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Histocytological Changes in the Wistar Rat Ovary at Different Pubertal Stages and Pregnancy

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ABSTRACT

The ovary is a vital organ in the female reproductive system, responsible for housing follicles, which contain oocytes essential for procreation. Although many studies have examined specific aspects of ovarian development in Wistar rats, there is a need to compare microanatomical features of the Wistar rat ovary across developmental stages. This study provides comparative microscopic details of the ovary across three key developmental stages: pre-puberty, puberty, and pregnancy. A total of twenty Wistar rats were procured. Three of the female rats gave birth; their pups were nurtured and then assigned to the pre-pubertal and pubertal groups. Ovarian tissues were collected at day 21, post-vaginal opening, and designated gestational days. Pre-pubertal ovaries were predominantly composed of primordial, primary, secondary, and tertiary follicles, with the absence of corpora luteum or pre-ovulatory follicles; the stroma was dense, cellular, showing spindle to plump cells with oval nuclei. In contrast, pubertal ovaries exhibited a full spectrum of follicular development, presence of pre-ovulatory follicles and corpora lutea indicative of ovulation; the stroma was loose and showed predominance of plump cells. During pregnancy, the ovarian structure was characterized by large corpora lutea occupying most of the ovarian space, accompanied by reduced folliculogenesis and pronounced vascular expansion. The corpora lutea cells were large, polygonal; their cytoplasm was abundant, eosinophilic to clear, and varied from granular to vacuolated as the pregnancy progressed. This study highlights changes in the histoarchitecture and cytoarchitecture of the ovary across the three main developmental stages of female reproductive life.

Keywords: microanatomy, Wistar rat, ovary, pre-puberty, puberty, pregnancy

INTRODUCTION

The ovary is a key organ in the female reproductive system that undergoes significant structural and functional changes throughout different developmental stages. As the primary female gonad, it is responsible for the production of oocytes and sex hormones that regulate reproductive function and maintain secondary sexual characteristics[1,2].

The Wistar rat (*Rattus norvegicus*) has been extensively used as an experimental model in reproductive research due to its relatively short reproductive cycle, well-documented physiological characteristics, and anatomical similarities to human reproductive systems[2,3]. Understanding the microanatomical changes in the Wistar rat ovary across different developmental stages provides valuable insights into mammalian reproductive health and has significant implications for comparative studies in human reproduction.

The microanatomical study of the ovary encompasses various structural components, including the germinal epithelium, tunica albuginea, cortex, medulla, stroma, follicles at different developmental stages, corpus luteum, and vasculature. Each of these components undergoes specific changes during pre-puberty, puberty, and pregnancy, reflecting the functional adaptations of the ovary to meet the reproductive demands at each stage[1,4,5].

During pre-puberty, the ovary undergoes initial follicular development. The pubertal period marks a critical transition characterized by significant hormonal changes, increased follicular development, and the onset of ovulatory cycles. Pregnancy introduces further dramatic changes to ovarian microanatomy, particularly in corpus luteum development and function[1,6,7].

Numerous studies have examined specific aspects of ovarian development in Wistar rats; however, there is a need for a comprehensive study that examines and compares the microanatomical features of the Wistar rat ovary across these three critical developmental stages. This is essential for establishing baseline histological parameters, understanding normal developmental processes, and providing reference data for experimental studies investigating reproductive disorders or the effects of various substances on ovarian structure and function. This study aimed to provide a detailed comparison of the microanatomical features of the ovary in the female Wistar rat across the three main developmental stages of the reproductive life cycle.

MATERIALS AND METHODS

Fifteen mature female rats with regular 4- to 5-day estrous cycles were selected for breeding. Females were paired overnight with the fertile male rats (3 females to 1 male), and the presence of a vaginal plug or sperm in the vaginal smear the following morning was considered evidence of successful mating, and that day was designated as day 1 of pregnancy[8]. Pregnancy was subsequently confirmed by progressive weight gain and gradual abdominal distension. Animals were maintained under standard housing conditions throughout the pregnancy period. On days 1, 3, 6, and 12 of gestation, the pregnant rats were euthanized with 20 mg/kg ketamine intramuscularly.

