DOI:10.22059/IJVM.2023.367617.1005464 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554

To Try to Understand the Dynamic Process of Rat Ovary Through Histological Sections and Cell Culture Studies

Murat Serkant Ünal [1], Seyedmahdi Tabatabaei [1,2,*]

[1] Department of Histology and Embryology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

[2] Young Researchers and Elite Club, Borujerd Branch, Islamic Azad University, Borujerd

Abstract:

Background: Primordial follicles, which are the structural units of the ovary and represent the ovarian reserve, continue their development by entering into paracrine, endocrine, and cell-cell interactions with the structures and cells in the niche.

Objectives: To examine the development of cells and structures in the ovary both in cell culture medium and in histological sections, and to observe the effects of the niche formed by them on folliculogenesis.

Methods: Three 3-week-old Wistar Albino rats as the first group for cell culture, and three 8-10-week-old (early reproductive period) and three 12-14-month-old (late reproductive period) Wistar Albino rats as the second and third groups for histologic sections were included in our study. To prepare histological sections, ovarian tissues of early breeding period rats and late breeding period rats were excised and passed through routine histological tissue process steps.

Result: Ovarian surface epithelium and stromal cells were observed to proliferate in cell culture. Primordial follicle-like structures were observed between the surface epithelial cells. Histological examination revealed periovary adipose tissue around the ovary, separated from the surface epithelium by a thin fibrous sheath. It was observed that primordial follicles were located in the tunica albuginea layer and the fibroblast-like mesenchyme in the tunica albuginea of the corpus luteum formed after ovulation, thickens this layer by proliferation of cells and creates a suitable environment for the development of primordial follicles, also the tubules of the rete ovarii, which emerge during the formation of the genitourinary system in the embryological period, continue to exist in the ovaries and mesovarium in rats in the early and late reproductive period.

Conclusions: The presence of tubules of the rete ovarii in the ovary and mesovarium during the reproductive period may indicate that these structures are also important in folliculogenesis.

Keywords: Epoophoron structures, Folliculogenesis, Graaf follicle, Ovary, Rete ovarii

Introduction

Ovary consists of cortex and medulla layers. The folding of the ovary during the embryonic period contributes to the formation of these layers. The border between medulla and cortex is not clear. The surface of the ovary is single-layered cuboidal epithelium and pseudostratified epithelium in some areas, and in some other areas, it is paved with squamous epithelium, and there is a basement membrane underneath. The cellular layer, known as the germinal epithelium, continues with the mesothelium outside the ovary (Auersperg et al., 2001; McKey et al., 2022). Tunica albuginea, an irregular tight connective tissue layer, is located between the surface epithelium and cortex, and there are fibroblast-like mesenchyme cells within the layer. The Tunica albuginea also contains a significant amount of type I and type III collagen fibers (Lind et al., 2006; Can, 2014).

Ovarian follicles are located in the stroma of the cortex. Follicles as the structural units of the ovary form a microenvironment for oocytes (Ross and Pawlina, 2006). The development of follicles in the ovary occurs with cell-cell interactions, endocrine, autocrine, and paracrine factors, especially FSH secreted from the pituitary. The most common type of follicle in the ovary is the primordial follicle, and this structure represents the reserve of the ovary. Primordial follicles contain a primary oocyte surrounded by a single row of squamous follicle cells. After puberty, primordial follicles are selected and begin to grow. They continue to develop as primary, secondary, and Graff follicles, respectively (Ünal and Seçme, 2022). In the medulla layer that is surrounded by loose connective tissue, there are blood and lymph vessels, and also nerves (Kinnear et al., 2020). In rats, parts of the ovary have a thin fibrous sheath with fibroblasts that is exactly next to the surface epithelium. This fibrous sheath is located

between the surrounding adipose tissue (peri-ovarian adipose tissue) and the ovarian surface epithelium. It has been reported that peri-ovarian adipose tissue provides the development of follicles in the ovary, and it has been shown that this tissue is important for the functions of the ovary. There is no periovarian adipose tissue in humans (Yang et al., 2018; Zhu et al., 2020; Zhang et al., 2021; Ünal and Seçme, 2022).

Ovulation is the process of releasing the secondary oocyte from the Graaf follicle. After ovulation, the basement membrane destroys, and the granulosa and theca interna cells of the follicle rearrange to form a temporary gland called the corpus luteum(Ross and Pawlina, 2006). The ovary is a dynamic complex organ that develops follicles according to estrus stages. We aim to examine the development of cells and structures in the ovary both in cell culture medium and in histological sections and to try to observe the effects of the niche formed by them on folliculogenesis.

Material and method

Approval for this study was obtained from Pamukkale University Animal Experiments Ethics Committee with the decision numbered -E60758568-020-402580 dated 24.07.2023. Three 3-week-old Wistar Albino rats as the first group for cell culture and three 8-10-week-old and three 12-14-month-old Wistar Albino rats as the second and third groups for histologic sections were included in our study. For cell culture, prepubertal rats that had not yet entered the estrous cycle and in which antral follicles have not seen were selected. Experimental steps of the study were carried out using Pamukkale University Experimental Surgery Application and Research Center and Cell Culture Laboratory infrastructure of the Histology-Embryology Department.

Cell culture process

The ovaries of three prepubertal (3 weeks old) female rats were collected under sterile conditions. First, the surrounding adipose tissue was excised under a stereo microscope. Then, the ovarian tissue was divided into small pieces in a petri dish, and an explant cell culture was created. In mixed cell culture, surface epithelial cells proliferating with ovarian stromal cells were locally labeled, and these cells were isolated by applying local trypsinization.

After the adherent of the ovarian surface, epithelial cells proliferated and became confluent (70-80%), the cells were removed with trypsin enzyme 0.25% (Hyclone, USA) and inoculated into two new flasks with a complete medium. The complete medium contains Dulbecco's Modified Eagle's Medium DMEM (Capricorn Scientific, Germany), Fetal Bovine Serum FBS (Capricorn Scientific, Germany), Penicillin-Streptomycin (Pan Biotech, Germany). Since the cells proliferated very rapidly, the media of the flasks were changed every day and optimal culture conditions were created. To evaluate the viability of proliferating cells, they were stained with Trypan blue, and the number of cells was determined with the Neubauer Improved counting chamber. All these stages have done using an inverted microscope (CKX41 Olympus, Japan).

Preparing tissue sections and taking histological images

Ovarian tissues of rats in the early breeding period (8-10 weeks old), and rats in the late breeding period (12-14 months old) were excised, and routine histological tissue process steps were performed. Serial sections with 5 µm thickness were taken from paraffin blocks by a microtome. Images of ovarian tissue sections that were stained with hematoxylin-eosin and Masson's trichrome were taken under a light microscope (BX51 Olympus, Japan).

Results

Cell culture

Cells began to migrate approximately 3 days after tissue fragments were taken from the ovary. On the 7th day, it was observed that ovarian stromal cells and ovarian surface epithelial cells (mixed cell culture) filled the petri dish. The morphological appearance of the ovarian stromal cells was observed to be fibroblast-like cells, and the ovarian surface epithelium was in the form of cobblestone. In addition, primordial follicle-like structures were observed among the ovarian surface epithelial cells (Figure-1 and Figure-2). Counting under phase contrast microscopy revealed that, respectively 1.5x106 and 2x106 ovarian surface epithelial cells (OSE) were grown in the culture dishes.

Figure-1 A-B Migration of cells from tissues(X10). C-D Ovarian stromal cells and ovarian surface epithelium(X10).

Figure-2 A-B-C: Proliferation of ovarian surface epithelium(X10). D: primordial follicle-like formed between cells(X10).

Histological view

It was observed that the ovarian surface epithelium was single-layered squamous epithelium in some areas, single-layered cuboidal epithelium in other parts, and stratified in some places. While the ovarian surface epithelium generally develops as a single-layered squamous and cuboidal epithelium in early breeding period rats (2nd group), it has been observed that it is stratified cubic epithelium in some regions in late breeding period rats (3rd group) (Figure 3). It was also observed that the peri-ovarian

adipose tissue adjacent to the surface epithelium of the ovary forms the macro environment of the oocytes (Figure 4).

