

LYMPHATIC AND BLOOD VASCULATURE OF THE FORMING CORPUS LUTEUM

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ABSTRACT

The process of lymphatic vascularization of the corpus luteum was examined during luteinization following the first and second ovulations in young rats.

In the corpus luteum formed after the first ovulation, blood vessels permeate the luteal parenchyma which at this stage shows laminar organization. Thereafter, a nest of connective tissue appears in the central portion of the corpus luteum. At this time lymphatics are not found.

Luteinization following the second ovulation is similar to that of the first generation until the later stages when lymphatic vessels start to appear initially in the peripheral zone of the corpus luteum and subsequently in the central connective tissue nest. These lymphatic vessels are lined with endothelial cells that stain darkly with toluidine dye.

The ovary is not only a highly vascularized organ but undergoes tremendous demands on its blood supply as it oscillates through several stages and sizes to carry out diverse functions. Similar changes occur within the ovary as follicles go through several stages from immature to mature including luteinization. During the luteal phase, moreover, there is a sustained heightened blood flow and luteal blood capillaries are exceptionally permeable to plasma protein. In the sow, for example, luteal blood capillaries are 1500 times more permeable than hind limb capillaries and 50 times more porous than hepatic sinusoids. High capillary permeability and increased extravasation of plasma proteins into the corpus luteum probably account for the high rate

of ovarian lymph formation. In this context, the lymphatic system is indispensable to clearance of extravasated plasma protein and fluid, and appropriately, the ovary is endowed with a notably rich lymphatic network (1,2). Relatively large lymph vessels are abundant in the medulla of the ovary and form extensive anastomoses both within the medulla and adjacent cortex. In the latter, lymphatic connections vary widely in diameter and are associated primarily with developing follicles. In the theca, small lymph vessels are found in conjunction with the blood vessels. The richest supply of lymphatics is associated with graafian follicles and corpora lutea (1,3).

Changes in the distribution of ovarian lymphatics during different stages of the estrous cycle have been carried out in smaller mammals including rats, a commonly used laboratory subject. Accordingly, we considered it worthwhile to pursue the morphology of ovarian lymphatics in the rat especially in the developing corpus luteum. Because, however, corpora lutea of adult rat ovaries at several different developmental stages belong to 4 to 5 consecutive generations, morphological analysis is particularly difficult. Thus, we opted to examine the first and second estrous cycles following vaginal opening where only one or two stages exist and thereby render the staging of the corpus luteum in question much easier.

MATERIALS AND METHODS

Fifty-six Wistar rats were used. At the time of vaginal opening (3.00-4.00 a.m. on 36th-37th post-natal days) (5), a vaginal



Fig. 1. Mature follicle about to ovulate. Note the undulating boundary (arrow) of the theca and the follicular epithelium. B: Blood capillaries are dispersed along the boundary (x650).

smear was examined, and those demonstrating a cornified vaginal epithelium were considered as immediately post-first ovulation. The ovaries were excised from the rats at prescribed time intervals after the first ovulation. The numbers of rats examined at each time interval were as follows: 4 rats at 0, 3, 6, 12, and 18 hours after the first positive vaginal smear, and 2 rats on each day of 1 to 15 postovulation.

The excised ovary was cut into 4 pieces and immersed in chilled aldehyde fixative (2.0% glutar- and 4.0% paraformaldehyde in 0.1M phosphate buffer solution). After several hours of aldehyde fixation, they were post-fixed in 1% osmium tetroxide for 2 hours and then embedded in epoxy-resin (Quetol 651, Nisshin-EM).

Serial semithin (1.5μ) sections were cut and stained with toluidine blue. The semithin sections suspected by light microscopy to contain lymphatics were further trimmed, and ultrathin sections were taken from the pertinent part of the semithin sec-

tion. Electron microscopy confirmed the lymphatic nature of the "suspected" vessels after double staining with uranyl acetate and lead tartrate.

For measurement of sizes and numbers of tissue constituents of interest, photomicrographs of the areas of concern were enlarged to x1200.