Three pregnant rats were transferred to individual cages for parturition. Following delivery, six pups were successfully weaned at postnatal day (PND) 14. These pups were then assigned to either the pre-pubertal or pubertal experimental groups. All animals were provided with standard laboratory chow and tap water *ad libitum*.

The following inclusion criteria were applied:

1. Pre-pubertal Group: Pups aged 14 days (PND 14) exhibiting an absence of vaginal opening and no signs of illness or developmental abnormalities.
2. Pubertal Group: Rats exhibiting recent vaginal opening (within the preceding 7–10 days) and a body weight between 120g and 140g, consistent with normal developmental parameters for this stage[9].

Animals meeting these specific criteria were humanely euthanized with 20mg/kg ketamine intramuscularly. A midline abdominal incision was made to expose the reproductive organs. The ovaries were collected, fixed in 10% neutral buffered formalin, and processed for hematoxylin and eosin staining. Slides were examined using a light microscope (Olympus CH-2), and regions of interest were captured with a camera (Hayear HY-1139) attached to the microscope using AmScope software. White balance correction was performed using Adobe Photoshop Lightroom (v1052). The study protocol was reviewed and approved by the University of Ilorin Ethical Review Committee (UERC/ASN/2024/3012).

RESULTS

The microanatomical examination of pre-pubertal Wistar rat ovary revealed distinctive structural features characteristic of this developmental stage. Different populations of follicles (including primordial, primary, secondary, and tertiary follicles) were present in the cortex (Figure 1). The primordial follicles were characterized by a single layer of flattened granulosa cells, with spindle nuclei, surrounding the oocytes (Figure 1B). Primary follicles were characterized by 2 to 3 cell layers of granulosa cells surrounding large ova (Figure 1B). Secondary follicles were characterized by 4 to 5 cell layers of granulosa cells without an antral cavity (Figure 1C). Early tertiary (antral) follicles were characterized by the presence of small fluid-filled spaces within the stratified granulosa cell layers (Figure 1D). The cytoplasm of the granulosa cells was scanty and eosinophilic. Notably, no preovulatory follicles or corpora lutea were observed in any of the pre-pubertal ovarian specimens. Limited vascularity was observed. The ovarian stroma consisted of spindle to plump cells with oval nuclei and abundant eosinophilic cytoplasm (Figure 1E).

The pubertal Wistar rat ovary showed structural differences when compared to the pre-pubertal stage. The cortical region of the pubertal ovary contained follicles of all stages of development, including primary, secondary, tertiary, and preovulatory follicles, but not primordial follicles (Figure 2A). Tertiary (antral) follicles were more numerous compared to the pre-pubertal stage. A distinctive feature of the pubertal ovary was the presence of corpora lutea. These corpora lutea were composed of polygonal cells with abundant eosinophilic cytoplasm (Figure 2B). The ovarian stroma in the pubertal ovary was loose and showed increased vascularity compared to the pre-pubertal stage (Figure 2C).

On gestational day 1, a few antral follicles were present (Figure 3A). The granulosa cells were stratified and polygonal, with round-to-oval nuclei (Figure 3C). Theca interna cells were luteinized. Their cytoplasm was abundant and granular. The corpora lutea of pregnancy were seen as focal collections of luteinized cells surrounded by thin fibrovascular septae (Figure 3A). The luteinized cells were large, polygonal with round to oval nuclei and abundant eosinophilic to pale granular cytoplasm (Figure 3B). There was increased vascularization, with congested vascular channels.

On gestational day 3, the population of corpora lutea increased progressively. The cytoplasm of the luteal cells was eosinophilic and granular (Figure 4B). Corpus luteum cysts, i.e., cystic spaces containing eosinophilic material lined by luteinized granulosa cells, were seen.