Figure-3 Ovarian surface epithelium (OSE). A: Single-layered squamous epithelium(X100). B: Single-layered cuboidal epithelium(X100). C-D: Multilayered epithelium(X100).

Figure-4 A: Periovarian adipose tissue(X10). B: Fibroblast-like mesenchyme cells and concentrically arranged collagen fibers in the tunica albuginea(X100). C: Tunica albuginea layer of corpus luteum (Masson's Trichrome) (X20). D: Primordial follicles seen in the tunica albuginea layer (X20).

When the corpus luteum was formed from the ovule Graaf follicle, it was seen that the volume of this structure increased considerably. However, at this time, the surface epithelium is flat and few in cell number, and the tunica albuginea which is located under the surface epithelium is quite thin. Later, it was observed that fibroblast-like mesenchyme cells in the surface epithelium and tunica albuginea proliferated and showed a concentric arrangement by increasing the amount of collagen fibers they contained. The squamous surface epithelium proliferates and turns into cuboidal epithelium in some places. The tunica albuginea layer thickens, new vascular structures (capillary vessels, venules) are formed and restructured, and primordial follicles are seen in this reconstructed tunica albuginea layer (Figures 5, 6).

Figure-5 A: Graafian follicle(X10). B: Ovule Graaf follicle(X100). C: Enlargement and proliferation of granulosa cells(X10). D: Granulosa cells proliferated and completely closed the lumen(X10).

Figure 6 A-B: The appearance of the surface epithelium and tunica albuginea in the corpus luteum. It is seen that the surface epithelium is of the squamous epithelial type, and the tunica albuginea is composed of 1-2 rows of fibroblast-like mesenchyme cells(X20). C: It is seen that there are follicles in the tunica albuginea that have begun to thicken(X20). D: It is seen that fibroblast-like mesenchyme cells in the tunica albuginea proliferate and thicken this layer and contain vascular structures(X20).

The expanding layer of tunica albuginea adjacent to the corpus luteum provides a favorable environment for the development of follicles in this region. In addition, primordial follicles were found not only in the tunica albuginea of the corpus luteum but also in the tunica albuginea layer of the entire ovary. In some areas, the tunica albuginea with the ovarian surface epithelium, makes crypt-like extensions into the cortex. Primordial follicles were also observed in these regions in the sections. In some cases, after ovulation, some of the ovarian surface epithelium invaginates into the cortex region and continues its existence with parts of tunica albuginea. Primordial and primary follicles were also seen in these structures, known as inclusion cysts.

It was observed that spiral arterioles were common in the whole ovary (cortex and medulla layer). Also, it has been seen that the follicles developing in some corpus luteum structures form a cystic appearance in some places (Figure 7).

Figure 7 A: Invaginated surface epithelium causing the formation of inclusion cysts after ovulation and a part of the tunica albuginea(X10). B-C: Spiral arterioles

(X40- X20). D: Follicle that develops inside the corpus luteum and makes a cystic appearance(X4).

It is observed that the rete ovarii found in the ovaries exist in both early-breeding period rats and late-breeding period rats (Figure 8). In the medulla layer of the ovary, vascular structures (small arteries-arterioles and small veins), adipose tissue (adipocyte cells), and primordial-primary follicles were observed, albeit very rarely.

Figure 8 A-B: In the cortex layer of the ovary (X10- X20). C: In the medulla(X20). D: In the mesovarium Rete ovarii and epoophoron structures(X4).

As a result of apoptosis of granulosa cells in atretic follicles that cannot continue their development, the theca interna layer dominates. It was also observed that these structures, which turned into the thecal glands, were located in the cortex and medulla layer. Apart from these, they have been seen in the medulla layer of the ovary in structures called ganglia, which belong to the autonomic nervous system (Figure 9).

Figure 9 A: Primordial follicle, adipose tissue, and vascular structures in the medulla layer(X20). B: Primary follicles in the medulla layer(X10). C: Atretic follicles in the medulla layer(X20). D: Ganglion structure in the medulla layer(X40).

Discussion

Primordial germ cells originate from the epiblast, migrate along the primitive streak, and embed in the endoderm cells in the wall of the yolk sac near the allantois in the third week. In the fourth week, they move along the dorsal side of the hindgut mesentery with ameboid movements and reach the primitive gonads at the beginning of the 5th week, where they are called oogonium. The primary sex cords extend from the coelomic

epithelium into the mesenchyme. Later, the cells in the primary sex cords degenerate and replace by the secondary sex cords surrounding the oogonium (Sadler, 2022).

Rete ovarii develops as a result of differentiation of mesonephric cells that migrated to the developing gonad during the embryological period. Rete ovarii is the homolog of the male rete testis. Rete ovarii usually appear as groups of anastomotic tubules lined with cuboidal or columnar epithelium. Rete ovarii undertakes important functions in the control of meiosis in the ovary. Studies have shown that in the embryonic period with the arrival of mesonephric-origin somatic cells that make up the rete ovarii, some germ cells enter meiosis. Observation of secretory substances in the lumen of the rete tubules may indicate that they have a secretory ability. Three different parts have been described in the rete ovarii: extraovarian rete, connecting rete, and intraovarian rete. We have little data about the function and structure of the rete ovarii throughout adult life. Studies show that this structure is not a vestigial part but is a dynamic system that plays a role in regulating the functions of the ovaries in the postnatal period (Wenzel and Odend'Hal, 1985; McNatty et al., 1995; Smith, 2012, Apperson et al., 2017; Mfoundou et al., 2021).

The epoophoron, another structure that develops in the embryological period, remains in the mesovarium between the ovary and the tuba uterina as mesonephric duct remnants. The Epoophoron, which consists of several blind-ending tubules and ducts, can appear as the equivalent of the duktuli efferentes and epididymis in males (Moore et al., 2008; Apperson et al., 2017).

Signaling pathways that play a controlled activating and suppressive role in the transition from primordial follicle to primary follicle and protect the follicle pool, operate independently but in a balanced way. In cases where the balance is disturbed, massive activation of the follicles and premature depletion of the follicle pool occur (Kabasakal, 2023). Primordial follicles, which are intensely located in the cortex and medulla of the ovary until the first four weeks of birth in rats, begin to appear mostly in the cortex

region and in the tunica albuginea layer, which is more resistant to environmental factors during the reproductive period (Picut et al., 2015).

During ovulation, collagen fiber bundles in the theca externa and tunica albuginea layers continue the collagenolysis. The proteolytic activity in the ovarian surface epithelium contributes to the restructuring, and breakdown of the ovarian surface epithelium and the ovarian cortex during ovulation. Ovarian surface epithelium produces proteolytic enzymes such as urokinase plasminogen activator (uPA) and matrix metalloproteases 2 and 9 (Okamura et al., 1980; Ahmed et al., 2007). In addition, matrix metalloproteinases 1 (MMP-1) and 3 (MMP-3) are proteolytic enzymes involved in the remodeling of the extracellular matrix of the ovary in the menstrual cycle (Bogusiewicz et al., 2000). In some cases, after ovulation, a part of the ovarian surface epithelium invaginates towards the cortex region and forms inclusion cysts by maintaining its presence together with parts of the tunica albuginea (Auersperg et al., 2001; Ahmed et al., 2007). The ovaries are innervated by the autonomic ovarian plexus and receive both sympathetic and parasympathetic nerves. Parasympathetic ganglion cell groups in the ovary are distributed in the medulla (D'Albora and Barcia, 1996; Pastelín et al., 2017).

The ovaries are innervated by the autonomic ovarian plexus and receive both sympathetic and parasympathetic fibers. In the ovary, parasympathetic ganglion cell groups are distributed in the medulla (24,27).