The profiles of lymphatic vessels encountered in the corpus luteum were very few in number and consequently no systematic stereological analysis such as numerical density and volume ratio was made.

RESULTS

Preovulatory mature follicles

In some of the ovaries taken immediately after confirmation of cornified vaginal epithelial cells (within an hour after vaginal opening), mature follicles were about to ovulate. In these follicles, the tex-

ture of the theca interna consisting mainly of round cells of 5μ diameter darkly stained with toluidine blue and the theca externa composed of spindle form cells of 7μ diameter containing lutein granules, seemed less dense as compared with that of the premature follicles. The intercellular space and the blood capillaries of the theca were increased. The boundary between the theca and the follicular epithelium was undulating, and many blood capillaries were seen beneath this boundary (Fig. 1). Mitoses were sporadic among the follicular epithelial cells.

First stage of forming corpus luteum

Near the site of the rupture of the follicle, the blood vessels (venules) of the tunica albuginea were interspersed among granulosa cells (Fig. 2a). In the basal portion of the corpus luteum (i.e., opposite to the site of rupture) thecal blood capillaries were continuous with the fascicular structures which represented newly forming blood vessels. Although lymphatic vessels were visible in the tunica albuginea near the rupture site, they were not traceable into the parenchyma of the corpus luteum (Fig. 2c). The outer zone of the corpus

luteum looked heterogeneous and was composed of light and dark cells. Both types of cells were smaller ($5-6\mu$) than the granulosa lutein cells ($7-8\mu$) in the corpus luteum of the later stages. Some of these cells were undergoing mitosis (Fig. 2b), and a small fraction of the cells adjoining the peripheral boundary of the corpus luteum contained lutein granules. The granules (9-15, average 12 in number per cell) were about 1μ in diameter and stained yellowish in toluidine blue stained sections.

3-6 hours after ovulation

The parenchymal cells of the corpus luteum began to show laminar organization (Fig. 3a). The outer zone adjoining the theca consisted of large ($7-8\mu$) cells with many lutein granules (20-35/cell) (Fig. 3d). In the intermediate zone, cells ($6-7\mu$) with lutein granules (10/cell) intermingled with cells without such granules (Fig. 3c). In the innermost zone, smaller cells ($5-6\mu$) without granules and weakly stained cytoplasm predominated, and a small acellular lumen lined with epithelioid cells was seen in the central part of the corpus luteum (Fig. 3b). Mitotic figures were frequent throughout the three zones.