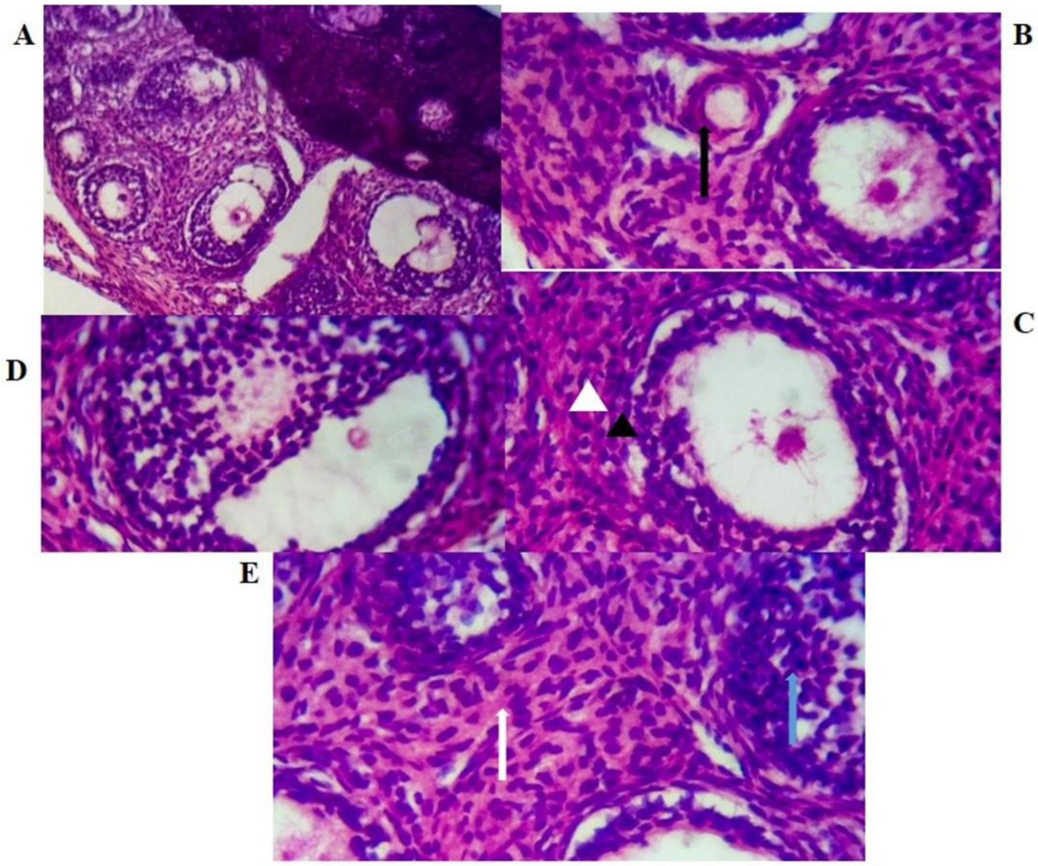

Figure 1. A: Photomicrograph of the pre-pubertal ovary showing different populations of follicles (H&E, x40) B: A primordial follicle (black arrow) with a layer of flattened granulosa cells surrounding the oocytes (H&E x400). C: Secondary follicle with multiple layers of granulosa cells (H&E x400). Theca interna (black arrowhead) & theca externa (white arrowhead) D: Tertiary follicle with antrum (H&E x400). E: Pre-pubertal ovarian stroma, showing spindle-shaped to plump cells with oval nuclei (white arrow); granulosa cells (blue arrow) (H&E x400).