Polycystic ovary syndrome (PCOS) is a disease that causes infertility and leads to low oocyte and embryo quality. The incidence of ovarian hyperstimulation syndrome (OHSS) in patients with PCOS is considerably higher than in patients without PCOS. Ovarian hyperstimulation syndrome (OHSS) is a serious complication that poses a threat to patients undergoing ovulation stimulation. In PCOS, thickening of the tunica albuginea layer of the ovary is observed (Alhilali et al., 2022). In their experimental animal study, Omairi et al. investigated the histologic changes of commonly used drugs in ovarian tissue and the effects of these drugs on follicle structures (Omairi and Al

Qaisi., 2022). In another study, Elahinia et al. examined the changes in the effects of the drugs administered depending on the phases of the estrous cycle. As a result of the study, they found that the drugs showed different effects depending on the stages of the cycle (Elahinia et al., 2023). Fazlelahi et al. found the optimal dose of different doses of drugs depending on the estrous cycles in the ovary (Fazlelahi et al., 2023). In our study, we tried to show the histologic appearance of the cells and their structures in the ovaries of early reproductive rats (8-10 weeks) and late reproductive rats (12-14 months) in a way to contribute to the literature. In addition, phase contrast microscopy was used to observe the interactions and proliferation of stromal cells and surface epithelium known to be present in the niche of follicles in mixed cell culture.

Conclusion

The development of some follicles in the developing follicle pool, as well as the stopping of the development of other follicles, are associated with hormones secreted from some of them. In addition, we think that antral follicles and corpus luteum, especially with their enlarged volumes, do not leave an area (niche) for primordial and primary follicles to develop in some cases and trigger their transformation into atretic follicles (which leads to apoptosis and autophagy). Adipose tissue around the ovary contributes to the development of follicles by secreting hormones and paracrine factors.

After ovulation, it was observed that the ovule Graaf follicle underwent remodeling of the ovarian surface epithelium and tunica albuginea (surface epithelium and tunica albuginea adjacent to the future corpus luteum). This occurs not only in the stigma, where ovulation occurs, but also along the entire tunica albuginea and surface epithelium adjacent to the ovulated Graaf follicle. In addition, vascular structures (capillaries and venules) were also observed along the tunica albuginea adjacent to the corpus luteum.

The appearance of primordial follicles in the tunica albuginea layer, which is more resistant to environmental factors, suggests that their niches are fibroblast-like mesenchymal cells to support their development happen. The presence of primordial follicles in the tunica albuginea layer of the ovary provides them with significant advantages in terms of better protection. It is thought that the follicles in this layer differentiate from the oogonium, dispersed as a result of the breakdown of the ovarian cords and settle here.

Since the structure and functions of the tunica albuginea have not been investigated sufficiently so far, our knowledge about this layer is limited. In addition to all these, the presence of tubules of the rete ovarii, which develop during the embryological period of the genitourinary system in the ovary and mesovarium during the reproductive period, indicates that these structures may also be important in terms of folliculogenesis.

Conflict of Interest

The authors declare no conflict of interest.

References

Ahmed N, Thompson EW, Quinn MA. Epithelial-mesenchymal interconversions in normal ovarian surface epithelium and ovarian carcinomas: an exception to the norm. J Cell Physiol. 2007;213(3):581-8. https://doi.org/10.1002/jcp.21240. PMID: 17708542.

Alhilali MJ, Parham A, Attaranzadeh A, Amirian M, Azizzadeh M. Polycystic Ovary Syndrome Develops the Complications of Assisted Reproductive Technologies. Arch Razi Inst. 2022 Aug 31;77(4):1459-1464. doi: 10.22092/ARI.2022.358889.2329. PMID: 36883162; PMCID: PMC9985778

Apperson KD, Bird KE, Cherian G, Löhr CV. Histology of the Ovary of the Laying Hen (Gallus domesticus). Vet Sci 2017 11;4(4):66. https://doi.org/10.3390/vetsci4040066. PMID: 29232906.

Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology and pathology. Endocr Rev. 2001;22:255-88. https://doi.org/10.1210/edrv.22.2.0422. PMID: 11294827.

Bogusiewicz M, Rechberger T, Jakimiuk AJ, Skorupski P, Jakowicki JA, Postawski K. Evaluation of matrix metalloproteinases-1 and -3 concentrations in the tunica albuginea, the apical wall of atretic follicles and the corpus luteum of normal human ovaries. Gynecol Endocrinol. 2000:14(1):25-31. https://doi.org/10.3109/09513590009167656. PMID: 10813103.

Can A. Kök Hücre;Biyolojisi, Türleri Ve Tedavide Kullanımları, Akademisyen Tıp Kitabevi, Ankara;2014. https://doi.org/10.37609/akya.259

D'Albora H, Barcia JJ. Intrinsic neuronal cell bodies in the rat ovary. Neurosci Lett. 1996 16;205(1):65-7. https://doi.org/10.1016/0304-3940(96)12361-2. PMID: 8867022.

Elahinia A, Hassanpour S, Ghotbitabar Z. Effects of Ginsenosides on Pentylenetetrazol-Induced Convulsions during Estrus Cycle in Rat. Arch Razi Inst. 2023;78(4):1359-1364. doi: 10.32592/ARI.2023.78.4.1359.

Ernst LM, Ruchelli ED, Carreon CK, Huff DS. Color Atlas of Human Fetal and Neonatal Histology. Springer 2019 Second Edition Cham, Switzerland.

Fazlelahi Z, Kaboutari J, Zendehdel M, Panahi N. Effects of Intracerebroventricular Injection of the Steroidal and Non-Steroidal Anti-Inflammatory Drugs on the Seizures during the Estrous Cycle in Rat. Arch Razi Inst. 2023 Jun 30;78(3):807-813. doi: 10.22092/ARI.2022.360115.2553. PMID: 38028823; PMCID: PMC10657946.

Kabasakal G, Turan E, Ünal MS. Effect of mesenchymal stem cells on ovarium tissue in experemental ovarian failure cases. Kocatepe Med J 2023:24:2;249-253.

Kinnear HM, Tomaszewski CE, Chang FL et al. The ovarian stroma as a new frontier. Reproduction 2020;160(3):25-39. https://doi.org/10.1530/REP-19-0501. PMID: 32716007. PMCID: PMC7453977.

Lind AK, Weijdegård B, Dahm-Kähler P, Mölne J, Sundfeldt K, Brännström M. Collagens in the human ovary and their changes in the perifollicular stroma during ovulation. Acta Obstet Gynecol Scand. 2006;85(12):1476-84. https://doi.org/10.1080/00016340601033741. PMID: 17260225.

McKey J, Anbarci DN, Bunce C, Ontiveros AE, Behringer RR, Capel B. Integration of mouse ovary morphogenesis with developmental dynamics of the oviduct, ovarian ligaments, and rete ovarii. Elife. 2022 Sep 27;11:e81088. doi: 10.7554/eLife.81088. PMID: 36165446; PMCID: PMC9621696.

McNatty KP, Smith P, Hudson NL, Heath DA, Tisdall DJ, O WS et al. Development of the sheep ovary during fetal and early neonatal life and the effect of fecundity genes. J Reprod Fertil Suppl. 1995;49:123-35. PMID: 7623307.

Mfoundou JDL, Guo YJ, Liu MM, Ran XR, Fu DH, Yan ZQ *et al.* The morphological and histological study of chicken left ovary during growth and development among Hy-line brown layers of different ages. Poult Sci. 2021:100(8);101191. https://doi.org/10.1016/j.psj.2021.101191. Epub 2021 Apr 16. PMID: 34242943.

Moore KL, Persaud. TVN Torchia MG. The Developing Human: Clinically Oriented Embryology 11th Ed. United States: Elsevier, 2020.

M,Shen Q, Li X, Kang J. Removal of peri-ovarian adipose tissue affects follicular development and lipid metabolism. Biol Reprod. 2020;103:1199–208. https://doi.org/10.1093/biolre/ioaa144. PMID: 32813010.

Okamura H, Takenaka A, Yajima Y and Nishimura T 1980. Ovulatory changes in the wall at the apex of the human Graafian follicle. J Reprod Fert 58 153–155. https://doi.org/10.1530/jrf.0.0580153. PMID: 7359471.

