Research Article

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Changes in structure during the corpus luteum's formation

Korpus luteum oluşumu sırasında meydana gelen yapısal değişiklikler

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Abstract

Purpose: Our study aims to investigate whether new follicles form among fibroblast-like cells in the region after the restructuring of the surface epithelium and tunica albuginea adjacent to the corpus luteum. Additionally, another goal is to demonstrate the structures developed from the follicles and the histological changes in the ovary.

Materials and methods: Histological sections of the corpus luteum, formed from the Graafian follicle after ovulation, were prepared from ovarian tissues of 12-14 months old Wistar albino rats.

Results: When the corpus luteum reaches a high volume, the number of fibroblast-like cells in the tunica albuginea is quite low. Subsequently, it has been observed that the number of fibroblast-like cells in the tunica albuginea rapidly increases, and these cells form concentric arrangements of collagen fibers. Additionally, the formation of primordial and primary follicles between the surface epithelium and the tunica albuginea adjacent to the corpus luteum has been observed.

Conclusion: Examining the ovary as a whole and investigating the developmental processes of structures can assist in gaining a better understanding of the dynamics of this organ.

Keywords: Corpus luteum, ovary, tunica albuginea, follicles.

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Öz

Amaç: Çalışmamız, korpus luteum'un bitişiğindeki yüzey epiteli ve tunika albugineanın yeniden yapılanmasından sonra bu bölgedeki fibroblast benzeri hücrelerin arasında yeni foliküllerin oluşup oluşmadığını araştırmayı amaçlamaktadır. Ayrıca, foliküllerden gelişen yapıları ve ovaryumdaki histolojik değişiklikleri göstermek de bir başka hedefimizdir.

Gereç ve yöntem: Ovulasyondan sonra graaf folikülünden oluşan korpus luteum'un histolojik kesitleri, 12-14 aylık Wistar albino tipi sıçanların ovaryum dokularından alınarak hazırlanmıştır.

Bulgular: Korpus luteum yüksek bir hacime ulaştığında, tunika albugineada bulunan fibroblast benzeri hücrelerin sayısı oldukça düşüktür. Daha sonrasında, tunika albuginea bulunan fibroblast benzeri hücrelerin sayısının hızla arttığı ve bu hücrelerin oluşturduğu kollajen liflerin konsantrik dizilim oluşturdukları gözlemlenmiştir. Ayrıca, korpus luteumun bitişiğindeki yüzey epiteli ve tunika albuginea tabakası arasında primordial ve primer foliküllerin oluştuğu izlenmiştir.

Sonuç: Ovaryumu bir bütün olarak ele almak ve yapıların gelişim süreçlerini incelemek, bu organın dinamiklerini daha iyi anlamamıza yardımcı olabilir.

Anahtar kelimeler: Korpus luteum, ovaryum, tunika albuginea, folikül.

Ünal MS, Tan S, Seçme M. Korpus luteum oluşumu sırasında meydana gelen yapısal değişiklikler. Pam Tıp Derg 2024;17:285-301.

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Introduction

The ovary is an organ with endocrine and exocrine functions and is covered by an epithelium that varies from single-layered squamous epithelium to cuboidal epithelium. In the lower part of the basement membrane, there is a tight connective tissue called the tunica albuginea. Tunica albuginea is rich in collagen fibers and is restructured after ovulation. Follicles are structural units located in the stroma of the cortex layer in the ovary consisting of the cortex and medulla. In embryonic life, primordial germ cells appear in the endoderm of the dorsal wall of the embryonic yolk sac, migrate towards the gonad outline, and are called oogonium. After the formed oogoniums, the primordial follicle structures are formed. Folliculogenesis is regulated by some factors including endocrine, paracrine, and autocrine factors [1, 2].

The oocyte plays an active role in the growth of follicles by secreting paracrine growth factors (such as GDF9 and BMP15) and directing the differentiation of granulosa cells. On the other hand, granulosa cells regulate the development of oocytes by communicating between germ and somatic cells [3]. The outer surface of the follicle cells surrounding the oocyte is bounded by the basal lamina and surrounded by stromal cells. Type IV collagen is the main component of the basement membrane in the follicle. The oocyte formed in the primordial follicle is surrounded by a single layer of flat follicle cells. The number of primordial follicles in the ovaries determines how long the reproductive ability will continue, and these structures are considered reserves. After puberty, primordial follicles are selected and begin to grow. Since primordial follicles do not contain FSH receptors, FSH is not required for their activation. In each cycle, 6-12 follicles begin to develop and only one continues to develop as a mature follicle, while the others undergo atresia [4]. While atresia in preantral follicles generally occurs by autophagy, in antral follicles, atresia mostly occurs by apoptosis mechanism [5].

Follicles continue their development as primary, secondary, and Graaf follicles, respectively. It can also undergo atresia at any stage of its follicular development. In some atretic follicles, the granulosa layer degenerates and the theca interna layer expands, and these atretic follicles resembling the corpus luteum are called corpora lutea atretica [6]. Although there are views that there is a certain fixed number of oocytes in the ovary and no new follicle production occurs, there are also studies suggesting that postnatal oogenesis production occurs [6].

The endocrine functions of the corpus luteum and the hormones it secretes have been extensively researched. However, there are relatively few studies that focus on the changes in the adjacent tunica albuginea and the restructuring of the ovarian surface epithelium. The presence of capillary vessels in the tunica albuginea adjacent to the corpus luteum, along with the observation of primordial follicles, has raised suspicions regarding postnatal oogenesis. Therefore, a thorough examination of the morphological changes in the ovule, Graafian follicle, and corpus luteum is necessary [1, 7, 8]. The developing Graaf follicle changes its location towards the outer part of the ovarian cortex towards ovulation. The theca layer in the Graaf follicle is divided into two parts as theca interna and externa. Theca interna consists of secretory cells of epithelioid character and is rich in terms of vascularization. It contains fibroblasts and type III collagen fibers. In the theca externa layer, there are myofibroblasts, type I and type III collagen fibers. After ovulation, the theca externa layer of the Graaf follicle completely degenerates; significant morphological changes occur in the tunica albuginea and the surface epithelium. Collagen fibers found in the theca externa layer, collagen fibers and fibroblast-like cells in the tunica albuginea are broken down with proteolytic enzymes. Then, reconstruction occurs not only in the tunica albuginea and surface epithelium part where the oocyte-cumulus complex is separated, but also throughout the tunica albuginea and surface epithelium of the entire corpus luteum. The reconstructed surface epithelium is of the squamous epithelium type and the tunica albuginea is a thin layer consisting of fibroblast-like cells containing one-two rows of concentrically arranged collagen fibers. Afterwards, the tunica albuginea thickens; fibroblast-like cells proliferate and become a layer containing five-six rows of concentrically

arranged collagen fibers. At this time, the surface epithelium also differentiates towards the cuboidal epithelium. New capillary vessels are formed in the thickened tunica albuginea [1, 7, 8].

The appearance of primordial follicles in the tunica albuginea adjacent to the corpus luteum in the sections suggests that VSELs cells, whose existence has been shown in previous studies, may come to this area from the bone marrow through capillary vessels and differentiate. VSELs cells in the bone marrow head towards the relevant organ by coming out to peripheral vessels in situations that cause tissue damage such as stroke, acute myocardial infarction, hypoxia where the inflammatory process is intense [9]. A similar inflammatory process emerges with the degeneration of collagen fibers and fibroblast-like cells in the theca externa and tunica albuginea as the Graaf follicle differentiates into the corpus luteum after ovulation. There are claims that VSELs cells coming out to peripheral vessels can reach the newly formed capillary vessels in the tunica albuginea of the corpus luteum and can turn into primordial follicles by receiving signals from the surrounding fibroblast-like cells. The finding supporting these views is that primordial follicles, which are densely located in the cortex and medulla of the ovary in the first four weeks after birth in rats, start to be seen mostly in the cortex region and the tunica albuginea during the reproductive period [10].

The aim of our study is to investigate the source of the primordial follicles seen in this region during the formation of the corpus luteum and the reconstruction of the tunica albuginea. On the other hand, we see it as a correct approach to examine the changes the structure undergoes, the cell types it contains, and its secretions with a holistic approach in order to better understand the functions of the corpus luteum.

Materials and methods

Ethical approval for our study was obtained from Pamukkale University Animal Experiments Ethics Committee with the decision no PAUHDEK. The ovaries of 6 Wistar albino-type rats (12-14 months old) in the ovarian aging process were removed under sterile conditions and general anesthesia. The right ovaries of all rats were fixed in 10% neutral buffered formaldehyde for 72 hours. After fixation, the routine tissue follow-up protocol was applied and embedded in paraffin blocks (FFPE). Hematoxylin-eosin staining was performed by taking 5 µm sections from FFPE ovarian tissues. Masson's trichrome staining was performed according to manufacturer instructions (Bio-Optica, Milano, Italy). Images of the ovarian surface epithelium were taken under light microscopy (BX51 Olympus, Japan). Using the explant culture method, a mixed cell culture was produced from the left ovaries [11]. Surface epithelial cells that were proliferating alongside ovarian stromal cells in a mixed cell culture were locally tagged, and these cells were extracted by using local trypsinization. The adhering ovarian surface epithelial cells were removed using trypsin enzyme 0.25% (Hyclone, USA) and injected into two fresh culture dishes with full media after they had multiplied and reached confluence (70-80%). Dulbecco's Modified Eagle's Medium (DMEM) (Capricorn Scientific, Germany), Fetal Bovine Serum (FBS) (Capricorn Scientific, Germany), and Penicillin-Streptomycin are all included in the complete medium (Pan Biotech, Germany).

