



## Histomorphological Study of the Ovaries and Follicles Growth in Adult Female Albino Rats

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### Abstract

The Ovary plays a vital role in the reproductive process and this study was carried out in order to investigate the structure of ovaries of the female mature rat. Ovaries were obtained by direct incision on the abdominal cavity and were then divided into two sections and fixed in buffered formalin. The sections (5-7  $\mu\text{m}$ ) were cut in paraffin blocks, stained with Mayer's haematoxylin stain, periodic acid Schiff (PAS) technique. In histological examinations it was composed of two main zones, cortex and medulla. The surfaces of the ovaries were wavy and lined by simple cuboidal or columnar epithelium. The tunica albuginea consisted of connective tissue fibres. There were connective tissue cells in the cortex, as well as interstitial cells containing. Primordial, primary, secondary, tertiary follicles, corpus luteum and atretic follicles were seen in the cortex of ovaries. Mean diameters of these follicles varied between 80 and 1600  $\mu\text{m}$ . Medulla consisted of loose connective tissue which contained blood and lymph vessels of varying sizes. The diameter of ovarian follicles in the cortex was measured as primordial  $36.5 \pm 1.31 \mu\text{m}$ , primary single layer  $60.58 \pm 2.68 \mu\text{m}$ , primary multi-layer  $167.84 \pm 5.59 \mu\text{m}$ , secondary with C-shaped antrum  $193.83 \pm 75.71 \mu\text{m}$  and graafian  $389.62 \pm 9.55 \text{mm}$ .

### Introduction

Ovaries a pair of ovary is located in the pelvic region whose primary function is to nurture and prepare oocytes (eggs) for the process of ovulation. These processes of ovulation and fertilization are controlled largely by cells of the ovaries that produce and secrete hormones namely estrogen [1]. The ovary is situated within peritoneum. It is also called as "broad ligament". Usually close to the lateral wall of the pelvic cavity [2]. Because, the ovary extends into the peritoneum cavity; ovulated eggs briefly reside within the peritoneal cavity before the capture by the oviducts [3]. A large bursa ovarica surrounded the ovary completely [4].

Rat ovary is divided into an outer cortex and inner medulla. But, in general, ovary development stages or ovary maturation stages can be evaluated [5]. Morphological development of the ovaries is often different with histological analysis because ovarian development cannot be described accurately [6]. The ovaries are responsible for the differentiation and release of the mature oocyte for fertilization and successful propagation of the species [7]. The ovary was dividing into three functional units: the follicles, corpora lutea and interstitial

stromal tissue. All three units of ovary under effect of gonadotropin of anterior pituitary gland. All three units possessed the capacity to synthesize hormonal substances, especially steroids hormones, that secret from the [8]. The development of ovarian follicles is considered to be regulated by various factors such as gonadotropin that released from anterior Pituitary. In mammals, the ovarian follicles consisted of an innermost oocyte, which surrounding by granulosa cells layer and outer layer of thecal cells. The oocyte grew and matured inside within the follicular fluid [9].

Type of ovarian follicles, Primordial follicles The origin of oocytes and primordial follicles during early embryonic development has been reviewed, [10, 11]. Primordial follicles and their oocyte when grew, they moved deeper into the cortex and each composed of a small immature and quiescent oocyte and surrounded by single layer of flattened granulosa cells (pre-granulosa cells) that enveloped by a basal lamina [12, 13]. The primary follicles were contained primary oocyte that surrounded by single layer of cuboidal follicular epithelial cells. The primary follicle was seen at the onset of the

growth of the pre granulosa cells, which were the simple squamous epithelium rest on basement membrane opposite the stromal cells that gives origin to the theca cells [14] Secondary follicles The development of the primordial follicle to a preantral follicle basically involves cellular growth, proliferation and differentiation.

But, the mechanisms that control the differentiation of pregranulosa to granulosa cells during activation of the primordial follicles are unknown, [15]. The columnar cell monolayer is proliferates and forms a multilayered zone of granulosa cells (zonagranulosa), around the oocyte.

### Graffian Follicle

The beginning of the formation of the antrum, the formation of the zonapellucida and the differentiation of the theca externa marks a tertiary follicle, [16].The tertiary follicles may be subdivided into small antral follicles which contain more than six layers and over 250 granulosa cells, [17].It was characterized by a follicular antrum, which surrounded by interstitial cells of both theca interna and externa layers. These cells that surrounded the oocyte and near the zona pellucid were called corona radiata cells [18].

## Materials and Methods

### Animals and Study Design

Twenty female albino rats were selected to process this study. The collected animals were mature rats.