Omairi S, Alyodawi K, Al Qaisi T. Histological Changes in Ovary Treated with Dexamethasone and Cefotaxime Sodium. Arch Razi Inst. 2022 Jun 30;77(3):999-1005. doi: 10.22092/ARI.2022.357511.2050. PMID: 36618313; PMCID: PMC9759227.

Pastelín CF, Rosas NH, Morales-Ledesma L, Linares R, Domínguez R, Morán C. Anatomical organization and neural pathways of the ovarian plexus nerve in rats. J Ovarian Res. 2017 14;10(1):18. https://doi.org/10.1186/s13048-017-0311-x. PMID: 28292315.

Picut CA, Dixon D, Simons ML, Stump DG, Parker GA, Remick AK. Postnatal ovary development in the rat: morphologic study and correlation of morphology to neuroendocrine parameters. Toxicol Pathol. 2015;43(3):343-53. https://doi.org/10.1177/0192623314544380. Epub 2014 Aug 8. PMID: 25107574.

Ross MH, Pawlina W. Histology: A Text and Atlas with Correlated Cell and Molecular Biology, 6th ed. Philadelphia, United States: Lippincott Williams and Wilkins; 2010.

Smith PR, Quirke LD, Juengel JJ, Hurst PR. Development of the Rete Ovarii in the Sheep Ovary. Biology of Reproduction, Volume 81, Issue Suppl_1, 1 July 2009, Page 104. https://doi.org/10.1093/biolreprod/81.s1.104.

T.W. Sadler. Langman's Medical Embryology 11th Ed. Philadelphia, United States: Lipincot Wiliams & Wilkins,2010. https://doi.org/10.1097/00001416-201007000-00014.

Ünal, MS, Seçme M. Does the ovarian surface epithelium differentiate into primordial follicle and primary follicle precursor structures? Cukurova Med J 2022:47;1256-1262. https://doi.org/10.17826/cumj.1134852.

Yang L, Chen L, Lu X, Tan A, Chen Y, Li Y *et al.* Periovarian adipose tissue contributes to intraovarian control during folliculogenesis in mice. Reproduction. 2018;156:133-44. https://doi.org/10.1530/REP-18-0120. Epub 2018 May 29. PMID: 29844226.

Zhang L, An G, Wu S, Wang J, Yang D, Zhang Y. *et al.* Long-term intermittent cold exposure affects periovarian adipose tissue and ovarian microenvironment in rats. J Ovarian Res. 2021;14:107. https://doi.org/10.1186/s13048-021-00851-8. PMID: 34419111.

Wenzel JG, Odend'hal S.The mammalian rete ovarii: a literature review. Cornell Vet 1985 75(3):411-25. PMID: 4017592.

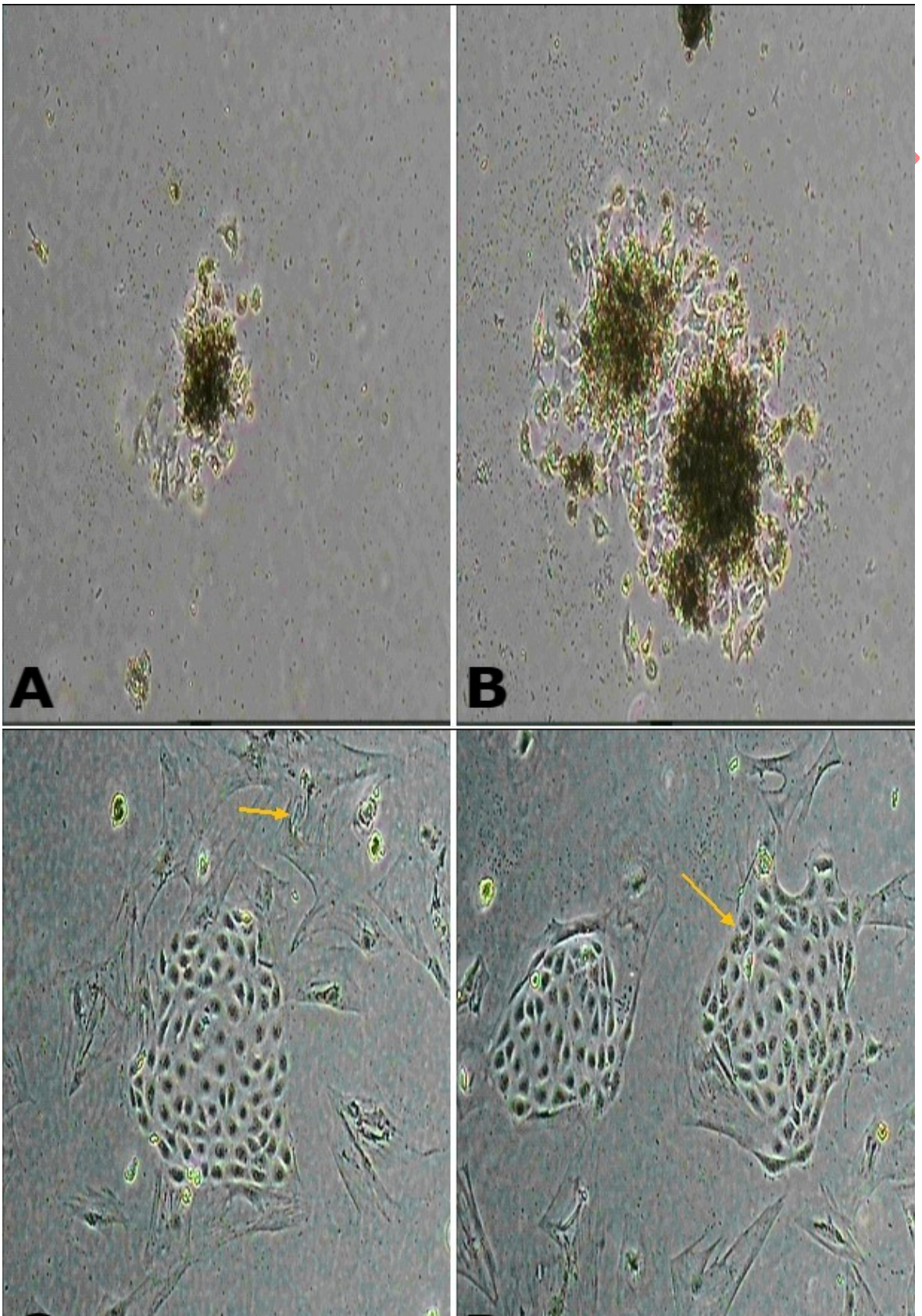
A
B

Figure-1 A-B Migration of cells from tissues(X10). C-D Ovarian stromal cells and ovarian surface epithelium(X10).