Results

After ovulation, the basement membrane between the mural granulosa cells and theca layer was destroyed and it was observed that the mural granulosa cells rapidly hypertrophied and filled the entire structure. In the formed corpus luteum, initially, the number of granulosa lutein cells is high, but later the migrating theca lutein cells seem to provide the majority. With the formation of new vessels in the structure, blood supply also increases. The corpus luteum volume is at its highest when it is newly formed (Figure 1-2). In some areas, the ovarian surface epithelium transforms into pseudostratified epithelium. Primordial follicles were observed to form in this remodeled tunica albuginea (Figure 3-11). Meanwhile, the surface epithelium is squamous, few in number, and the underlying tunica albuginea is rather thin. Later, fibroblast-like cells in the surface epithelium and tunica albuginea proliferate. The squamous surface

epithelium increases in number and turns into cubic epithelium in places. The tunica albuginea thickens and restructures (Figure 12-15). In some sections, vascular structures were observed in atretic follicles. In the lumen of another atretic follicle, a primary follicle was observed (Figure 16). It was observed that while the structure regressed, the presence of granulosa lutein cells did not continue and the darker stained theca lutein cells filled the structure. In the late stage, the corpus luteum is formed by theca lutein cells, which are known to secrete progesterone and androgen hormones (Figure 17). In the sections, it was observed that the granulosa cells in the primary and secondary follicles, which were partially atresia, degenerated, and the theca interna cells continued their functions in the structures that turned into glandular structures (Figure 7).

We consider that VSELs cells found in the bone marrow reach the tunica albuginea adjacent to corpus luteum via newly formed capillary vessels, and these cells develop by receiving signals from fibroblast-like cells and sometimes differentiate by entering among proliferating surface epithelia. When we isolate and culture the ovarian surface epithelium, the observation of structures similar to primordial follicles among proliferating ovarian surface epithelia has increased our suspicions in this direction (Figures 18).

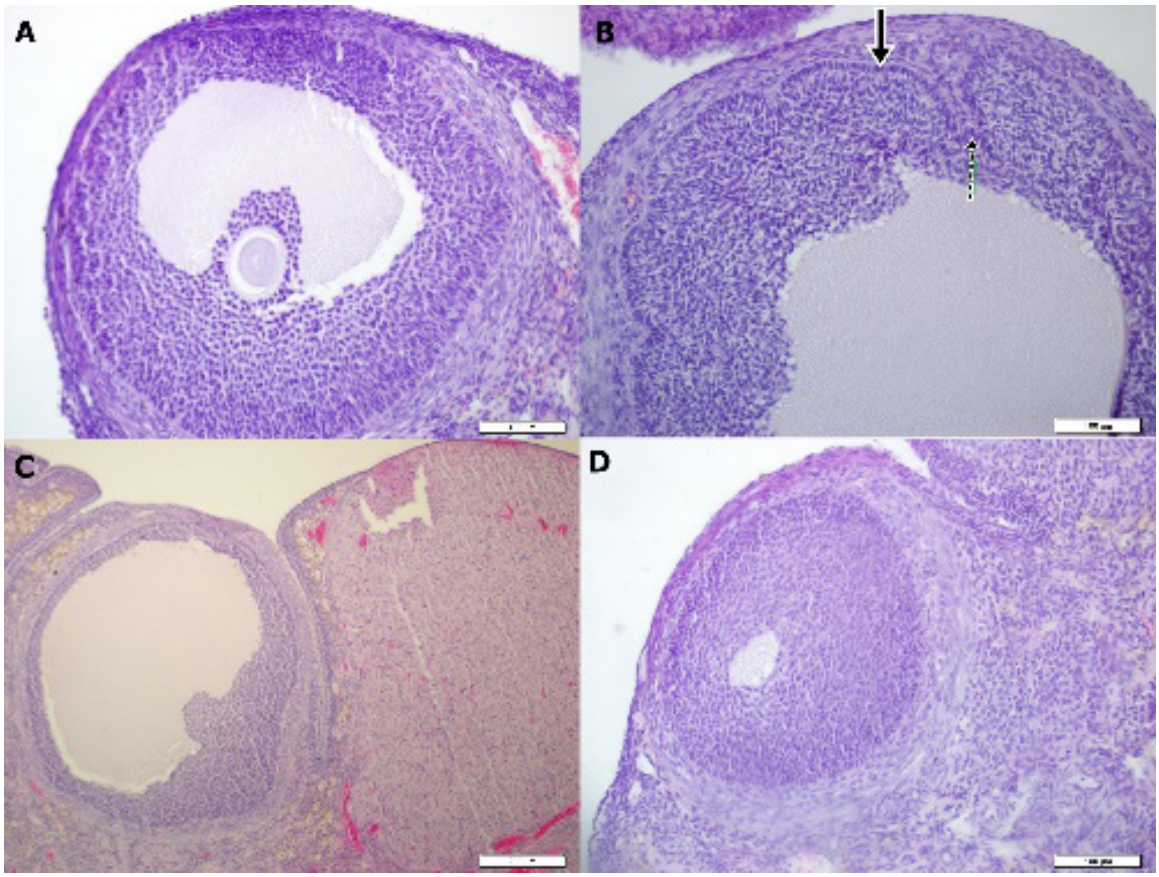

Figure 1. A- Graafian follicle B- (→) Granulosa cells, (- - >) Invading theca interna cells C- Ovulated graafian follicle D- Ovulated graafian follicle in which granulosa cells that have begun to hypertrophy fill the antrum (Magnification: 200X)

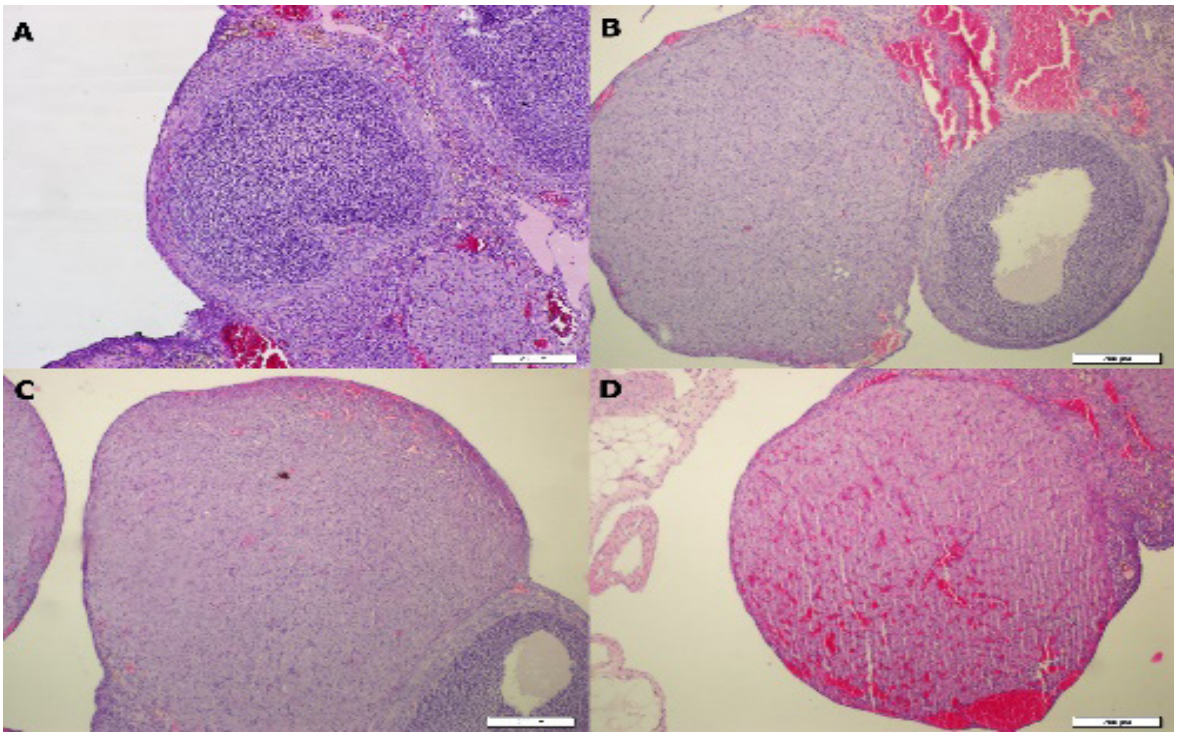

Figure 2. A- Ovulated graafian follicle, in which the antrum is entirely filled with granulosa cells that have started to hypertrophy. B- A large-volume Graafian follicle. C- The corpus luteum's developing vascularization. D- The corpus luteum, where vascularization has significantly enhanced (Magnification: 200X)