### Dissection of Animals

Each selected animals was euthanized by intramuscular injection of ketamin. The animal was placed on dorsal recumbency to view the ventral aspect, thereafter; a mid-line abdominal incision was made to expose the structures in the peritoneal cavity. The reproductive organ exposed and both ovaries were observed and photographed in situ and later dissected out. For the histological technique

### Preparation of Histological Specimens

The ovaries as a whole were dissected and washed with normal saline and then by 10% formalin fixed for 72 hrs. Next to fixation, specimens were dehydrated through ascending series of ethyl alcohol (70%, 80%, 90% and 100%) each for 2 hrs, then cleared with xylene for ½ hr. Specimens were

infiltrated with paraffin wax (58-60 °C ) then embedded with paraffin wax to obtain blocks of paraffin. Paraffin sections of six microns were obtained by using rotary microtome. General and special stains were used to stains the tissue sections such as hematoxyline-eosin (H & E), Periodic acid Schiff (PAS) [10].

### Micromorphometric Measurements

The diameter of the all type follicles were measure Follicles of ovary were photograph during the colour USB 2.0 digital image system (Scope Image 9.0) which is provided with image processing soft ware applied for the measurements of follicular diameters.

### Statistical Analysis

All data of both and micromorphometric measurements were analyzed by ANOVA and t - test using SPSS software (version 14).

## Results

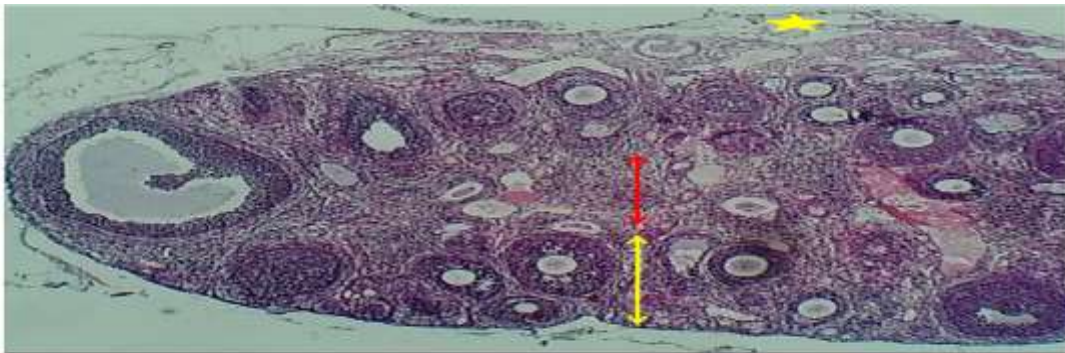
Generally, ovary was covered with a fibrous capsule and showed two compartments viz. peripheral zone or cortex and the medulla. The peripheral zone or cortex is very important part consist of many developing follicles of different sizes and morphology. The medulla forms a blood network. The ovary and oviduct connected by hilus region. In the Figure 4, degenerated follicles were observed. In the stroma, corpus luteum and albicans were scattered. Fig.1.

The ovary large in size and more elongated and covered by single layer of simple cuboidal epithelium cell represent in germinal epithelium and increase of the thickness of dense irregular connective tissue and continuous with tunica albugenia, numerous small primordial, primary follicles, early primary follicle secondary and preantral follicles and less amount for large follicle which is represent antral follicles filled with the fluid and single cavity were identified filling the outer cortex region of the ovary Fig 3 and Fig 5, graffian follicle identified at this age group about 2-3 follicles in the most section of mature ovaries. Fig 2.

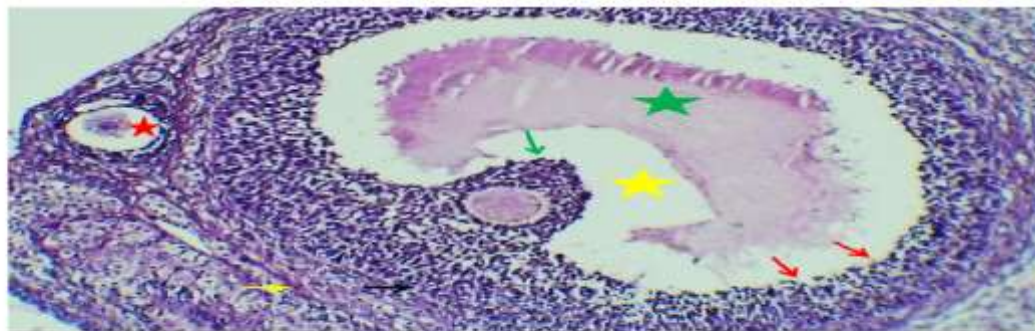
The ovarian follicles are classified into primordial, early primary, late primary and graffian follicle. We measured follicles at every stages of development. In adult stage, all four stages of follicles were observed. The size of primordial, early primary, late primary and graffian follicle were  $36.5 \pm 1.31$ ,