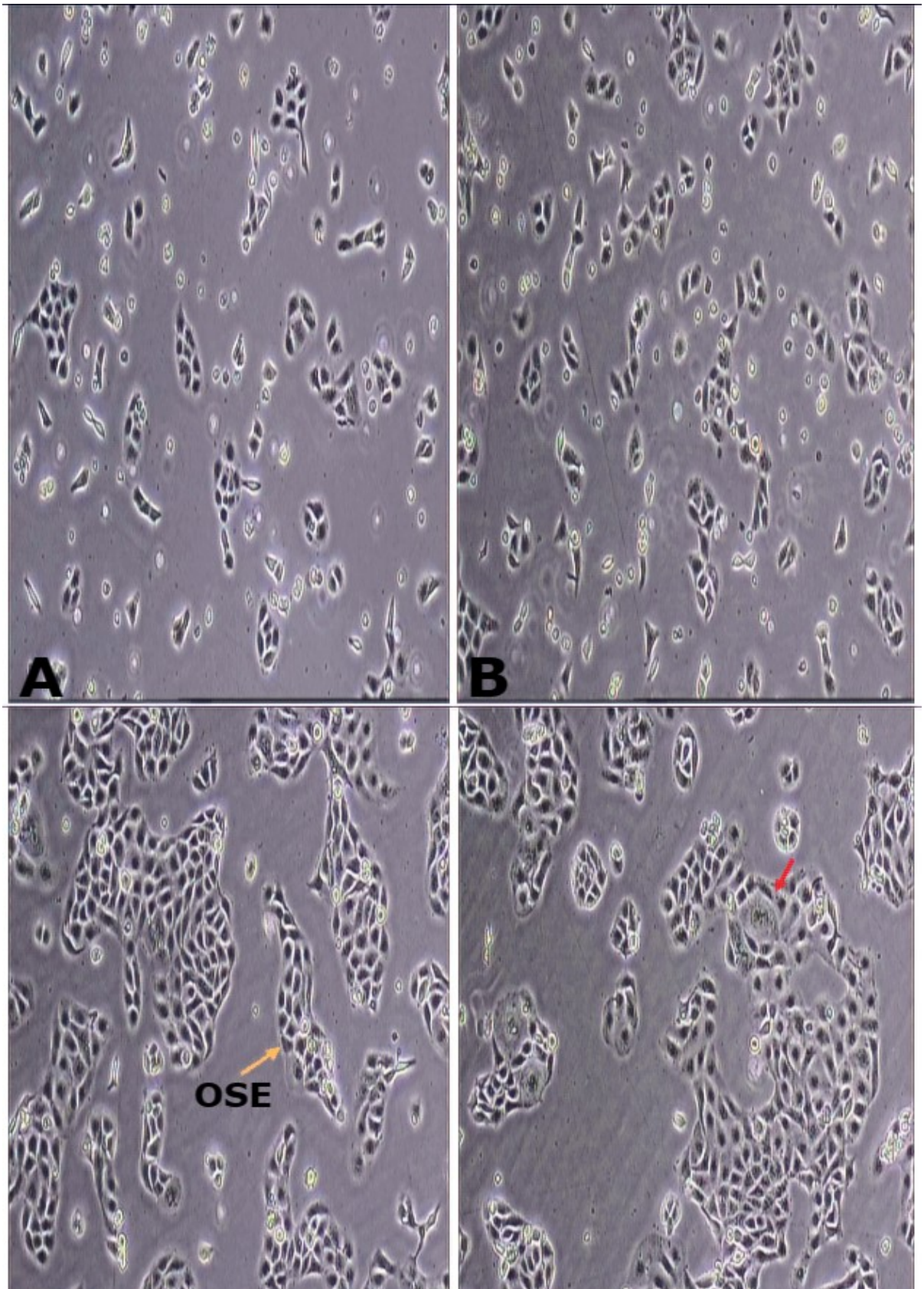
A
B
OSE

Figure-2 A-B-C: Proliferation of ovarian surface epithelium(X10). D: primordial follicle-like formed between cells(X10).

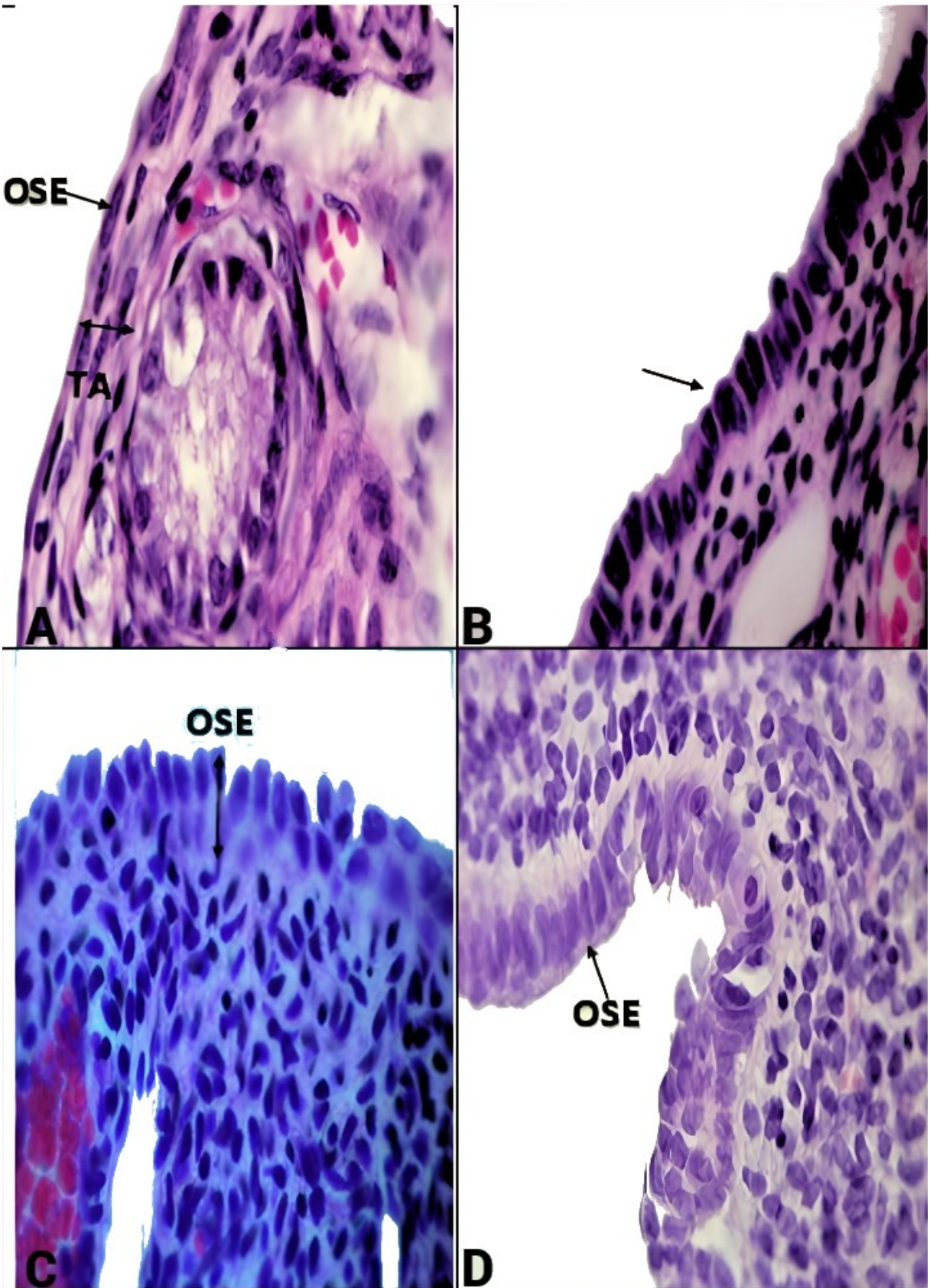
OSE
TA
A
B
OSE
C
OSE
D

Figure-3 Ovarian surface epithelium (OSE). A: Single-layered squamous epithelium(X100). B: Single-layered cuboidal epithelium(X100). C-D: Multilayered epithelium(X100).