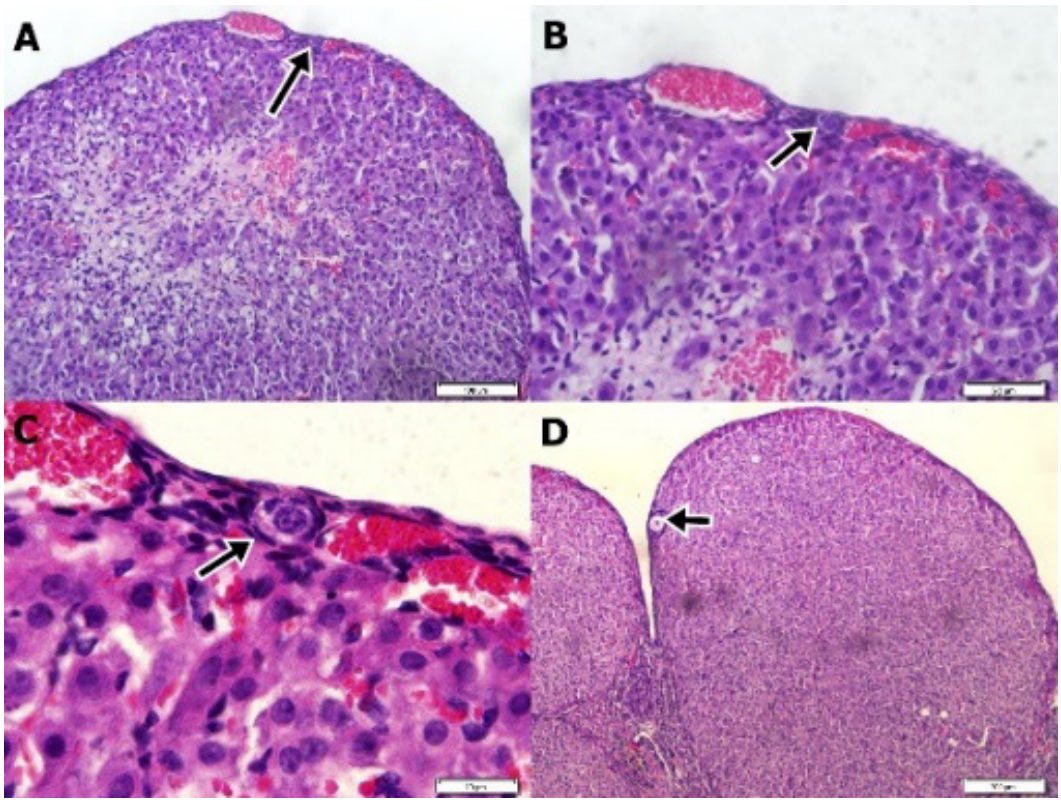

Figure 3. A (Magnification: 200X) - B (Magnification: 400X) - C (Magnification: 1000X) - D (Magnification: 200X)- (→) Primordial follicle that occurs between the completely regenerated tunica albuginea and the surface epithelium after the Graafian follicle has ovulated. D- (→) primary

follicle

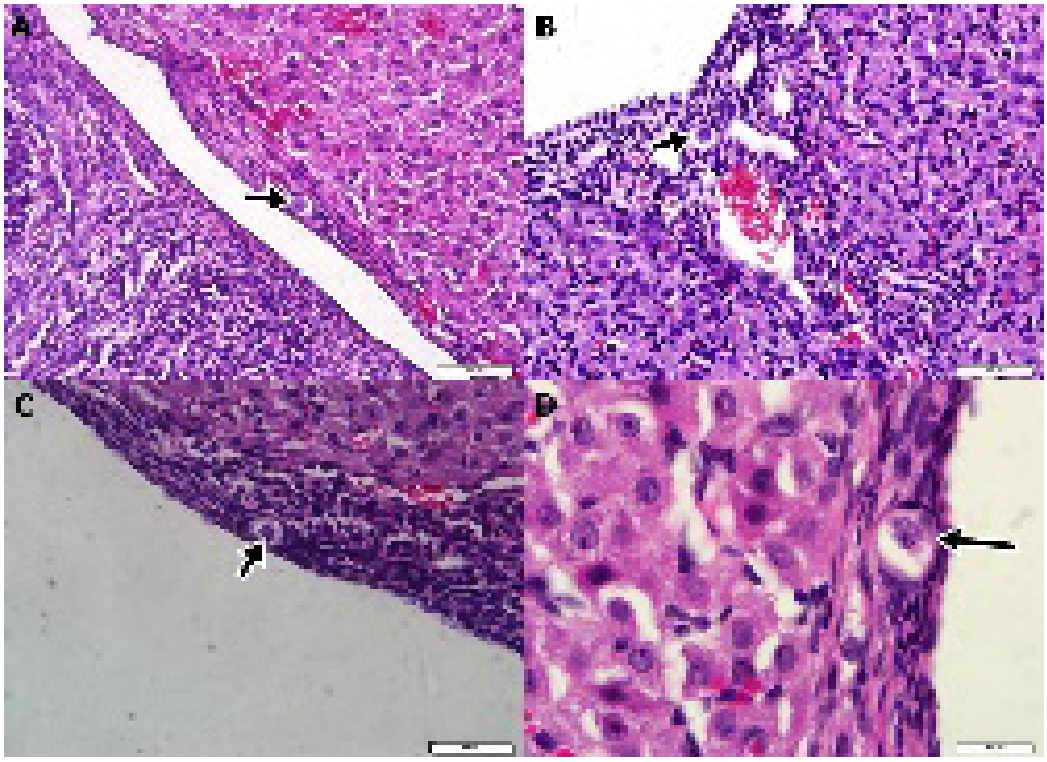

Figure 4. A- (→) Primordial follicle in the tunica albuginae of the corpus luteum (Magnification: 200X) B- (→) Primordial follicle in the tunica albuginae of the corpus luteum (Magnification: 200X) C- (→) Primordial follicle in the tunica albuginae of the corpus luteum (Magnification: 400X) D- (→) Primordial follicle in the tunica albuginea of the corpus luteum (Magnification: 400X)

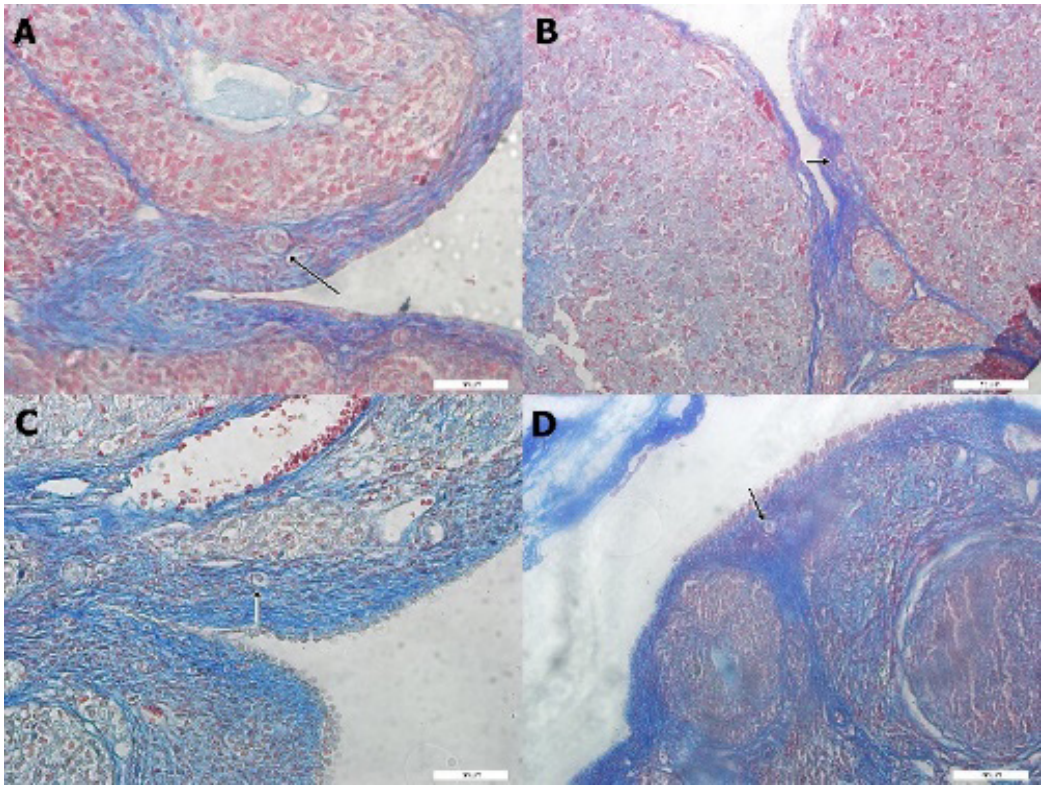

Figure 5. A (Magnification: 400X)- B (Magnification: 400X)-C (Magnification: 400X) - D (Magnification: 200X)- (→) Primordial follicles in the tunica albuginea (Masson trichrome staining)

