's Hematoxylin and Eosin (H & E) to show the general histological structure, Crossmon's trichrome stain for collagen and muscle fibers, Silver impregnation for reticular fibers, Weigert's stain for elastic fibers. (Suvarna, 2018). All the stained sections were examined with a standard light microscope (Olympus BX 21) and photographed by a digital Dsc-W 800 super steady cyper shot camera (Sony®, Japan) at the Department of Histology and Cytology, Zagazig University, Egypt.

4. Statistical analysis

The recorded data were analyzed by Graph Pad prism 8.0.2 (GraphPad Software, Inc). The results were reported as Mean \pm SE. Normality achieved by non-normal Shapiro-Wilk (W) $P > 0.05$. Independent samples *t*-test was run to test differences in weight, length, width, and thickness between mature and immature turkey ovaries. Statistical significance of results was considered to be at $P < 0.05$.

3. RESULTS

3.1. Anatomically

Dissected ovaries of immature and mature turkey hens were located in the Coelomic cavity. The color of the ovaries in immature turkey was pale yellow, while in mature turkey was dark yellow and in both age groups, ovaries were irregular shaped resembled to bunch of grapes due to different sized follicles on their surfaces. The ovary bounded cranially by left lung, caudally by oviduct, dorsally by cranial lobe of left kidney and ventrally by stomach (Figs. 1 & 2). The average weight of the ovary of 1.95 ± 0.11 and 15.45 ± 0.26 g in immature and mature turkey, respectively. The average length of the ovary was 2.45 ± 0.07 and 3.96 ± 0.04 in immature and mature turkey, respectively. The average width of the ovary was 1.82 ± 0.07 and 4.03 ± 0.08 in immature and mature turkey, respectively. The average thickness of the ovary was 1.02 ± 0.06 and 2.33 ± 0.06 in immature and mature turkey, respectively (Table 1).



Fig. 1 A photograph showing the topography of ovary and oviduct in immature turkey (ventral view): O. Ovary; Lk. Left kidney; R. Rectum Ll. Left lung; Ov. Oviduct and C. Cloaca

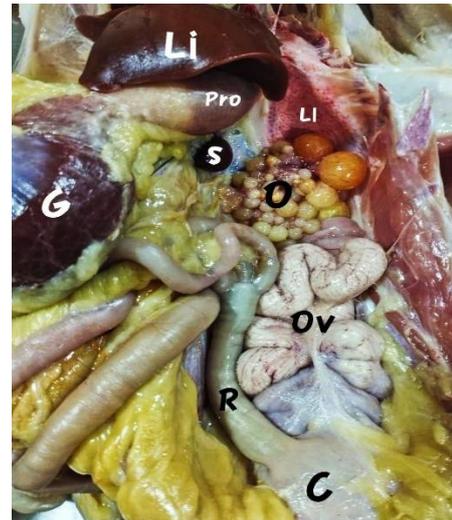


Fig. 2 A photograph showing the topography of ovary and oviduct in mature turkey (ventral view): O. Ovary; Lk. Left kidney; R. Rectum Ll. Left lung; Ov. Oviduct; C. Cloaca; Li. Liver; S. Spleen; Pro. Proventriculus and G. Gizzard.

Table 1 Changes in weight, length, width, and thickness of ovary in immature and showed that mature turkey.

Ovarian parameters	Immature turkey	Mature turkey
Weight	1.95 ± 0.11	$15.45 \pm 0.26^{***}$
Length	2.45 ± 0.07	$3.96 \pm 0.04^{***}$
Width	1.82 ± 0.07	$4.03 \pm 0.08^{***}$
Thickness	1.02 ± 0.06	$2.33 \pm 0.06^{***}$

*** indicates high statistical significance differences at $P < 0.001$.

3.2. Histologically:

3.2.1. At six months of age

The ovary of turkey was composed of cortex and medulla, there was a clear separation between them. The cortex was indented as it comprised ovarian follicles with different sizes in various developmental stages which bulged from it at different distances. The follicles included primordial, primary, secondary follicles, in addition atretic follicles were observed. The ovarian cortex was covered by a single layer of simple low cuboidal epithelium that was separated by a relatively thick connective tissue capsule "tunica albuginea" from the ovarian cortex (Fig 3A). The primary follicle was lined with one layer of follicular cells "cuboidal epithelium" and had central nucleus of the primary oocyte (Fig 3B). The secondary follicle was lined with two layers of cuboidal granulosa cells and appeared to be surrounded with two layers; flat squamous cells "theca interna" and fibrous layer "theca externa" which were developed from the cortex stromal cells. The granulosa cells were separated by an eosinophilic basement membrane from the theca interna. In addition, there were some large vacuolated cells with clear cytoplasm and dark nuclei distributed in the thecal layer surrounding the follicle, these cells are known as thecal gland. (Fig 3C). Interestingly, some secondary follicles had vacuoles that resemble pre-antral follicles in primates and other birds (Fig 3D). The ovarian medulla, the inner vascular part of the ovary, was composed mainly of vascular connective tissue including large blood vessels and strands of smooth muscle fibers. The medulla was characterized by interstitial pale stromal eosinophilic polyhedral cells with centrally rounded nuclei (Figs 4A & 4B).