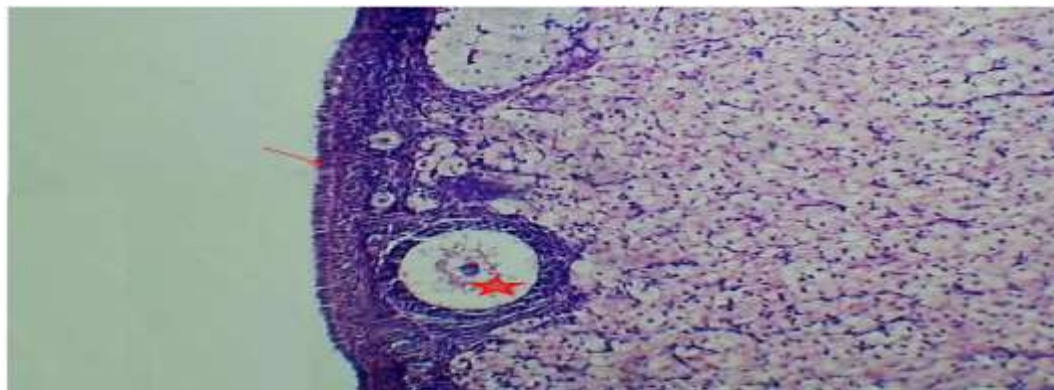
60.58±2.68, 167.84±5.59 and 389.62±9.55mm, respectively.



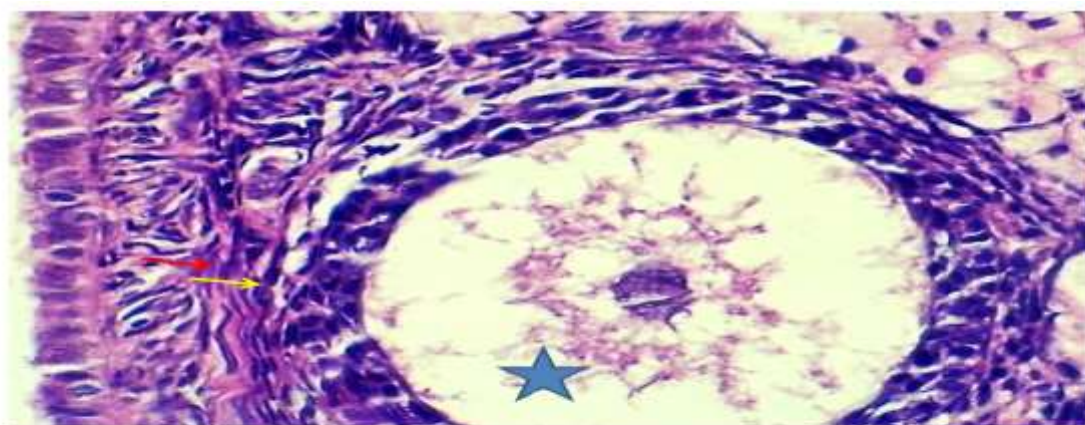
**Fig. 1 :** Ovary of female mature rat showed, part of mesovarium (yellow star), cortex (yellow double heads arrows) showed different types of follicles, medulla (red double heads arrows), blood vessels (blue arrows). H&E, X10



**FIG. 2 .** Ovary of mature rat showed graafian follicles in late stage follicle: corona radiata (green arrow), antrum (yellow star) follicular fluid green star , granulosa (red arrow), theca interna (black arrow), theca externa (yellow arrow), secondary follicle (red star). H&E, X10