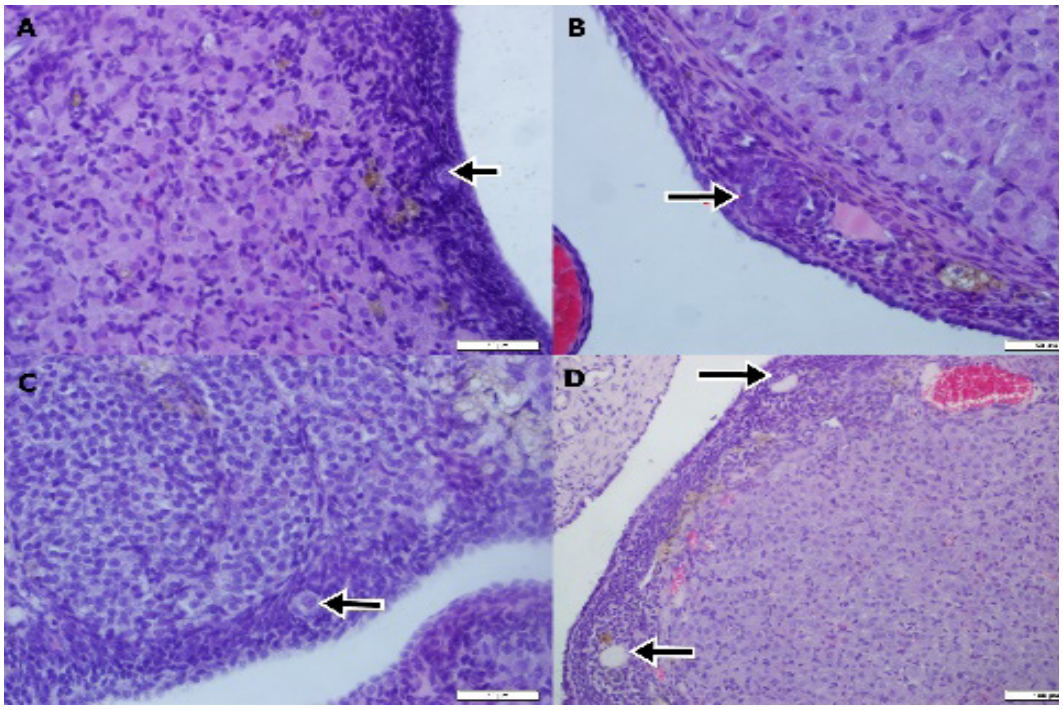

Figure 6. A- (→)Primordial follicle in the tunica albuginea part of the corpus luteum (Magnification: 400X), B- (→)Primary follicle in the tunica albuginea part of the corpus luteum (Magnification: 400X), C- (→)Primordial follicle in the tunica albuginea part of the regressed corpus luteum (Magnification: 400X), D- (→) atretic follicle in the tunica albuginea part of the corpus luteum (gland structures that develop with apoptosis of granulosa cells in primary-secondary follicles and proliferation of theca lutein cells, Magnification: 200X)

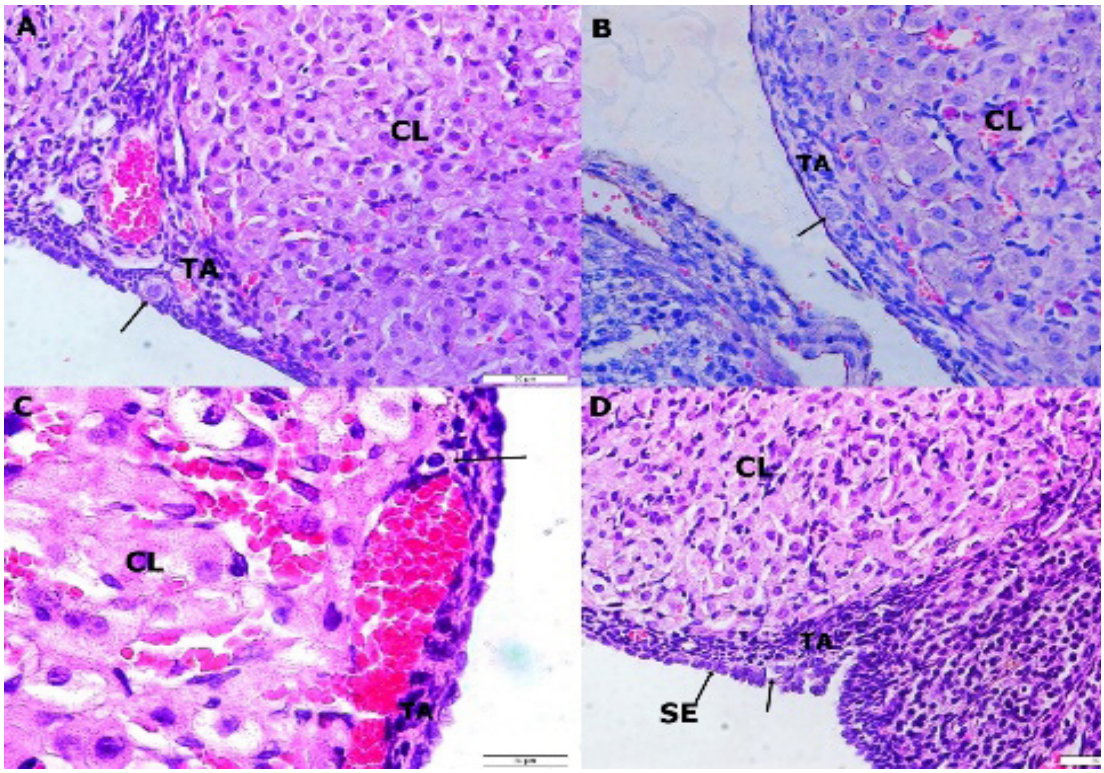

Figure 7. A-D- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium)

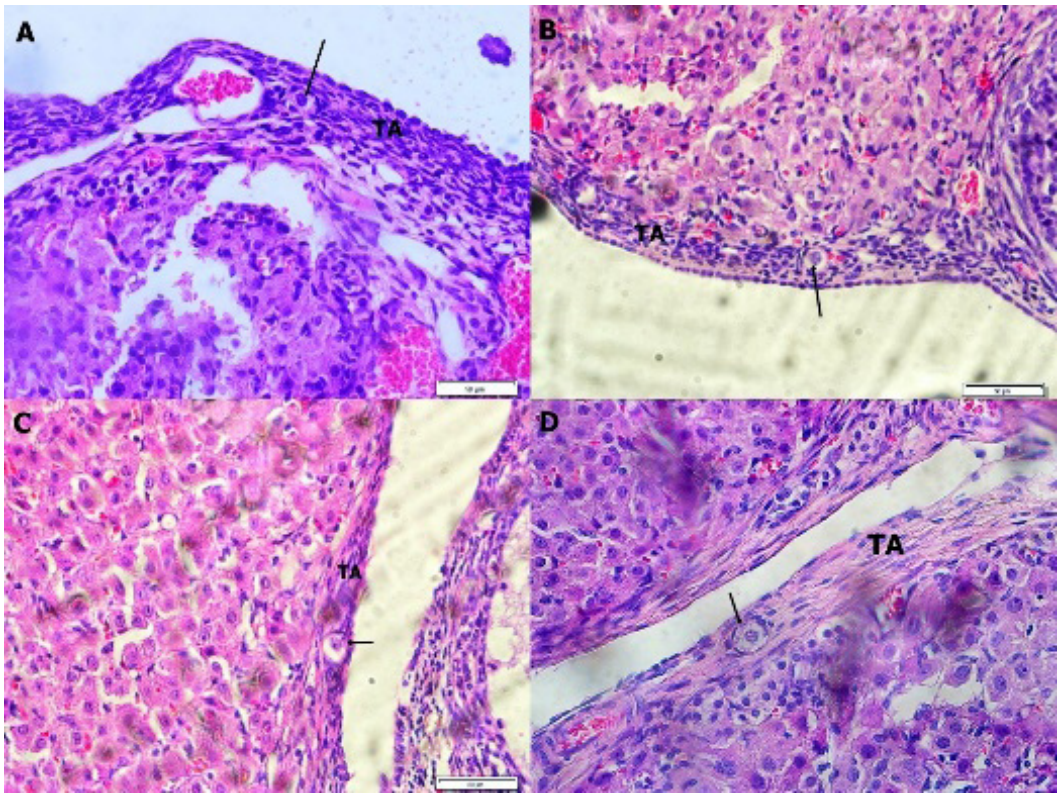

Figure 8. A-D- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(TA: Tunica albuginea)

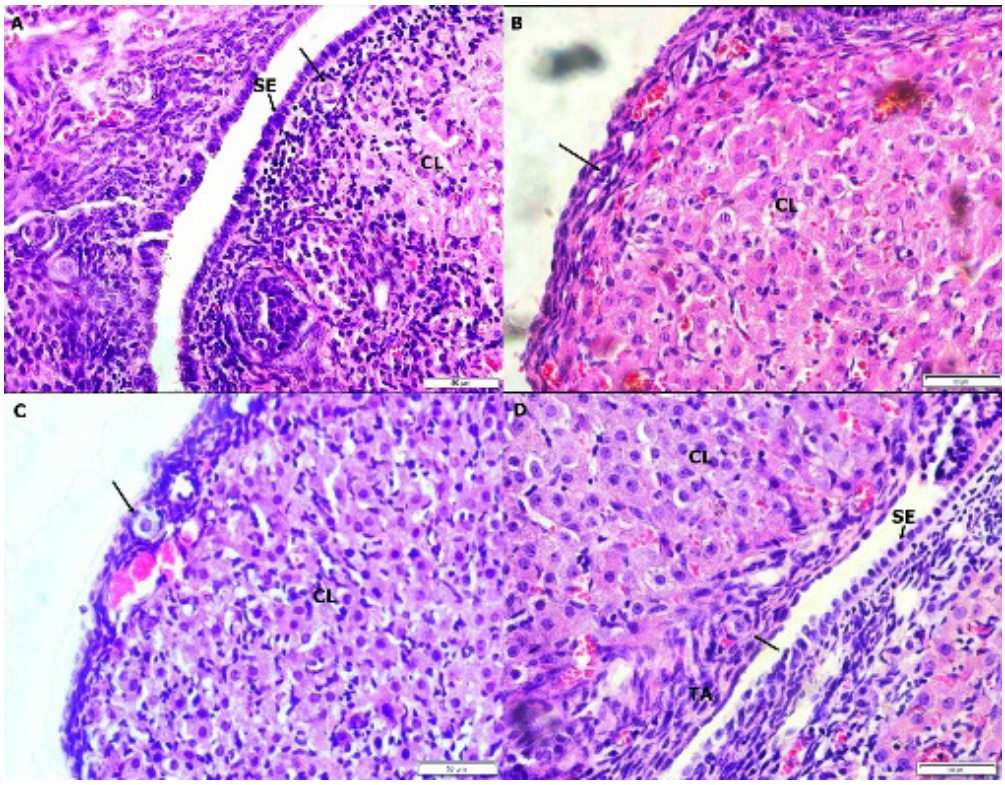

Figure 9. A-D- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(CL: Corpus luteum, SE: Surface ephitelium)