3.2.2. At one year of age

The ovarian follicles showed advanced stage of growth and increased in size leading to the expansion of the cortex, the interstitial connective tissue between the ovarian follicles decreased as a result of swelling of the follicles. The cortex and medulla were intermingled and the distinguishing

between them becomes hard (Fig 5A). The large growing follicles migrated to the ovarian surface and the covering ovarian epithelium become thinner and appear squamous, the granulosa cell and theca cells become more developed. The growing follicles showed an undifferentiated cytoplasmic area appeared anterior to the perivitelline membrane and the homogenous ovoplasm converted to yolk granules (Fig 5B). The yolk granules were dense albuminoid dark granules inside yolk spheres. Postovulatory regressed follicle that resembles corpus luteum was observed with central groups of vacuolated granulosa cells interrupted by thin strands of theca interna and surrounded with peripheral thickened theca externa (Fig 6A), the fibrous theca externa contained bundles of collagen fibers (Fig 6B)

At six months of age, the interfollicular connective tissue was thick and well developed and showed the presence of collagen, elastic, and reticular (Figs 7A-C). At one year of age, the amount of connective tissue fibers among large sized ovarian follicles was regressed and collagen, elastic, and reticular fibers were observed (Figs 8A-C).

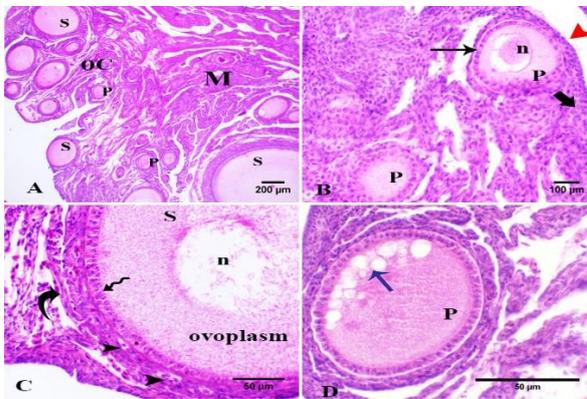


Fig. 3 A Photomicrograph of the six months female turkey ovary. (A) showing the ovary composed of outer ovarian cortex (OC) and inner vascular medulla (M), the cortex was occupied by different developmental stages of ovarian follicles: primary follicles (P), secondary follicles (S). B) showing the ovary covered by simple cuboidal epithelium (red arrowhead), the tunica albuginea (thick arrow), the primary follicle (P), the nucleus of the primary oocyte (n), the follicular epithelium (arrow). C) showing secondary ovarian follicle (S) with central nucleus (n) and lined with double layer of granulosa cells (zigzag arrow) and surrounded with theca layer (closed arrow) with thecal gland (black arrowhead). D) showing primary follicle with ovoplasm containing empty vesicles (blue arrow). Stain: - H&E.

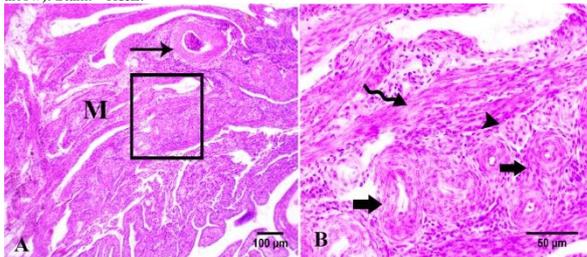


Fig. 4 A Photomicrograph of the ovarian medulla (M) of six months female turkey. A) showing ovarian medulla (M) with large blood vessel. B) represent the squared area in A and showing small blood vessels (thick arrows), bands of smooth muscle fibers (zigzag arrow), and interstitial pale eosinophilic polyhedral cell clusters (arrowhead). Stain H&E.

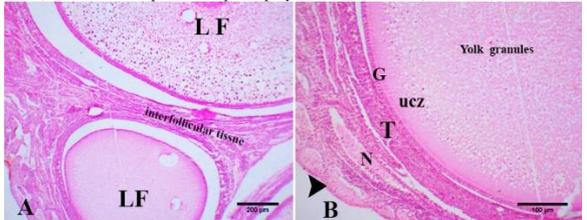


Fig. 5 A Photomicrograph of mature (one year) female turkey. A) showing large growing follicles protruding at the cortex (LF) and separated with narrow interfollicular connective tissue. B)- showing simple squamous ovarian surface epithelium (arrowhead). B) Showing the large growing follicle was lined with follicular epithelium "stratified granulosa cells" (G) and surrounded with theca interna and externa (T), the follicle contained yolk granules that were surrounded by undifferentiated cytoplasmic zone (UCZ), which separated the yolk granules from the follicular wall nerve bundle (N). Stain: A&B - H&E.