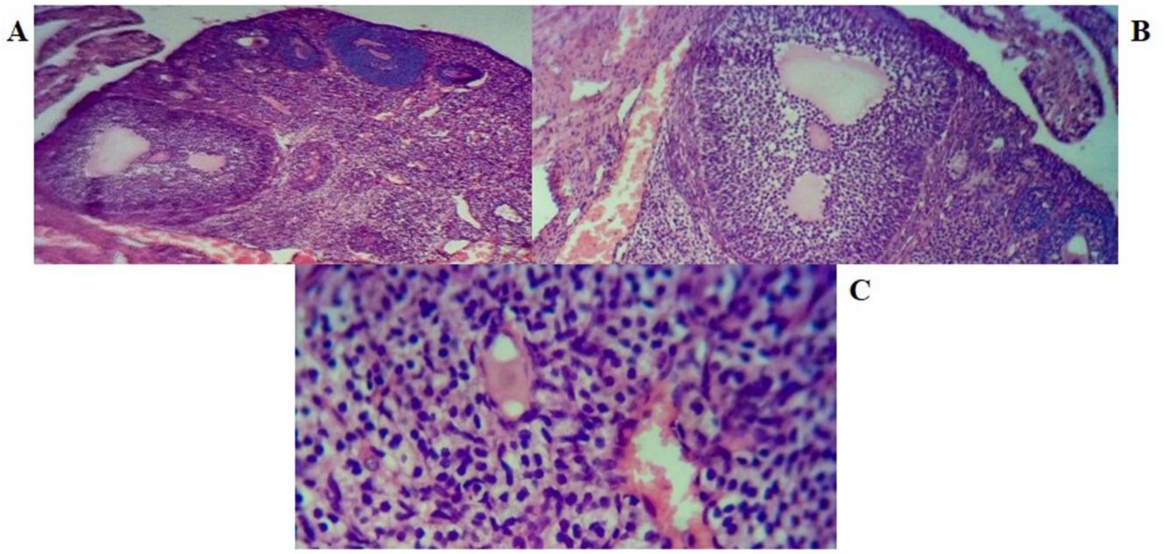

Figure 2. A: Photomicrograph of the pubertal ovary showing its cortical region (H&E x 40). B: Corpus luteum containing eosinophilic secretion (H&E x 100). C: Loose stroma of pubertal ovary with increased vascularity at (H&E x 400).

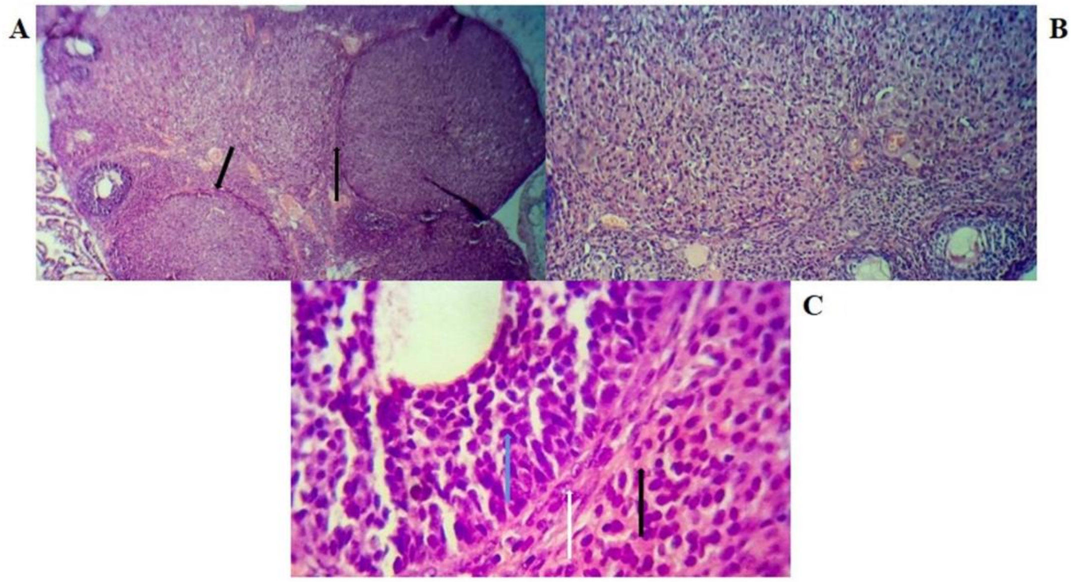

Figure 3. A Photomicrograph showing the ovary of a pregnant rat at day 1. Fibrovascular septae (black arrow) (H&E x40). B: Corpus luteum at day 1(H&E x100). C: Theca externa (black arrow) & interna (white arrow), granulosa cells (blue arrow) (H&E x400).