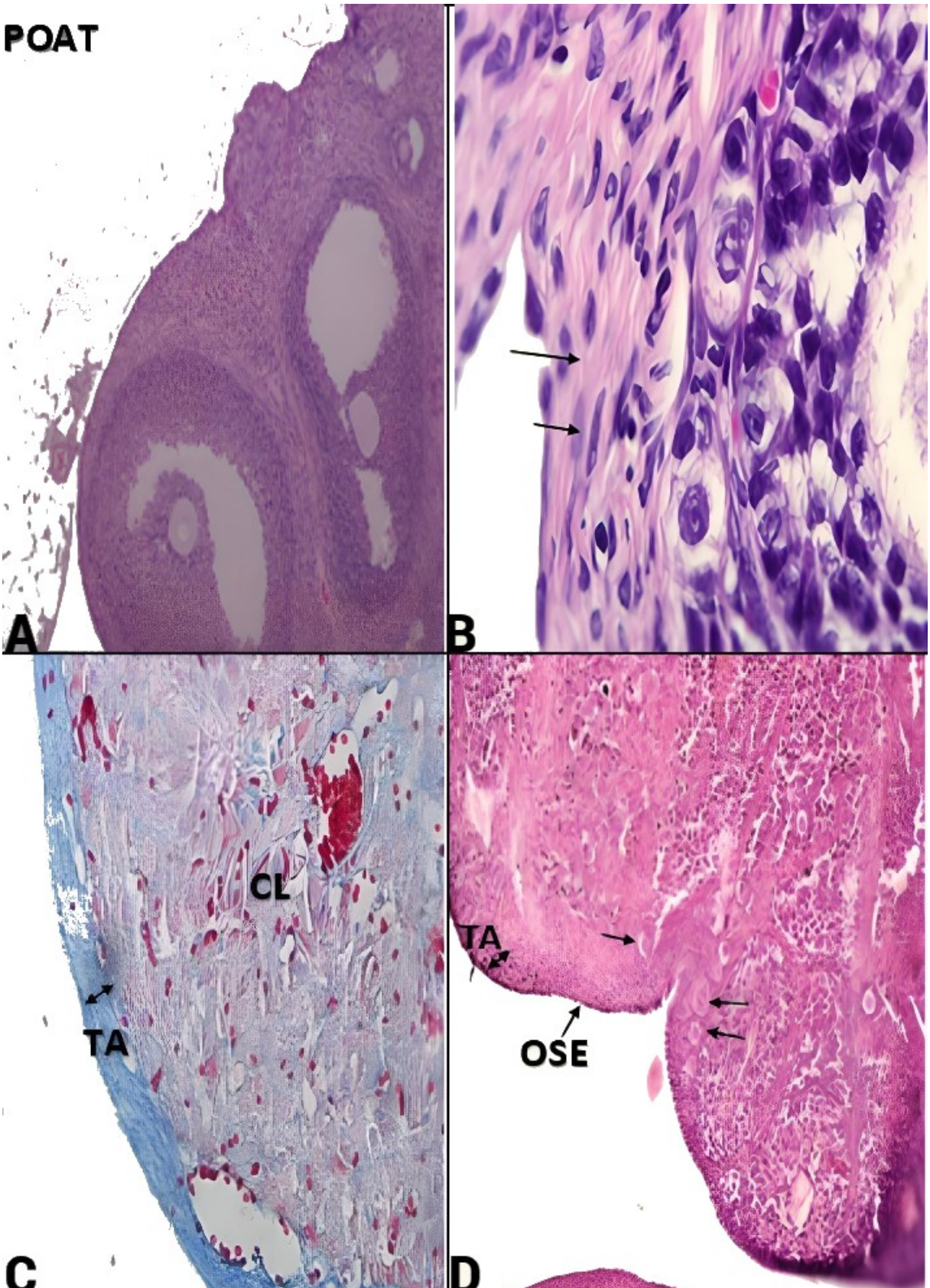
POAT
A
B
CL
TA
C
TA
OSE
D

Figure-4 A: Periovarian adipose tissue(X10). B: Fibroblast-like mesenchyme cells and concentrically arranged collagen fibers in the tunica albuginea(X100). C: Tunica albuginea layer of corpus luteum (Masson's Trichrome) (X20). D: Primordial follicles seen in the tunica albuginea layer (X20).

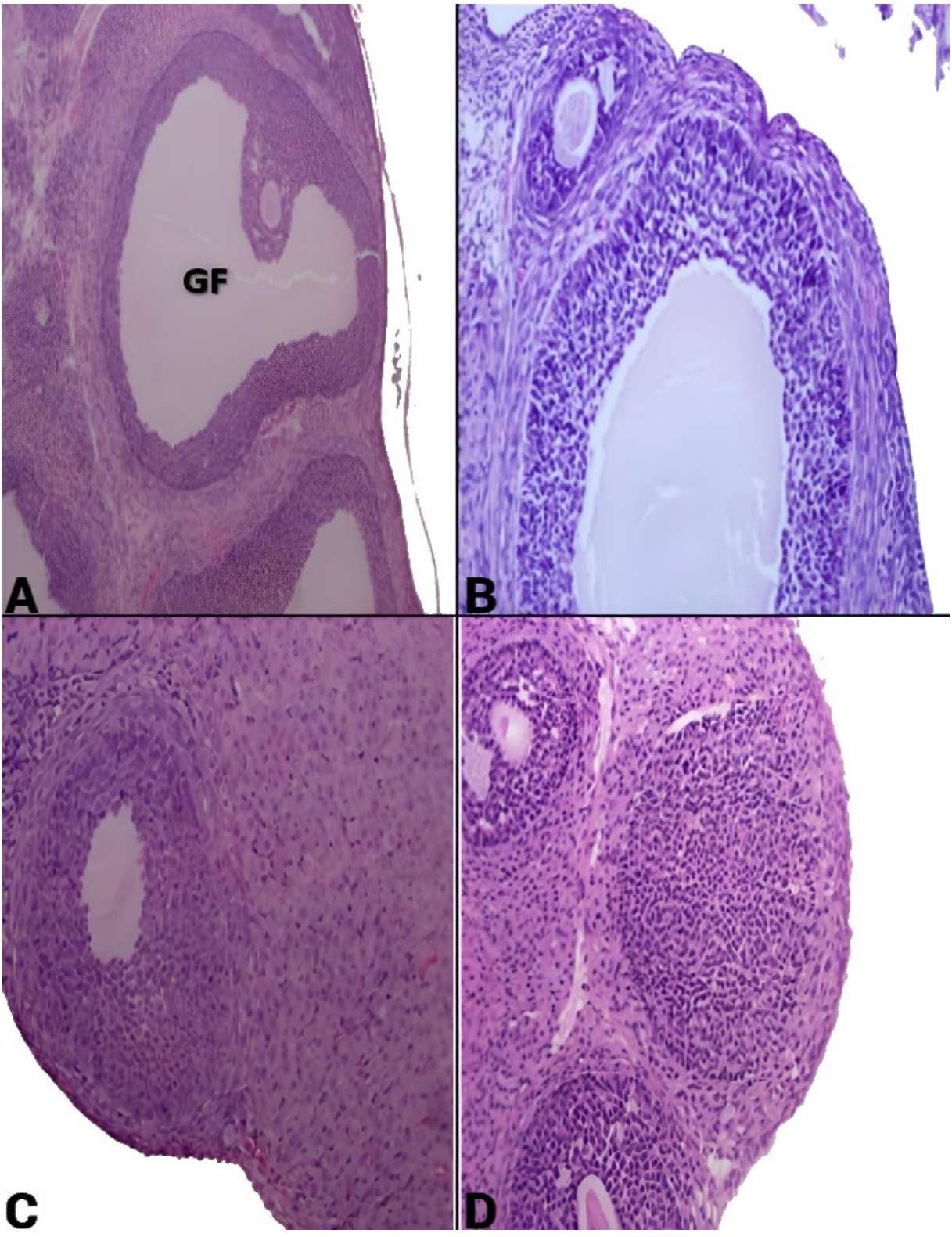
GF
A
B
C
D

Figure-5 A: Graafian follicle(X10). B: Ovule Graaf follicle(X100). C: Enlargement and proliferation of granulosa cells(X10). D: Granulosa cells proliferated and completely closed the lumen(X10).