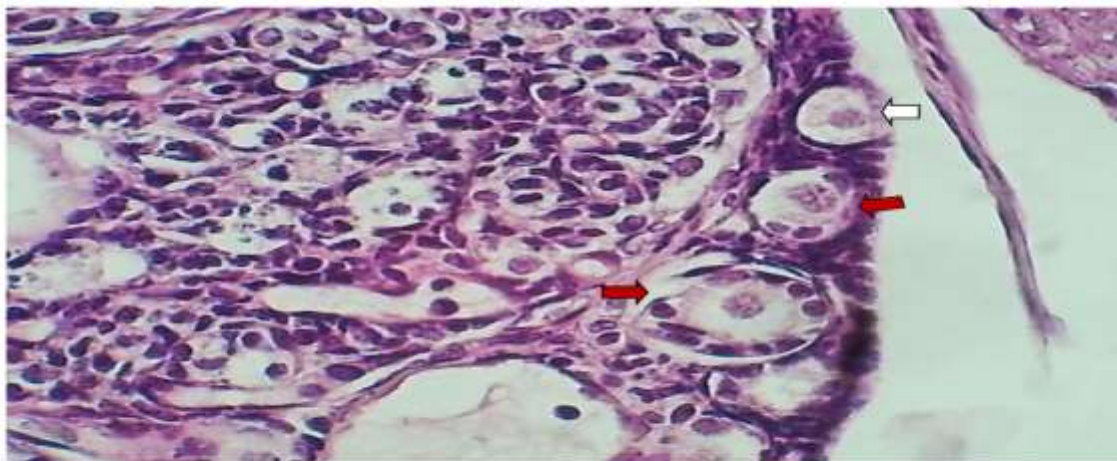


**Fig. 3: Ovary of mature showed epithelia (red arrow) secondary follicles (red star ) primary follicles, antrum red star , . H&E, 10 X**

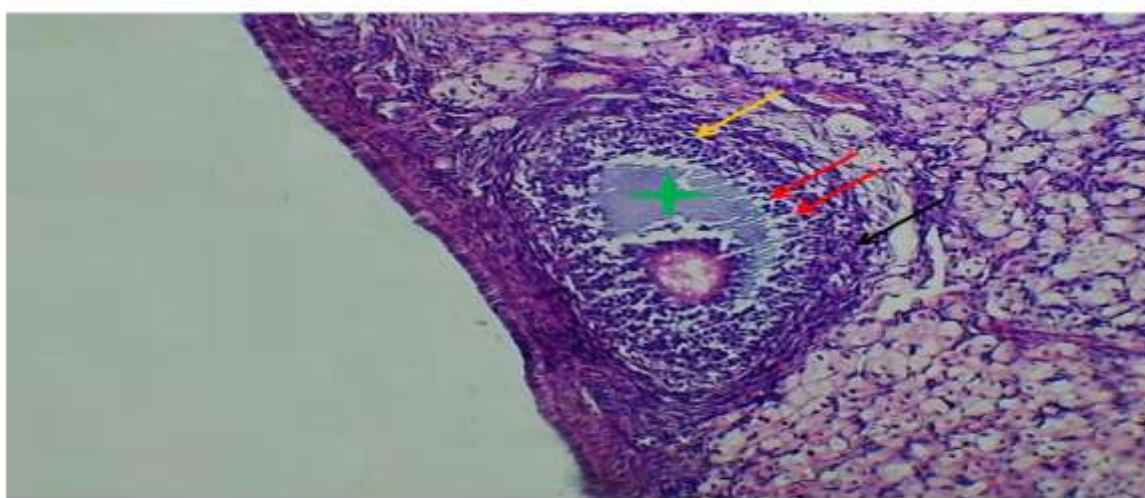


**Fig. 4 :** Ovary of mature rat showed secondary follicle granulosa , theca interna (yellow arrow), theca externa (yellow arrow) , cavities formation called antrum pockets (blue arrows), X40, H&E





**Fig.5 : Adult mature demale rat Different stage follicle white arrow primordial , red arrow early primary 40x H&E**



**Fig.6 : Ovary of mature female rat showed secondary large follicles last stage granulosa (red arrow), theca interna (yellow arrow), theca externa (black arrow), antral pocket formation (green star), antrum (blue star). X10, H&E**

**Primary Follicles**

They are observed in the Figure5. Oocytes are surrounded by the zona pellucida followed by a granulosa contents a single layer of cuboidal cells. Membrane of Slavjansky a basement membrane was appeared. The small primordial follicles (27.5µm diameter) were surrounded by 8-10 follicular cells. The diameters of oocytes were 16.3µm.

Few primary follicles appeared surrounded by some stromal cells. This envelope could be the undifferentiated theca cells, the Theca folliculi. Some primary growing follicles were recognisable by proliferating granulosa cells. Fig 1 .*Secondary follicle or preantral follicle* the follicles were centered by the many oocytes. Each oocyte contains a central nucleus with many nucleoli and germinal vesicle. The sizes of the oocyte dose not vary on the preantral follicle. When stained with

periodic acid-Schiff, the small oocytes, the granulosa was about 2-3cells thick and zona pellucida appeared clearly stained Fig 2. The enlarged granulosa was seen in the preantral follicles and they are homogeneous. The proliferating follicular cells showed an intense mitotic activity. As the preantral follicle nurtured, the division of ganulosa cell continued to form a stratified epithelium layer about 2-12 layer cells thick (Fig 3).