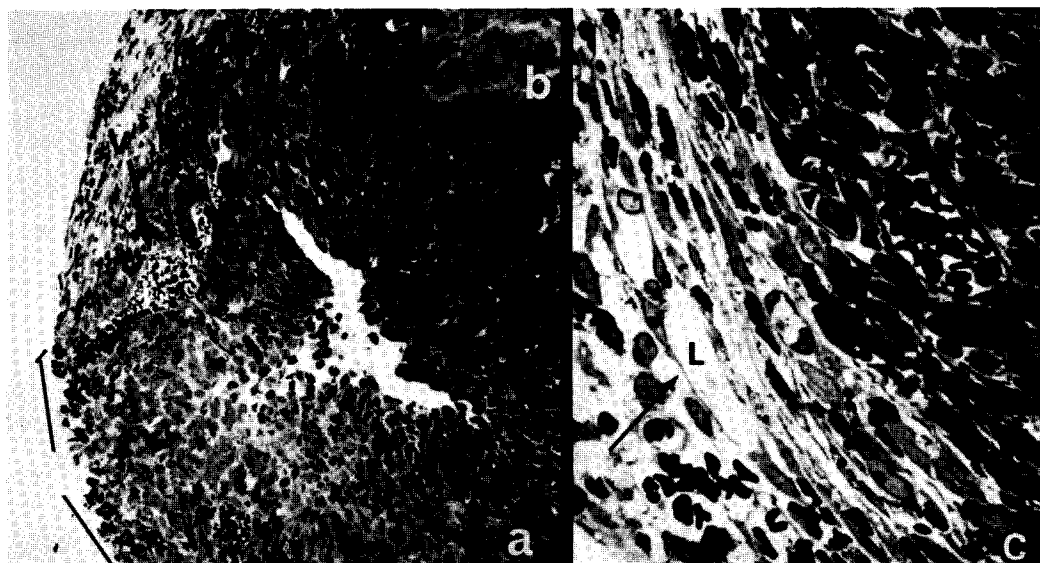


Fig. 2. The follicle at the time of ovulation. a: The region near the site of ovulation. A venule in the tunica albuginea (v) continues into the follicular parenchyma (x160). b: Mitotic cell (x650). c: Lymphatic (L) in the tunica albuginea near the site of ovulation.