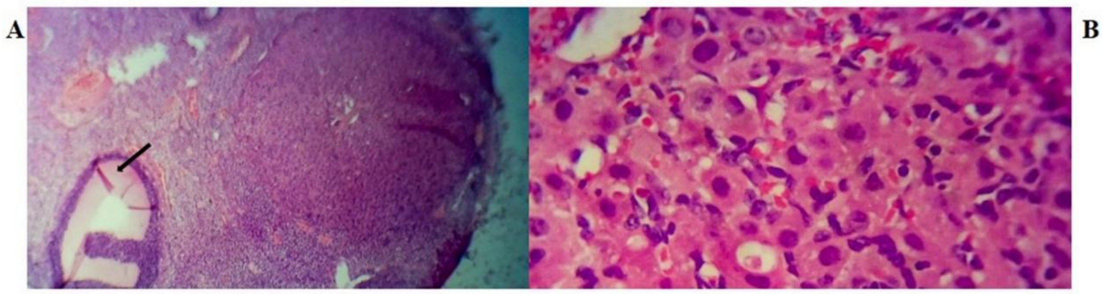

Figure 4. A: Photomicrograph showing corpus luteum and corpus luteum cysts (black arrows) at day 3 (H&E x100). B: Luteinized cells with abundant granular cytoplasm (H&E x400).

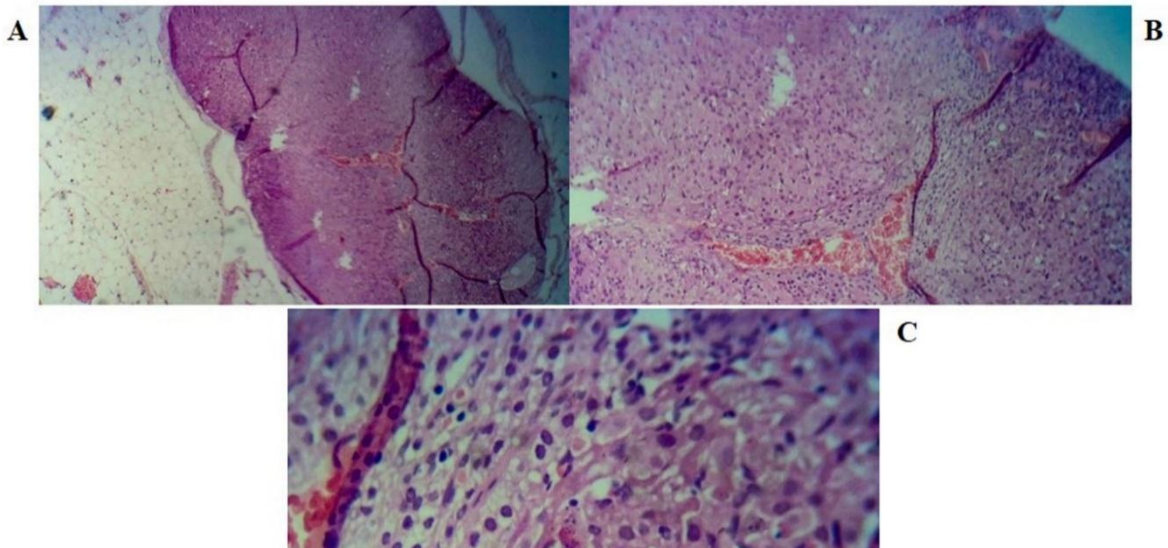

Figure 5. A: Photomicrograph showing corpora lutea at day 6 (H&E x40). B: Corpora lutea (H&E x100). C: Corpus luteum at day 6, some of the cells showing vacuolated cytoplasm (H&E x400).

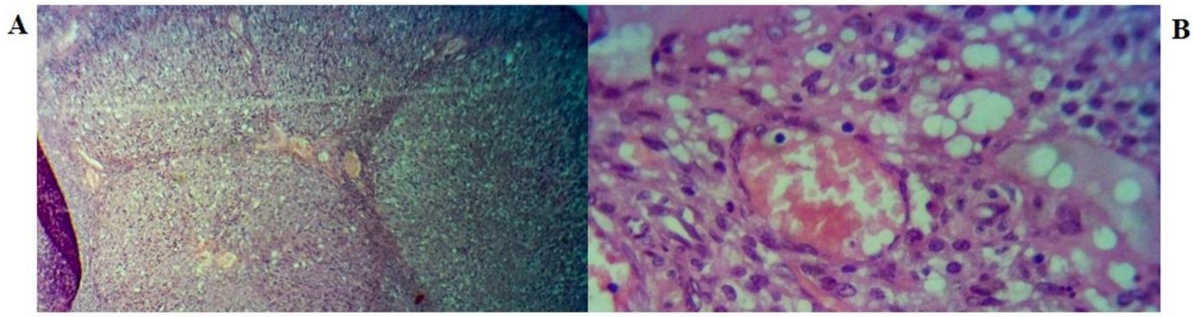

Figure 6. A: Photomicrograph showing corpora lutea at day 12 (H&E x100). B: A congested vascular channel surrounded by luteinized cells with vacuolated cytoplasm (H&E x400).