The theca interna differentiated when the dividing preantral follicle reached to225µm diameter with 6-12 cells and the antral cavity began to be formed. Inside the largest healthy follicles (275µm diameter), the inner cells of the theca folliculi were composed of large spindle shaped or polyhedral cells with elliptical or oval nuclei and forms an epithelioid layers of 3-4 cells layers. The outermost follicular cells were flattened, concentric arrangement and entangled in a network of connective fibers.

This part was well vascularized and formed the wall of the follicle; the theca folliculi differentiated into theca interna and theca externa. Irregular spaces appeared among the cells of granulosa, filled with follicular fluid.

### Tertiary Follicle or Antral Follicle

The largest healthy preantral follicles developed from the early small antral stage. The antrum, a single cavity, was formed by the proliferation of cells and the accumulation of fluid. Follicle morphology changed gradually Fig 2. The oocyte rapidly grew to about 70  $\mu\text{m}$ . The volume of antrum increased by confluence of the intercellular spaces. Some granulosa cells surrounding the oocyte showed close association between them, forms the cumulus oophorus, the compact cumulus cell-oocyte complex.

The mural granulosa cells were formed by the peripheral granulosa layer cells near the basement membrane. In the theca, the theca interna seemed more distinct than previously (Fig 6). The large, healthy antral follicles developed from the maturing follicles to form preovulatory follicles (Fig 6). In psammomys, healthy, largest antral follicle in terminal growth, were also observed. The preovulatory follicles were distinguished by large size (34000 $\mu\text{m}$  diameter) these oocytes occupied the centre of the follicle but the germinal vesicle became eccentric in position. The granulosa cells surrounding the oocyte formed a ring called the corona radiata. The oocyte was embedded in the cumulus area that became limited by the enlarging antrum. The mural granulosa was clearly seen.

Ovulatory follicle. Ovulatory follicles were observed during estrus. The healthy largest follicles in terminal growth were mature follicles or graafian follicles fig 2. They occupied a large part of the ovarian cortex and were nearly to the free surface of the gonad. In Psammomys, graafian follicles were characterized by the presence of secondary oocyte, indicating the germinal vesicle get the breakdown. This follicle was observed after LH surge. At this stage, the cumulus cell-oocyte complex formed a thick region protruding into the antrum and the connection with other granulosa cells was reduced. In the follicular wall changed many changes were occurred. The basement membrane became blurred; simultaneously

theca blood vessels were deeply penetrated in the theca interna. The follicle region was near the surface of the ovary become thinner. The germinal epithelium became squamous and ovulation will be take place at this level. In the psammomys obesus, the ovulation process occurred in a cyclic manner each 51 days in the normal estrous cycle. At this time, the oocyte surrounded with the cumulus oophorus was found in the oviduct lumen. The ruptured follicles were observed in the ovary with mural granulosa and the theca cells

### Discussion

The ovary of adult mammals is divided into outer highly cellular cortex and inner medulla which consisted mostly of a richly vascularized loose connective tissue. The cortex of rat ovary is covered by simple cuboidal epithelium (called germinal epithelium) [19]. The ovaries were covered with epithelium, usually consisted of simple cuboidal tissue. In the present study, ovaries showed cuboidal epithelial cells. Our findings are accordance with this report.

The cortex was occupied with different types and various, sizes of follicles [20]. The ovaries were covered with epithelium, usually consisted of simple cuboidal tissue. The cortex was occupied with different types. The ovarian cortex covered by simple squamous to simple cuboidal cells, which were used to be a germ cells source [21]. The underneath of this epithelium, tunica albuginea is present, which is fused into the ovarian stroma of loose connective tissue and ovarian follicles. The medulla contained both the blood and lymph vessels and nerves [22].

The cortex, which was surrounded on the outside by the surface epithelium, contained germ cell clusters, some primordial, primary, secondary, tertiary and atretic follicles, and dilated blood vessels. Primordial follicles in the ovary of the porcupine were found to be distributed under tunica albuginea and extended deeply for a short distance into the cortical tissue [23]. In the present study, histology of the ovary showed similar findings. Previous work done by [24] on female rats, it was found that the ovaries of adult rat consisted of various stages of the developed follicles which included the primary, secondary and graafian follicles in addition to degenerated corpus luteum.

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