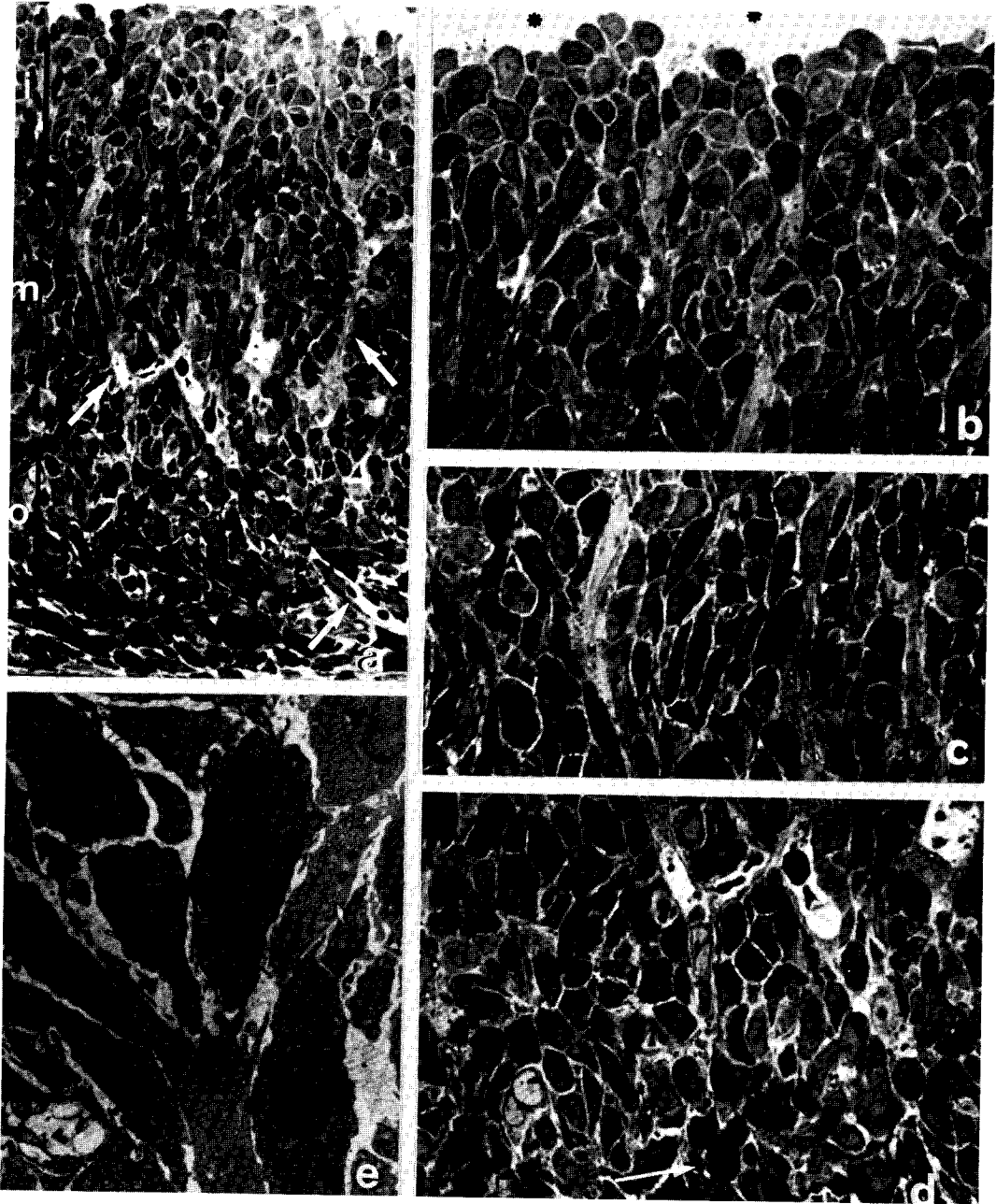


Fig. 3. A corpus luteum 3 to 6 hours after ovulation. a: Laminar arrangement of the luteal parenchyma. i: innermost zone, m: intermediate zone, o: outer zone. The arrow indicates a blood vessel within the parenchyma (x330). b: Innermost zone under higher magnification. Asterisk: small acellular lumen (x650). c: Intermediate zone highly magnified (x650). d: Outer zone containing larger cells (arrow) with luteal granules (x650). e: An electron micrograph showing the finger-like structure at the apex of the blood vascular strand (x3,000).