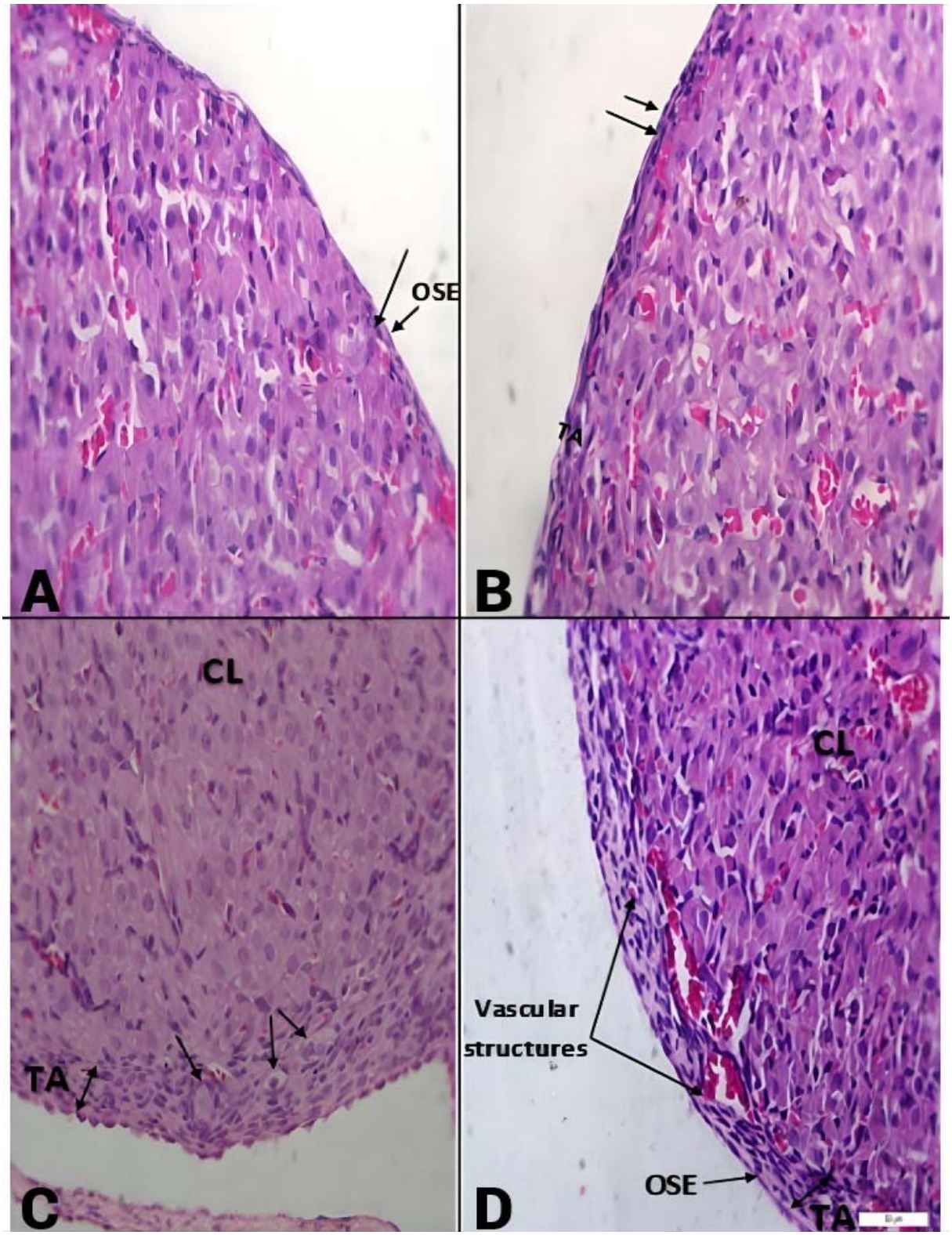
OSE
A
TA
B
CL
TA
C
CL
Vascular
structures
OSE
TA
D

Figure 6 A-B: The appearance of the surface epithelium and tunica albuginea in the corpus luteum. It is seen that the surface epithelium is of the squamous epithelial type, and the tunica albuginea is composed of 1-2 rows of fibroblast-like mesenchyme cells(X20). C: It is seen that there are follicles in the tunica albuginea that have begun to thicken(X20). D: It is seen that fibroblast-like mesenchyme cells in the tunica albuginea proliferate and thicken this layer and contain vascular structures(X20).

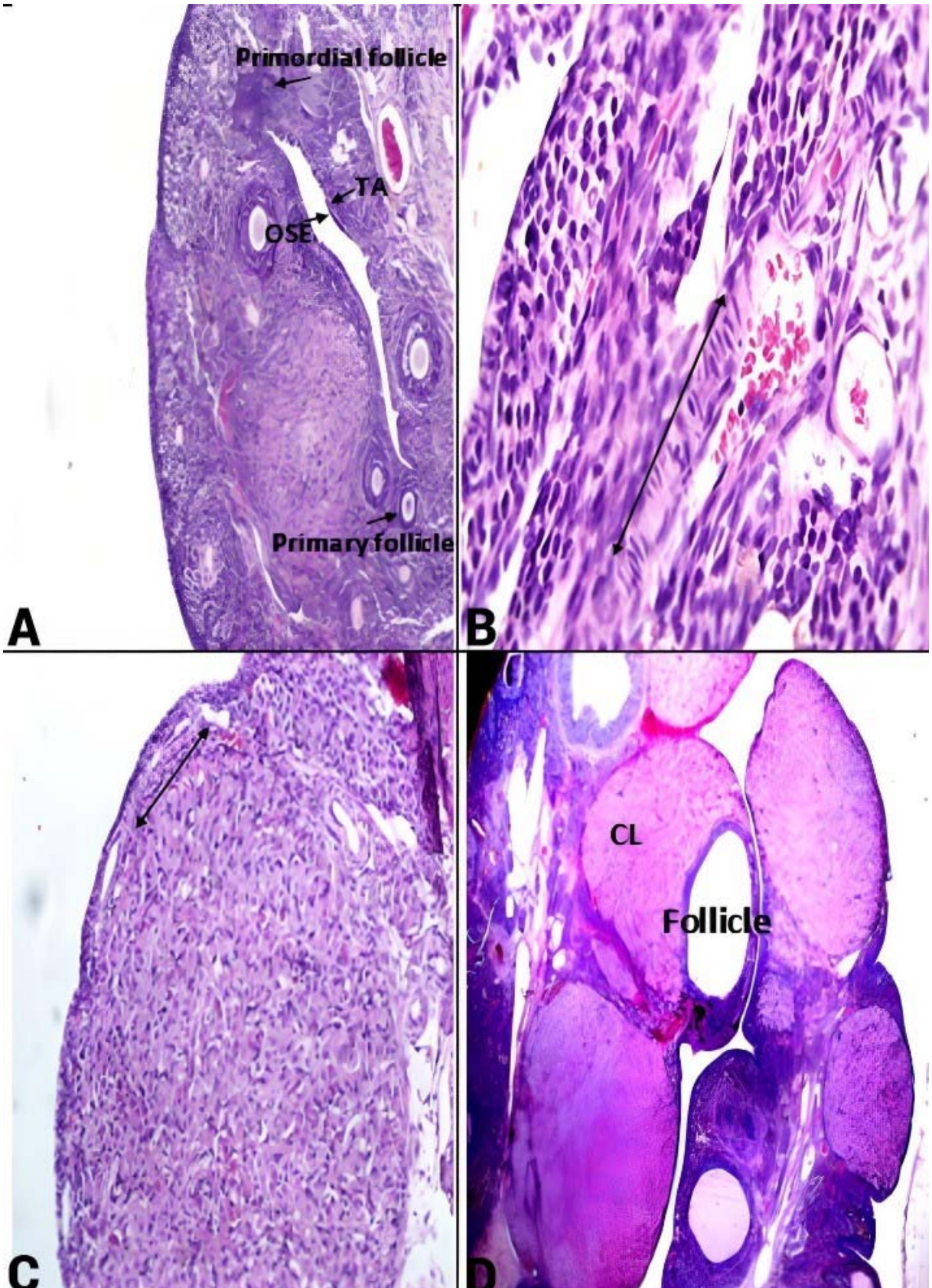
Primordial follicle
TA
OSE
Primary follicle
A
B
CL
Follicle

Figure 7 A: Invaginated surface epithelium causing the formation of inclusion cysts after ovulation and a part of the tunica albuginea(X10). B-C: Spiral arterioles (X40- X20). D: Follicle that develops inside the corpus luteum and makes a cystic appearance(X4).