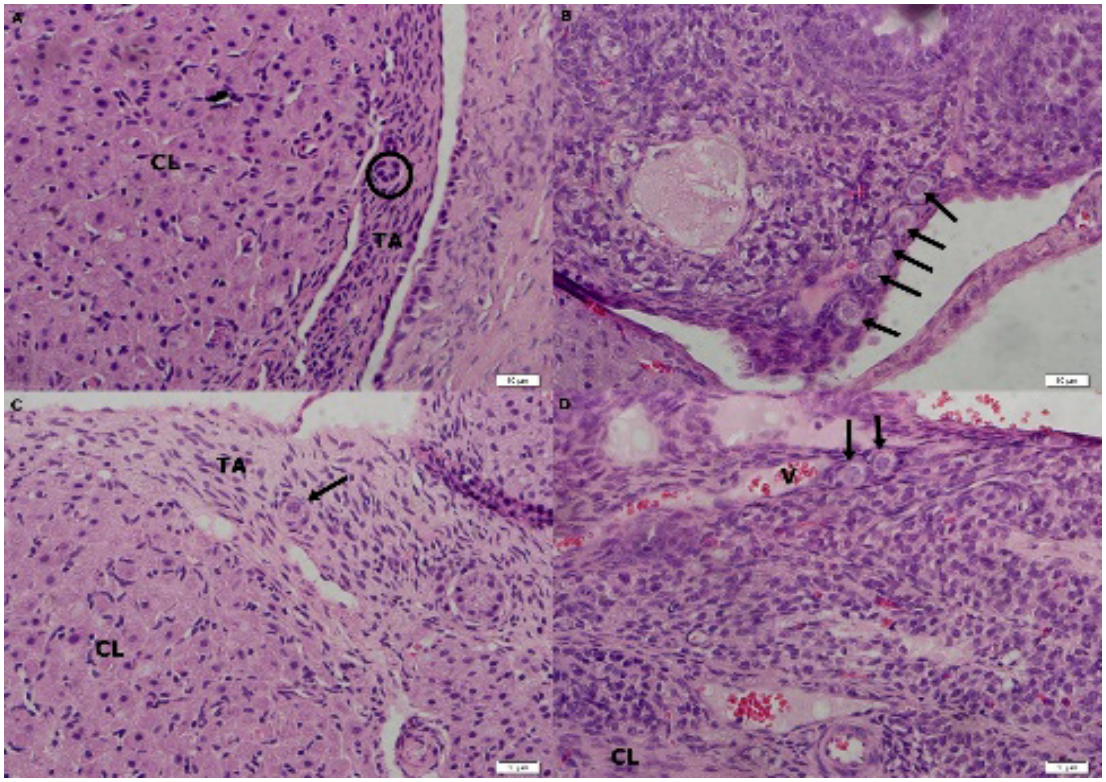

Figure 10. A- Primary follicles in the tunica albuginaea layer of the corpus luteum B- Primordial-primary follicles in the tunica albuginea C- Primordial follicles in the tunica albuginea formed by fibroblast-like cells just below the surface epithelium of the corpus luteum D- Primordial-primary follicles in the tunica albuginea, (Magnification: 400X)
(CL: Corpus luteum, TA: Tunica albuginea, V: Vessel)

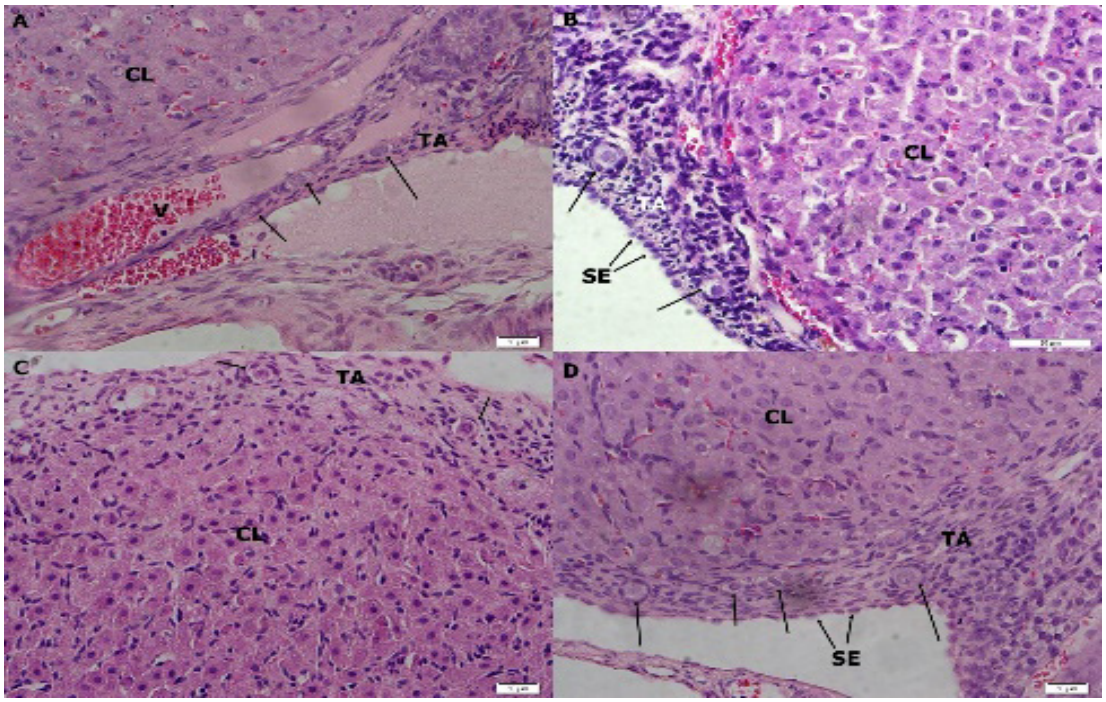

Figure 11. (A-D)- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium, V: Vessel)

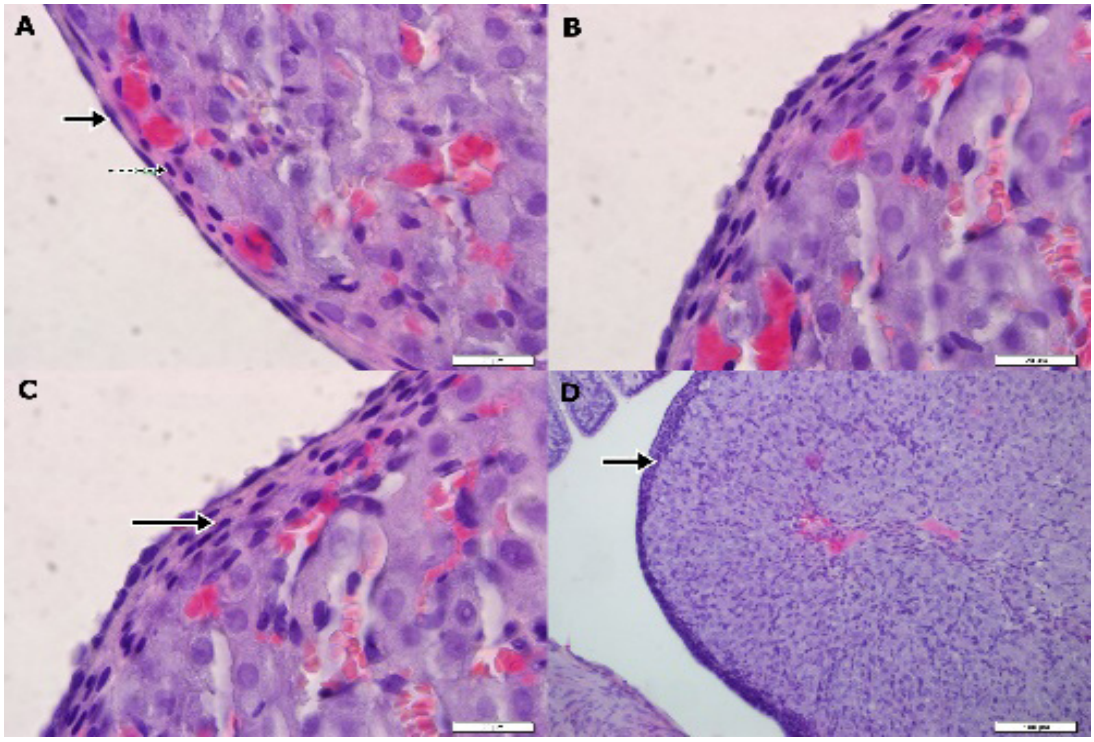

Figure 12. A- (→) Ovarian surface epithelium consisting of a single layer of squamous epithelium, (- - >) Fibroblast-like cell in the tunica albuginea B- Differentiation of surface epithelium into cubic epithelium C- (→) Proliferation of fibroblast-like cells D- (→) Thickening of the tunica albuginea Magnification (A,B,C:1000X, D:400X)