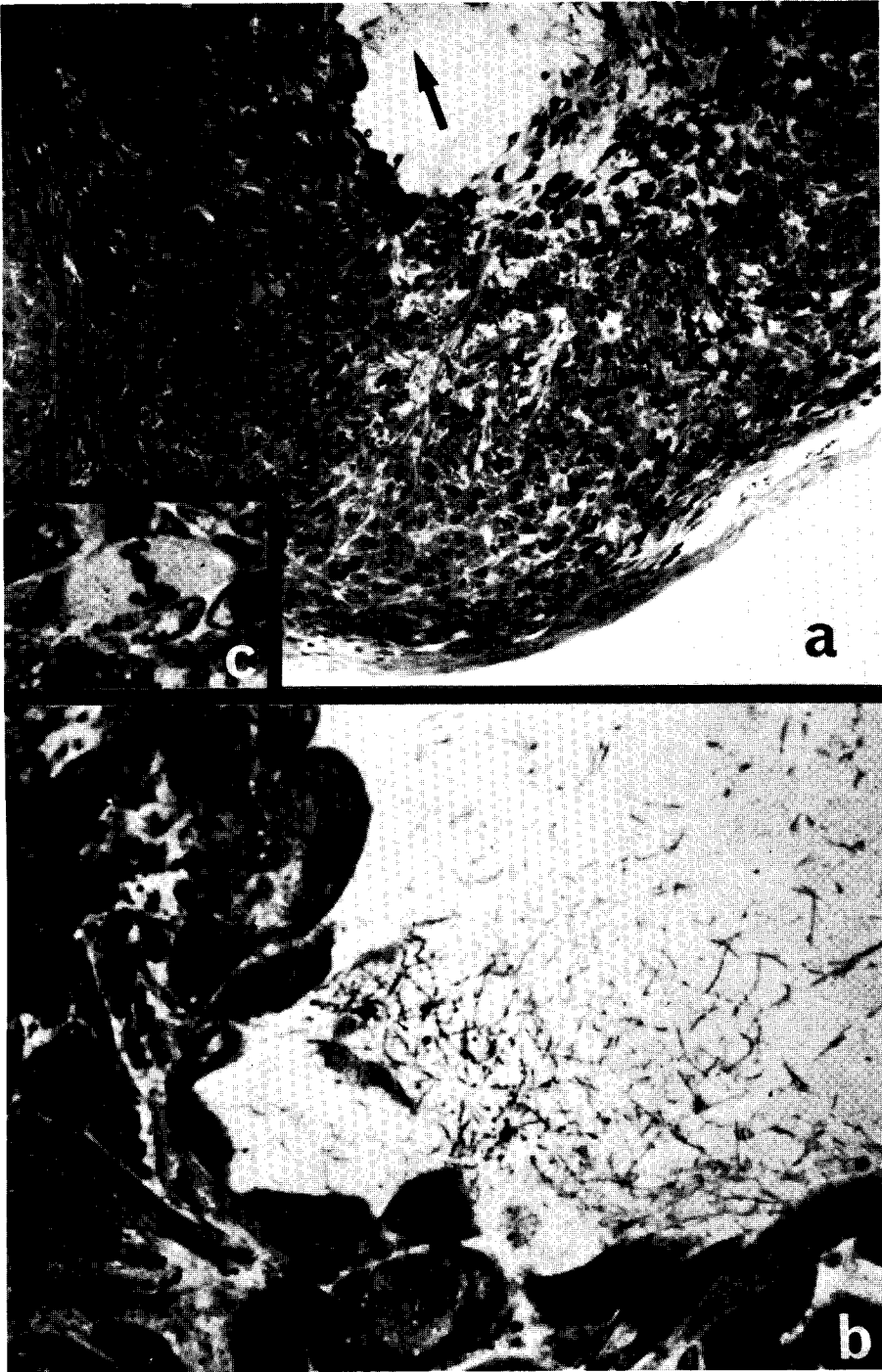


Fig. 4. A corpus luteum 18 hours after ovulation. a: Fibrinoid material (arrow) in the central lumen (x160). b: A part of Fig. 4a under higher magnification (x1,000). c: A cell in mitosis (x1,000).



Fig. 5. A corpus luteum 5 days after ovulation. a: lymph capillary (arrow) in the peripheral part of the parenchyma (x600). b: A part of Fig. 5a under higher magnification (x1,500).



Fig. 6. A corpus luteum 6 to 7 days after ovulation. Lymphatic capillary (L) in the connective tissue nest in the central portion (x600).

The blood vessels of the internal theca crossed into the radial vesicular structures of the corpus luteum parenchyma. The fascicles tapered toward the center of the corpus luteum and consisted mostly of connective tissue fibers. Among these fibers, narrow vascular lumina surrounded by endothelial cells stained darkly with toluidine blue. Occasionally these fascicles displayed small branches which ended in the intermediate zone of the parenchyma. Electron microscopy of the tip of these tiny branches revealed filopodia-like structures (Fig. 3e).

18 hours after ovulation

Fibrinoid material filled the central lumen seen in the previous stage (Fig. 4b), the laminar organization of the corpus luteum parenchyma became indistinct, and the constituent cells of the corpus luteum were homogeneous as to size, morphology of the nucleus and nucleolus, and number of lutein granules (Fig. 4a). Mitotic figures were still evident (Fig. 4c). Radial fascicular structures which contained the growing-in blood vessels disappeared, and blood vessels elements were diffusely distributed throughout the corpus luteum.

1 day after ovulation

No mitotic figure was found. Many fibroblasts were in the central part of the corpus luteum where fibrinoid material had been seen before, and in conjunction with vascular elements comprised the central connective tissue "core". Some of the blood vessels in the core were continuous with blood vessels in the parenchyma, but the diameter in the core vessels was larger (20 μ).

Throughout the process of luteal maturation after the first ovulation as described above, no lymphatic vessels were identified either in the parenchyma or in the core, but were seen in the follicular theca layer.

2-3 days after ovulation

The core of connective tissue became more densely populated by fibroblasts, leaving a smaller amount of intercellular space (Fig. 9a). The parenchymatous cells of the corpus luteum were similar to the previous

stage with regard to size and cytoplasmic stainability, but the nuclei became larger and lighter in appearance and the nucleoli more distinct. Though the parenchyma cells were packed densely, the boundaries among cells were readily discernable. The endothelial cells of the blood capillaries in the parenchyma continued to be darkly stained with toluidine blue.

4 days after the first ovulation

In addition to the corpora lutea already morphologically described, there appeared at this stage another type of corpus luteum. The latter was similar to the corpus luteum of the first stage (see *First stage of forming corpus luteum*, above) and probably represented a new corpus luteum following the second ovulation.