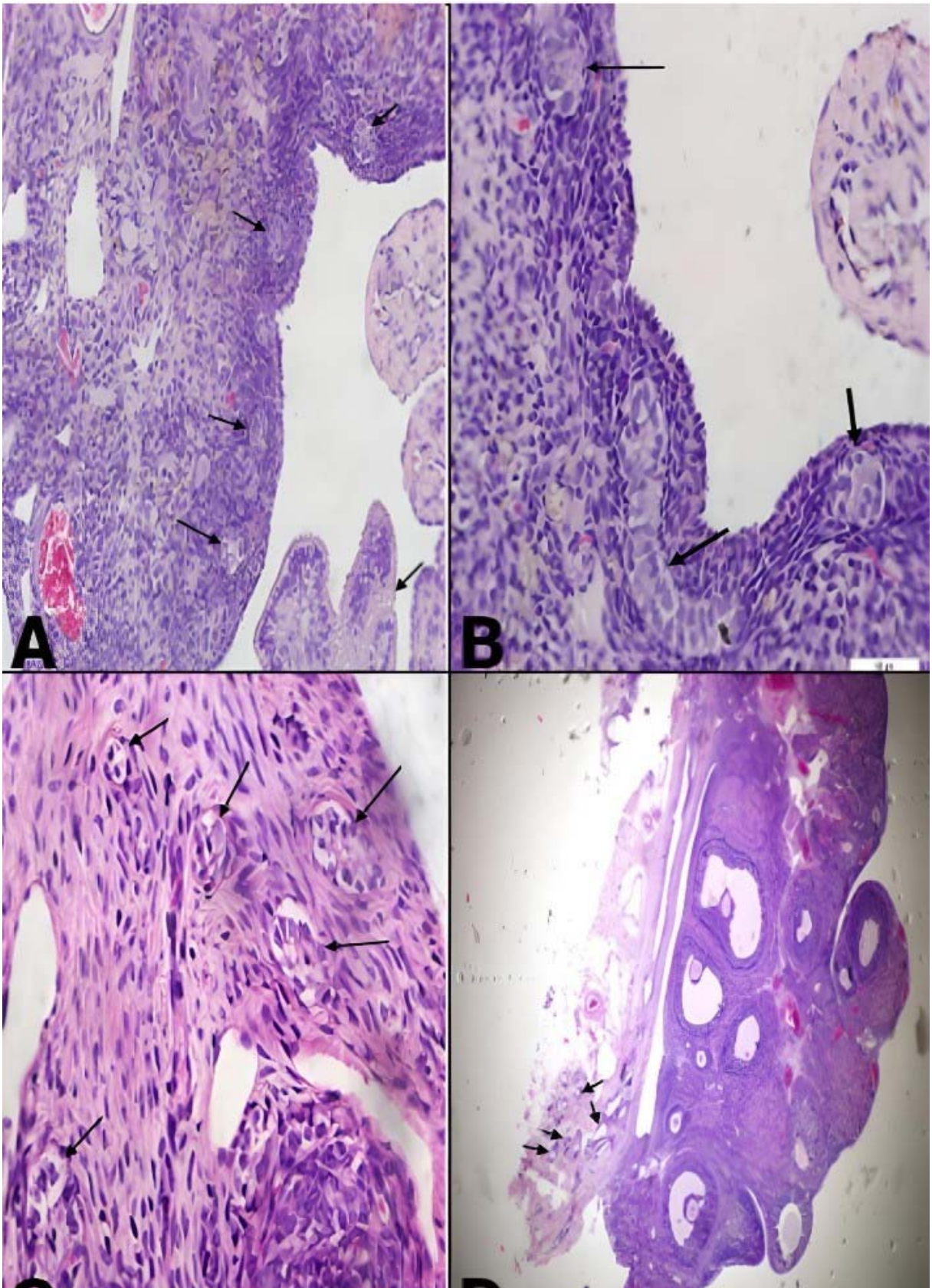
A
B

Figure 8 A-B: In the cortex layer of the ovary (X10- X20). C: In the medulla(X20). D: In the mesovarium Rete ovarii and epoophoron structures(X4).

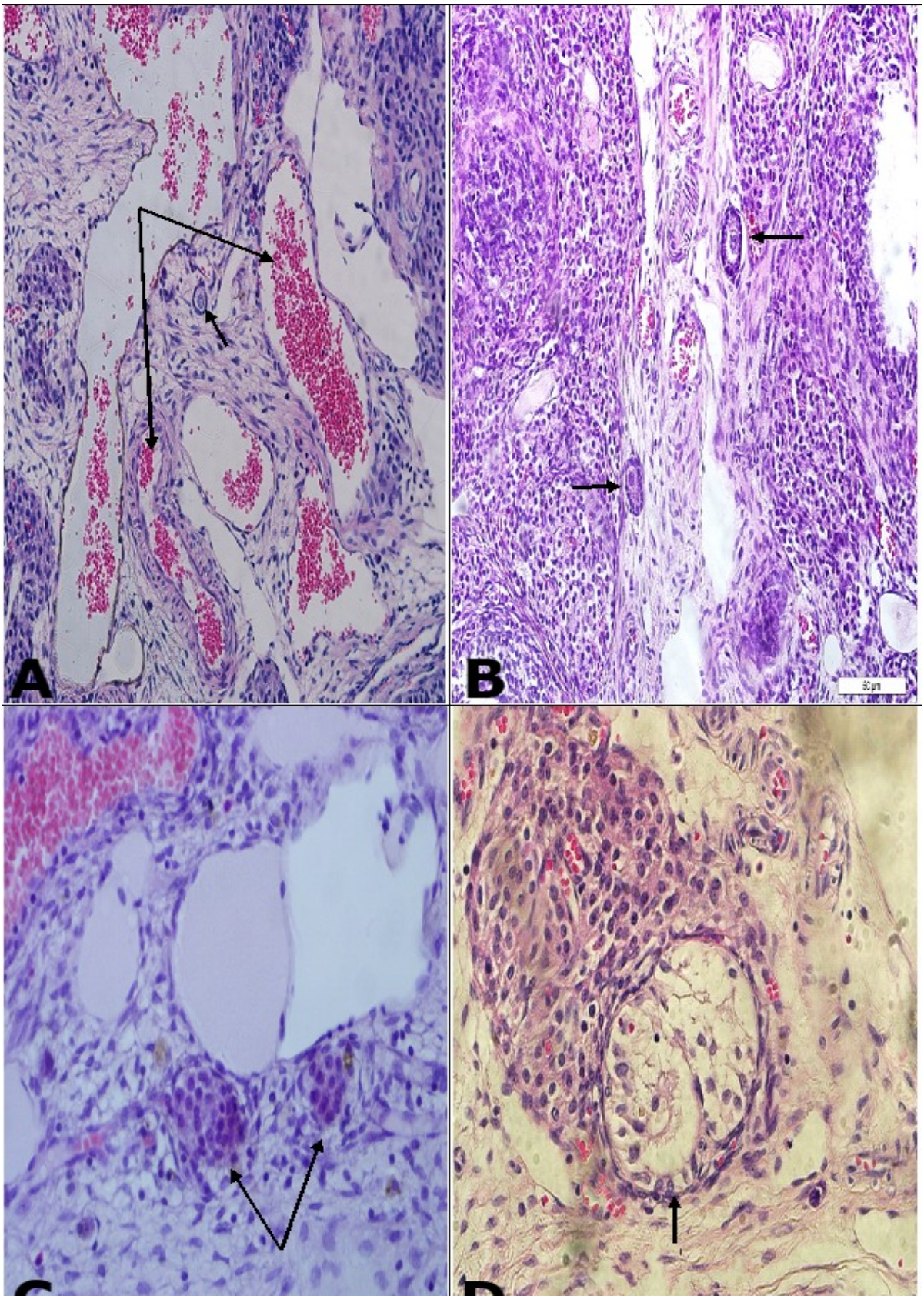
A
B
C
D

Figure 9 A: Primordial follicle, adipose tissue, and vascular structures in the medulla layer(X20). B: Primary follicles in the medulla layer(X10). C: Atretic follicles in the medulla layer(X20). D: Ganglion structure in the medulla layer(X40).