On gestational day 6, the corpus luteum cysts were absent. The corpus luteum population increased. The cytoplasm of the luteal cells was eosinophilic and vacuolated (Figure 5C). On gestational day 12, the corpora lutea occupied most of the ovary (Figure 6A). The cytoplasm of the luteal cells showed large vacuoles (Figure 6B).

DISCUSSION

The presence of primordial, primary, secondary, and early tertiary follicles in the pre-pubertal ovary indicates that initial follicular recruitment and development occur independently of the pubertal surge in gonadotropins. The early follicular growth is gonadotropin-independent and primarily regulated by intra-ovarian factors[10,11]. The absence of preovulatory follicles and corpora lutea further confirms the pre-reproductive status of these animals. The limited vascularization observed in the pre-pubertal ovary reflects the relatively low metabolic demands of this developmental stage. The stromal organization is critical for creating the microenvironment necessary for follicular maturation and for facilitating the physical changes associated with follicular growth and eventual ovulation. The presence of various populations of follicles is consistent with the concept that the ovary contains a finite pool of primordial follicles established during fetal development, which serves as the reservoir for future follicular recruitment throughout reproductive life[12].

The appearance of pre-ovulatory follicles in the pubertal ovary represents a key milestone in ovarian maturation. These follicles with their distinctive morphological features reflect the final stages of follicular development in response to the preovulatory surge of luteinizing hormone (LH). This indicates that the hypothalamic-pituitary-gonadal axis has matured sufficiently to support ovulation[12,13]. The presence of corpora lutea is another distinctive feature of the pubertal ovary. It provides definitive evidence that ovulation has occurred and that the post-ovulatory phase of the reproductive cycle has been established[12]. The observed enhanced vascularization reflects increased metabolic demands associated with follicular development and corpus luteum function.

The pubertal transition represents a period of profound microanatomical remodeling in the ovary, characterized by accelerated follicular development, the establishment of ovulatory function, corpus luteum formation, and enhanced vascularization. These changes reflect the integration of hormonal signals from the hypothalamic-pituitary axis with local regulatory factors within the ovary, thereby establishing the reproductive cycle and the capacity for fertility. The shift in follicular distribution, characterized by a relative decrease in primordial follicles and an increase in growing follicles, particularly tertiary and preovulatory follicles reflects the accelerated recruitment and development of follicles under the influence of rising gonadotropin levels[12,5].

The microanatomical features observed in the ovaries of pregnant Wistar rats reveal dramatic adaptations associated with the maintenance of pregnancy. The most striking feature of the pregnant rat's ovary is the presence of large corpora lutea of pregnancy, which dominate the ovarian parenchyma and occupy the ovarian volume. The significant hypertrophy of luteal cells and the cytoplasmic granularity/vacuolation reflect their enhanced steroidogenic capacity and are consistent with the high progesterone levels required to maintain pregnancy [14].

There are profound microanatomical adaptations in the ovary during pregnancy, characterized by luteal hypertrophy, enhanced vascularization, and suppressed follicular development. These changes reflect the shift in ovarian function from cyclical follicular development and ovulation to sustained luteal activity that maintains pregnancy. The remarkable plasticity of the ovary in responding to the hormonal signals of pregnancy underscores its critical role in reproductive success. It highlights the complex interplay between systemic hormones and local regulatory factors in modulating ovarian structure and function. The state of temporary anovulation in pregnancy is to prevent the establishment of additional pregnancies[15,16].

CONCLUSION

In conclusion, this study has compared ovarian microanatomy across key developmental stages in the female Wistar rat, documenting the progressive changes in tissue architecture, cellular composition, and functional organization that accompany reproductive maturation and adaptation.

Conflict of interest: All authors have no conflicts of interest to declare

Authors' contribution: KMI conceptualized and designed this study; KMI, RAL, SAA, NTI, and MAL collected and analyzed the data; RAL, KMI, and OAA wrote the manuscript. All authors proofread the manuscript.

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