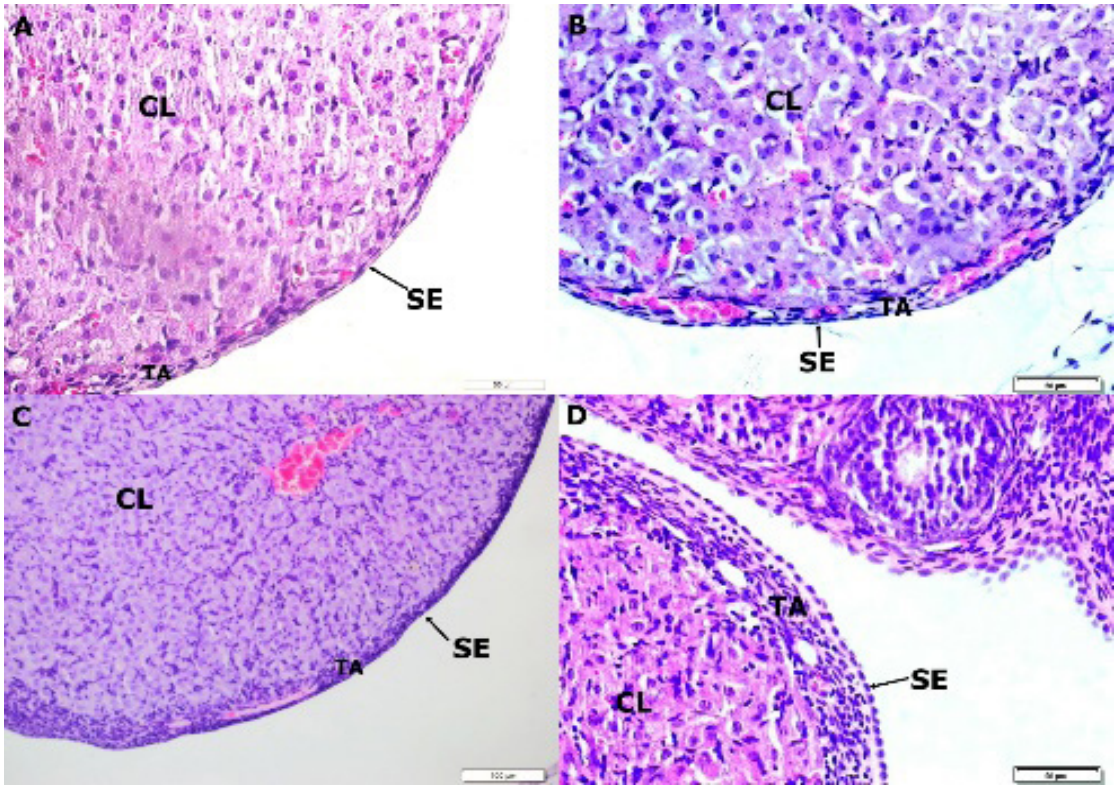

Figure 13. A-B- The surface epithelium of the corpus luteum and a thin layer of tunica albuginea are seen just below it. C-D- Fibroblast-like cells proliferate in the tunica albuginea and the layer thickens (CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium) (Magnification: 400X)

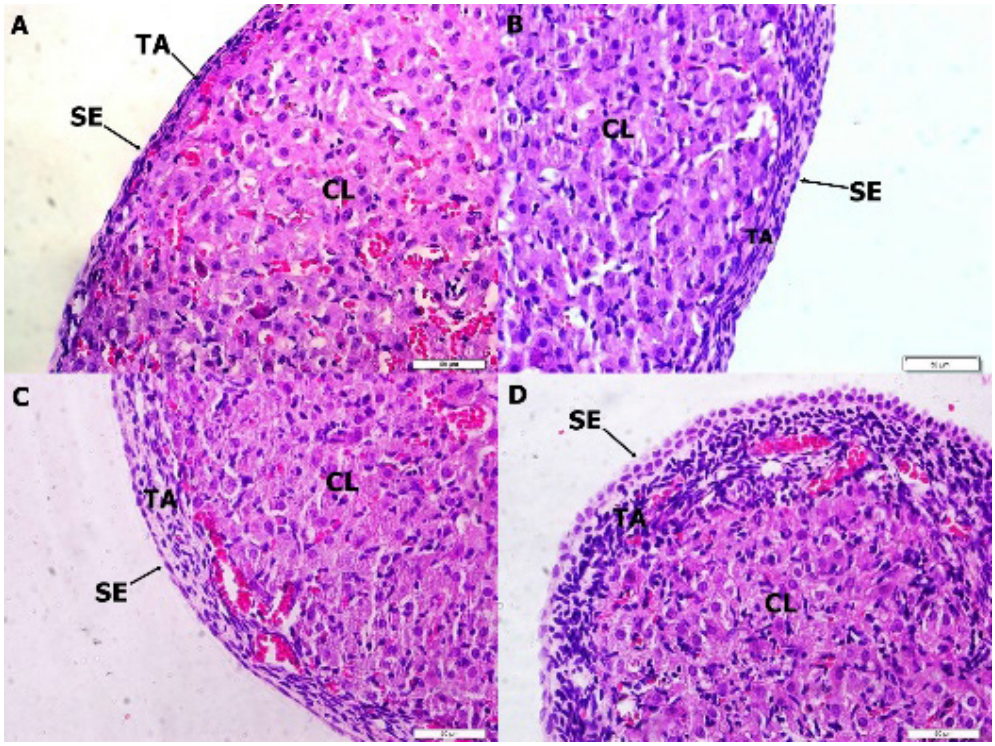

Figure 14. A-B- The surface epithelium of the corpus luteum and a thin layer of tunica albuginea are seen just below it. C-D- Fibroblast-like cells proliferate in the tunica albuginea and the layer thickens (Magnification: 400X), (CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium)

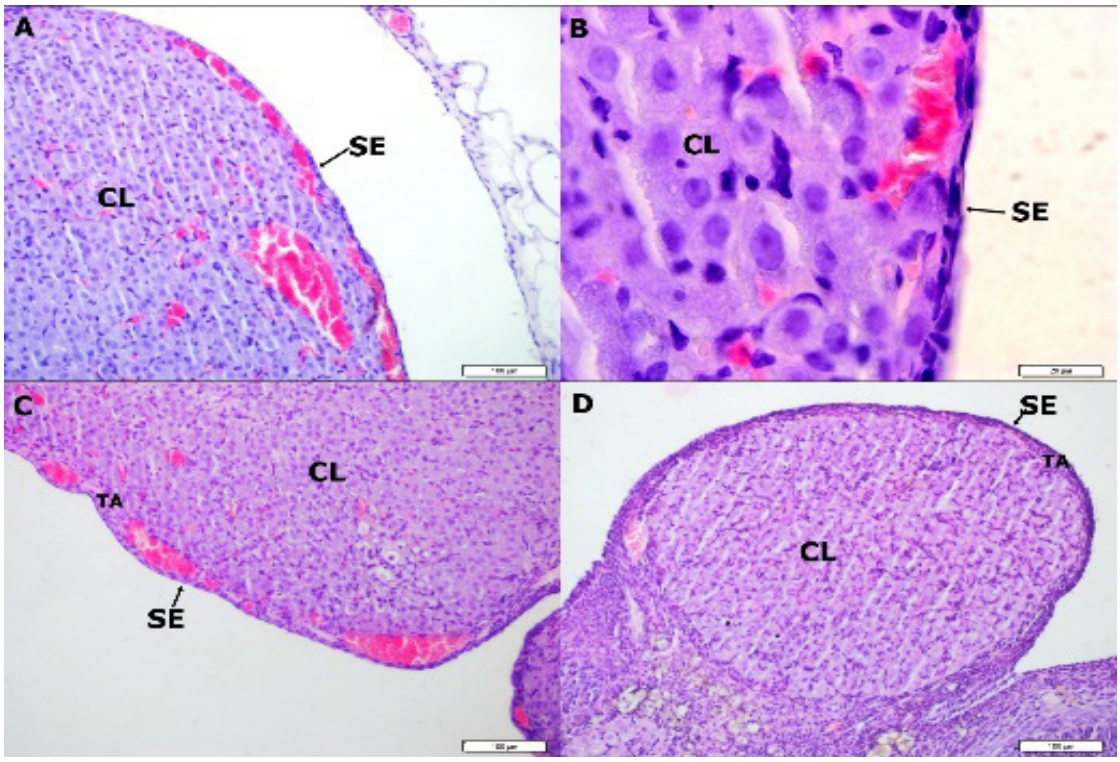

Figure 15. A (Magnification: 400X), B- The surface epithelium of the corpus luteum and a thin layer of tunica albuginea are seen just below it (Magnification: 1000X) C (Magnification: 400X), D- Fibroblast-like cells proliferate in the tunica albuginea and the layer thickens, (Magnification: 200X)
(CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium)