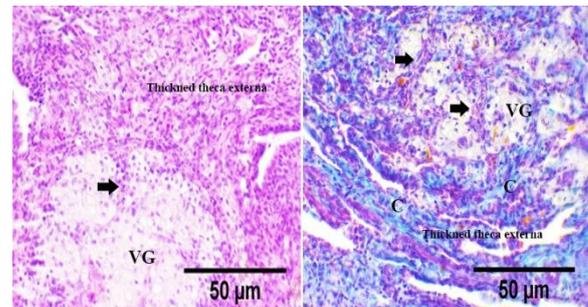


Fig. 6 A photomicrograph of the postovulatory regressed follicle in one-year female turkey. A)- Showing groups of central vacuolated granulosa cells (VG) interrupted with strands of theca interna (thick arrows) and surrounded with peripheral thickened theca externa layer. B)- Showing collagen fibers (C) in the thickened theca externa. Stain: - A)-H&E. B)- Crossman's trichrome

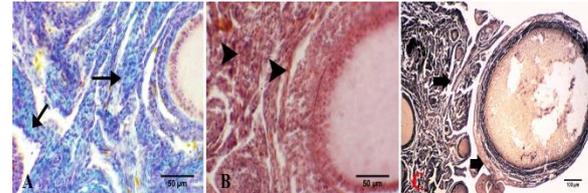


Fig. 7 A Photomicrograph of immature (6 months) female turkey showing the presence of thick interfollicular connective tissue fibers between ovarian follicles. A)- Showing collagen fibers (arrows). B)- showing elastic fibers (arrow heads). C)showing reticular fibers (thick arrows). Stains: - A)- Crossman trichrome. B)- Wiegert elastic. C)- silver stain.

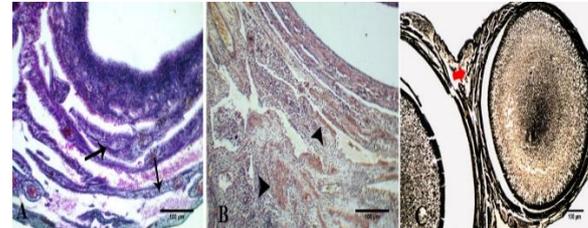


Fig. 8 A Photomicrograph of mature (one year) female turkey showing regression of the connective tissue fibers between large follicles. A), showing few collagen fibers (arrows). B), showing elastic fibers (arrow heads). C)- showing reticular fibers (red thick arrow). Stains: - A)- Crossman trichrome. B)- Wiegert elastic. C)- silver impregnation

4. DISCUSSION

In our result noticed that, well developed left ovary than right ovary. Due to ovarian tissue development .This results in disagreement with kheirabadi et. al. (2014), who reported that the left and right ovaries in ostriches were equally developed. The anatomical result becomes grossly clear at 26 days.

The histological results indicated that the right ovary has no cortex and only a medulla was noticed. These differences in size and structure between left and right ovaries confirmed the asymmetric development. The ovary of turkey, in our result was composed of different follicles in various stages of age (Immature, Mature) e.g., primordial, primary, secondary, tertiary (mature graafian) follicles, this agreed with anatomical observations of developing germ cells in several species (Pepling and Sprading, 1998; Mork et al., 2012).

In our result reported that, thick cortex in immature age than mature age which has thin cortex. This accord with the one-week-old chick left ovary with a thick cortex and mini medulla. In our result noticed that presence of postovulatory follicle which granulosa cells (white color internally) and theca cells (red color externally) become vacuolated and follicular wall regresses completely and resemble corpus luteum in primates and other birds, this resembles to luteal cells on the theca interna cells surrounding them. types of stromal endocrine cells: Some refer to them as theca gland (Hodges, 1974; Apperson et al., 2017). Phillips et al. (1985) related these cells to steroids' production. In our result noticed that presence of

atretic follicles at one year of age, this result agrees with Mfoundou (2021), who reported that, atretic follicles at different regressed stages were present as the chicks grew in age from 3-week-old. In our result noticed that presence of the ovarian follicles showed advanced stage of growth and increased in size leading the expansion of the cortex at one year age, this resembled to Mfoundou (2021), who mentioned that the cortex of the 3-months-old chick's left ovary continued to increase in size due to ovarian follicle development.

5. CONCLUSION

This study showed the ovary of female turkey at one year of age contained ovarian follicles at advanced stage of growth and also Postovulatory regressed follicle that resembles corpus luteum. So, the age of laying and maturation in turkey is nearly at one year of age.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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