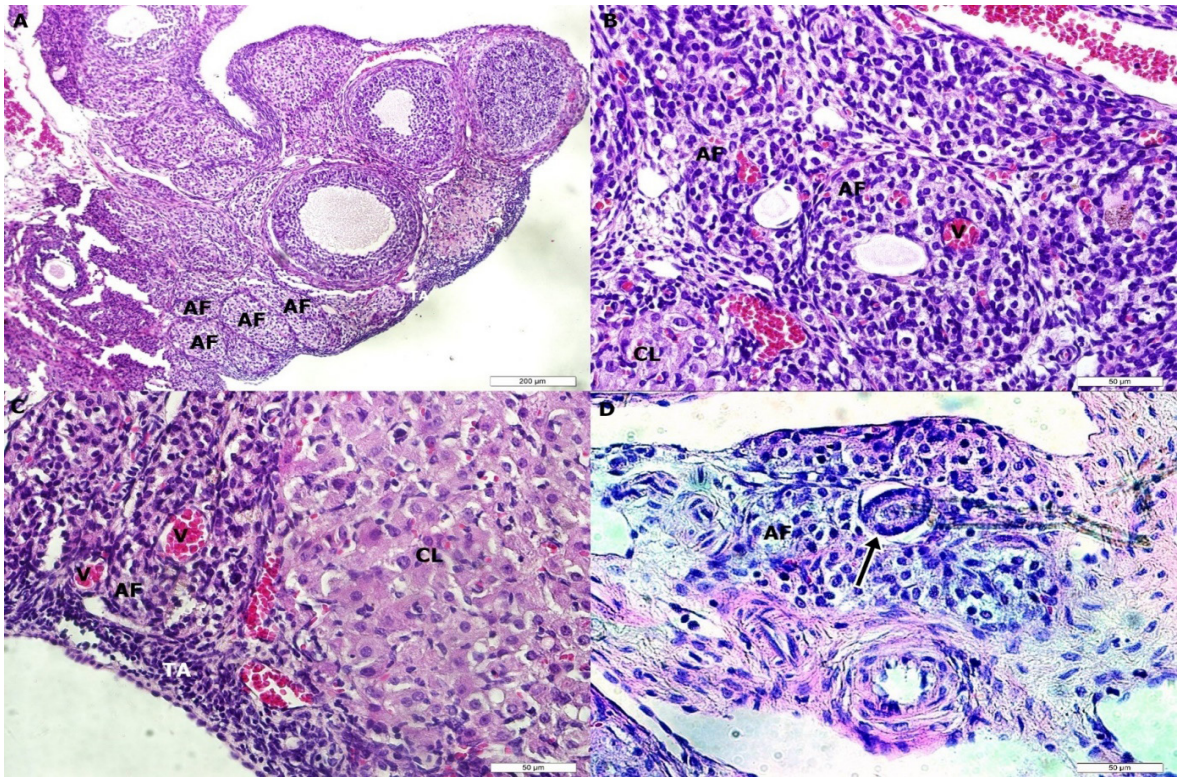

Figure 16. A (Magnification: 100X), B Atretic follicles C: Vascular formation in the lumen of the atretic follicle D: Primary follicle formed in the lumen of the atretic follicle, (B-D: Magnification: 400X) (AF: Atretic follicule)

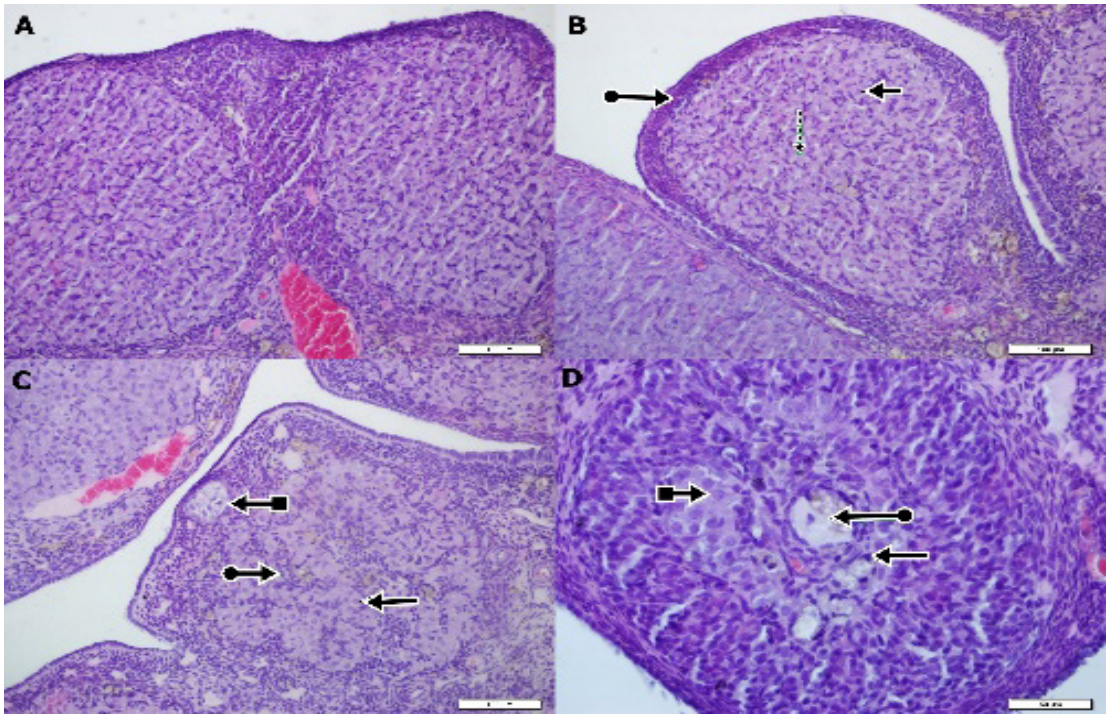

Figure 17. A- Theca lutein cells begin to appear in the corpus luteum as well as granulosa lutein cells B- **(●→)** Tunica albuginea, (- - >) Theca lutein cells, (→) Granulosa lutein cells C- **(■→)** Primary follicle formed in the tunica albuginea in the reconstructed corpus luteum, **(●→)** Theca lutein cells, (→) Granulosa lutein cells D- **(●→)** Lumen of corpus luteum, (→) Theca lutein cells, **(■→)** Granulosa lutein cells (A, B Magnification: 200X; C,D: Magnification: 400X)

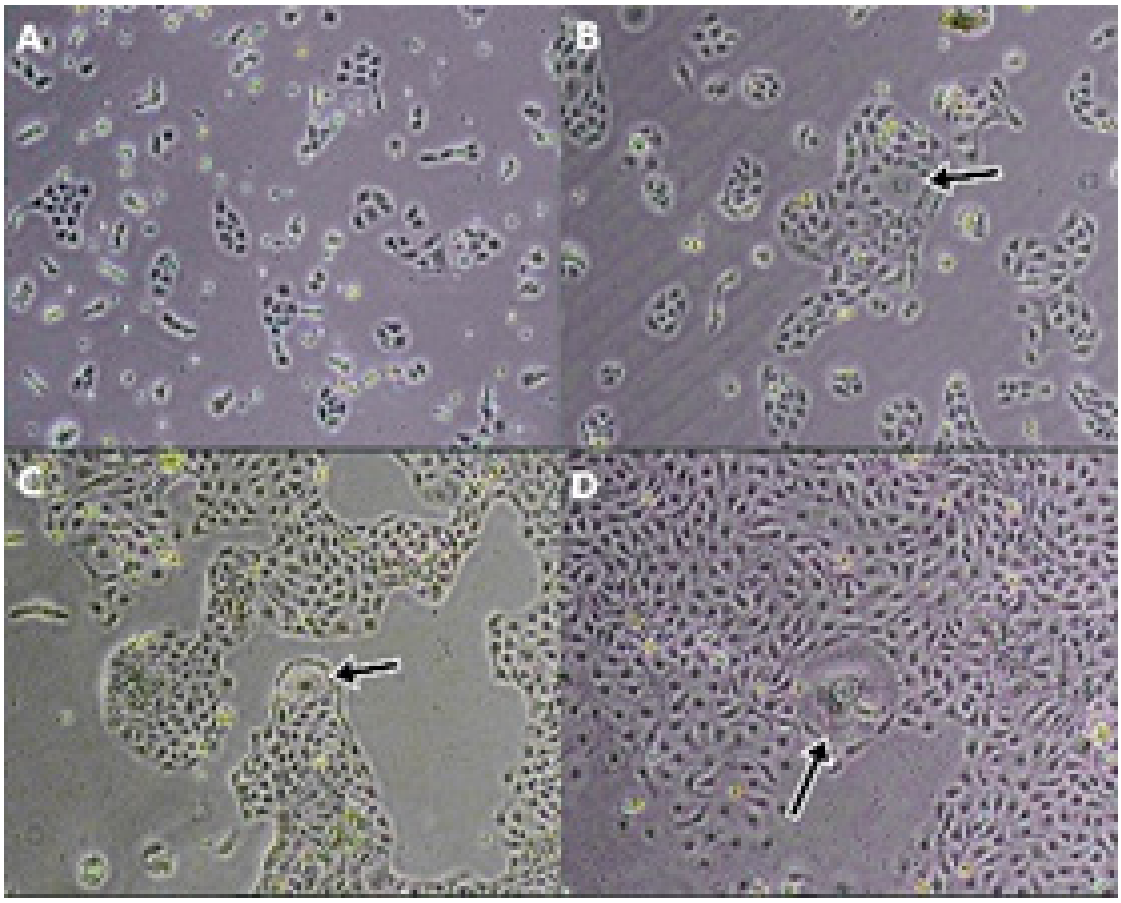

Figure 18. A- Morphological appearance of ovarian surface epithelium proliferating in cell culture (B-D) (→) Primordial follicle-like structures in ovarian surface epithelium (Magnification: 100X)

Discussion

The Graafian follicle is known as mature, and its wall consists of a granulosa cell layer and theca layer with basal lamina between them. In the region where it is associated with the oocyte, the cumulus granulosa cells form a mound called the cumulus oophorus [7]. In the theca interna layer adjacent to the mural granulosa cells, androstenedione, testosterone, and dihydrotestosterone are synthesized. Androstenedione is transported to granulosa cells and converted to estradiol (E2) by aromatase (CYP19A1). Mural granulosa cells do not have the enzymes necessary for the production of estrogens. The theca externa is a connective tissue containing collagen fibers and smooth muscle cells and is continuous with the stroma of the ovary. Granulosa cells in Graafian follicles acquire LH receptors as well as FSH receptors, which are necessary for the luteinization of the corpus luteum [4]. Ovulation is the process of expulsion of the secondary oocyte from the Graaf follicle. After ovulation, the basement membrane breaks down and the granulosa and theca interna cells are rearranged to form a gland called the corpus luteum. Blood vessels from the theca layer of the follicle invade the transforming granulosa cells. The cells of the epithelioid theca interna follow the blood vessels and become the theca lutein cells of the corpus luteum. Granulosa lutein cells are larger and centrally located. Theca lutein cells are smaller and located peripherally [1, 4, 12]. Immediately after ovulation, pericytes originating from the theca compartment are the first vascular cells invading the developing luteal parenchyma.

Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), endocrine-derived (EG-VEGF), and angiopoietins (Ang) are required for molecular regulation of angiogenesis in the corpus luteum. All findings show that the functional life of the corpus luteum depends on paracrine and autocrine mechanisms [13]. Then, a rich vascular network forms in the corpus luteum, and the

cells it contains begin to secrete estrogen and progesterone. During the luteal phase, theca lutein cells do not hypertrophy, while granulosa lutein cells undergo extensive hypertrophy and contribute significantly to the growth of the corpus luteum. In rodents, granulosa lutein cells and theca lutein cells differ in basal progesterone secretion rates, and granulosa lutein cells produce more progesterone than theca lutein cells [1, 13, 14].

Progesterone and estrogen hormones stimulate endometrial growth and differentiation to prepare for embryo implantation. In the case of pregnancy, the pregnancy corpus luteum is formed and degenerates after 4-5 months of existence. If pregnancy does not occur, the corpus luteum degenerates, and corpus Albicans is formed. Prostaglandin F2 alpha (PGF2α) secretion from the endometrium has an important role in the regression of the corpus luteum. Prostaglandin regresses the corpus luteum and stops the secretion of progesterone hormone. After the regression of the corpus luteum, the theca lutein cells released in the stroma can secrete corticosteroids and they are called interstitial glands [4, 15, 16].

The mesoderm-derived surface epithelium undergoes morphological changes due to cyclic changes, proliferates, and shows pseudostratification in some areas. Stem cell markers such as Oct 4, Stella, C-Kit, and Nanog are also expressed in the ovarian surface epithelium, along with epithelial (cytokeratin) and mesenchymal markers (vimentin). After ovulation, the ovarian surface epithelium helps repair damaged areas. The extracellular matrix also plays an important role in the development of the follicle and the formation of the corpus luteum [17-20]. The extracellular matrix (ECM) is a complex system consisting of a collagen network (types I, III, IV, VI) associated with proteoglycans and glycoproteins, elastic and reticular fibers, fibronectin, and laminin. It plays an important role in ovarian functions by participating in processes such as cell migration, proliferation, growth, and differentiation. The ECM provides structural support for the follicle, maintains cellular organization and connectivity, and provides signals that support follicle development and maturation [21-23].

Fibroblast-like cells in the externa layer of the graaf follicle have lysosome-like granules. While type I collagen fibres are found in the theca externa layer, both type I and type III collagen fibres are found in the theca interna. In the tunica albuginea, type I and type III collagen fibres form bundles with concentric arrangement.

Type I collagen fibres are mostly seen in the superficial part of the tunica albuginea, while type III collagen fibres are found in deeper regions. During ovulation, collagen fibre bundles in the theca externa and tunica albuginea undergo collagenolysis. Proteolytic activity in the ovarian surface epithelium causes the breakdown of the ovarian surface epithelium and ovarian cortex during ovulation and also contributes to their reorganisation. The ovarian surface epithelium produces proteolytic enzymes such as urokinase plasminogen activator (uPA) matrix metalloproteases 2 and 9 [7, 24, 25].

The corpus luteum is a temporary endocrine gland containing different cells such as granulosa cells, theca cells, endothelial cells, pericytes, fibroblasts, and macrophages [13, 15, 26]. These cells have different morphological, endocrine, and biochemical features. FSHRs play an important role during follicular development but have no function when follicular cells differentiate. Their expression decreases after the LH peak in the luteinization process. In rodents, both the estrogen receptor α (Erα) and estrogen receptor β (Erβ) genes are expressed in the ovary as a single (6.5 kb) and multiple transcripts (1.0 kb to about 10 kb). ERβ is abundantly expressed in the follicle, especially in the granulosa cell layer. However, ERα is a receptor found at higher levels than ERβ in the corpus luteum [13].

Angiogenesis is a crucial process that helps establish and maintain the normal structure and function of the corpus luteum. Luteal development can be considered as an inflammatory response. There are many activated leukocytes such as macrophages, neutrophils, and eosinophils in the corpus luteum, and these cells are commonly involved in neovascularization [27, 28]. Studies show that cytokines secreted by immune cells regulate both luteotropic and luteolytic processes [29].

Vascular endothelial growth factor (VEGF) has been found to have an important role in the corpus luteum. VEGF is secreted by granulosa

cells in the antral follicle and granulosa lutein cells in the corpus lutem and has two receptors, VEGFR-1 and VEGFR-2. Since it cannot pass through the basement membrane of blood vessels in the theca layer of the follicle, it cannot reach the granulosa cell layer. After the destruction of the basement membrane, blood vessels invade the corpus luteum together with cells in the theca interna layer. Perivascular cells (pericytes) found in the follicle in the preovulatory phase and seen in the capillaries of the corpus luteum in the early stages of the luteal phase show a high proliferation rate [27]. Studies have shown that the administration of HCG to human granulosa lutein (hGL) cells increases VEGF expression. In the follicles of the ovary, TGF-β1 protein expression occurs in both granulosa and theca cells, while TGFβ2 occurs specifically in the theca cells of the follicles. VEGF is expressed by the stimulating effect of TGF-β1 on granulosa cells [30].

Theca lutein cells and granulosa lutein cells express progesterone receptors. Studies have shown that progesterone receptors are expressed in steroidogenic cells at all stages of the luteal phase. Among all steroidogenic cell types examined, progesterone expressions were found to be variable during the luteal phase [31]. Studies have shown that progesterone alpha receptors (PRA) are expressed in the theca lutein cells of the regressed corpus luteum and the interstitial gland cells proliferating from theca cells in atretic follicles [32]. In addition, membrane progesterone receptor expression, unlike nuclear progesterone receptor, has been described in the corpus luteum of rats and sheep. However, the function of this receptor in luteal cell formation and its signaling mechanism is unknown [13]. In some studies, mural granulosa cells obtained from IVF were isolated and cultured to form an in vitro corpus luteum model. Studies with these cells may lead to the identification of signaling pathways related to regulating the corpus luteum and developing new treatment strategies [33].

On the other hand, numerous studies have been conducted regarding postnatal oogenesis. Johnson et al. [34] proposed that bone marrow or peripheral blood serves as a reservoir for ovarian stem cells, which migrate towards the ovary and facilitate germ cell renewal in the adult mouse ovary. In their studies, they demonstrated that oocyte production was restored following bone marrow transplantation in mice that had undergone chemotherapy and had ataxia telangiectasia mutations, revealing the presence of donor-derived oocytes [34]. Bukovsky et al. [35], in their studies, demonstrated that human ovarian surface epithelial cells could differentiate into oocytes and granulosa cells in a culture environment. They maintained the cells derived from the ovarian surface epithelium in environments containing estrogenic stimulants such as phenol red for 5-6 days. They reported that cells cultured in the presence of phenol red directly differentiated into large oocyte-like cells (180 microns).

Virant Klun et al. [36] claimed to have isolated ovarian stem cells in postmenopausal women and women with premature ovarian insufficiency in their study. They isolated small round cells, ranging from 2 to 4 mm in diameter, from the material obtained by scraping. They determined that these cells expressed transcription factors such as SSEA-4, OCT4, NANOG, SOX2, and C-KIT. Bhartiya et al. [37], in their study with mouse ovaries, identified two different cell populations. They noted that VSEL cells were 3-5 μm in size and expressed nuclear OCT4, while OKH cells were larger than 8 μm and expressed cytoplasmic OCT4.

There are also some limitations in our study. The cells and structures developing in the tunica albuginea adjacent to the corpus luteum are observed to not always be fully stained due to their very small size and sometimes their locations. In addition, even in serial sections, while cells and structures are seen in one section, they could not be detected in the next section and most of the time they could only be observed at x400 and x1000 magnifications. Due to the rare encounter with these cells, the chance of imaging can be increased by conducting immunohistochemistry techniques in a multicentric manner and including a large number of samples.

The corpus luteum differentiates from the structure left behind as a result of the expulsion of the oocyte from the Graaf follicle. The collagen fibers and the cells they contain in the external theca layer of the ovulated Graaf follicle completely degenerate. After the collagen fibers and fibroblast-like cells in the tunica albuginea are broken down by proteolytic enzymes, this

region is largely rebuilt. In the sections, new capillary vessels have been observed to form in the tunica albuginea, which was previously known to be avascular. It can be thought that VSEL cells such as monocytes, leukocytes, and mast cells, which pass from the bone marrow to the peripheral circulation, also reach the region via capillary vessels and develop by receiving signals from fibroblast-like cells located here. The appearance of primordial follicles in the tunica albuginea adjacent to the corpus luteum during reconstruction has increased suspicions towards neofolliculogenesis, and further studies are needed in this direction.

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Authors' contributions to the article

M.S.U. constructed the main idea and hypothesis of the study. M.S. and S.T. developed the theory and arranged/edited the material and method section. M.S.U. and S.T. has/have done the evaluation of the data in the Results section. Discussion section of the article written by all authors reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.