5 days after the first ovulation and the second day after the second ovulation

As in the previous day, there were two types of corpus luteum. One appeared as the corpus luteum derived from the first ovulation. The other resembled the corpus luteum 24 hours after the first ovulation and therefore was considered to be the corpus luteum derived from the second ovulation. Nonetheless, there were differences from the corpus luteum of the first ovulation in that novel capillaries were found in the peripheral portion of the luteal parenchyma where small ($\sim 6\mu$) pleomorphic cells prevailed (Fig. 5a). The endothelial cells of these capillaries were polygonal and wider than those of blood capillaries (Fig. 5b), were extremely tenuous except for the nuclear area, and were notably heterogeneous in cytoplasmic stainability with toluidine blue with some dark and others quite pale.

Electron microscopy revealed that wide gaps existed between the endothelial cells (both light and dark types), and the abluminal surface of these cells lacked basal lamina but were attached sporadically by anchoring filaments (6) (Fig. 8a).

In addition, the endothelial cells were interconnected in places by a specialized junctional apparatus resembling zonula adherens (Fig. 8b). Darkly staining endothelial cells on light microscopy were electron-

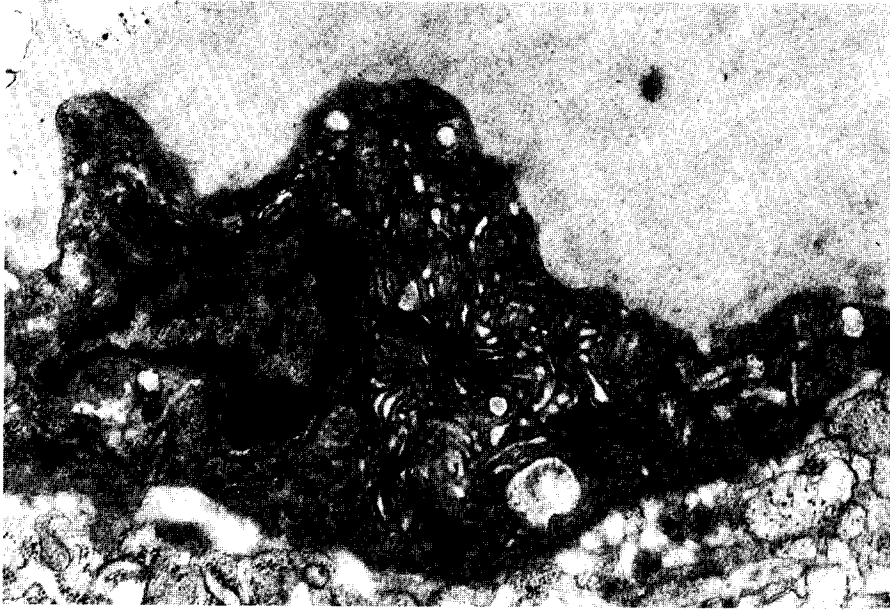


Fig. 7. An electron micrograph of the dark type of lymphatic endothelial cell from the corpus luteum on the fifth day after ovulation (x2,400).

dense and contained abundant free ribosomes in the cytoplasm on electron microscopy.

These light microscopic and ultrastructural features strongly suggest the lymphatic nature of these capillaries. Nonetheless, the perikaryal area of the "toluidine dark" endothelium of these "lymphatic" capillaries was taller and more electron-dense, and contained more free ribosomes and better developed Golgi apparatus than conventional lymphatic capillaries (Fig. 7). The central part of the corpus luteum from the second ovulation—now identifiable by the presence of lymphatic capillaries in the peripheral portion of the parenchyma—was occupied by dense connective tissue where venules with wide lumen joined blood capillaries.

6 to 7 days after the first ovulation

The central connective tissue area of the corpus luteum of the first generation lacking lymphatic capillaries widened and became less dense with increased intercellular spaces (Fig. 9b). The endothelial cells of the blood capillaries also became paler than in previous stages.

On the other hand, in the central dense connective tissue of the corpus luteum demonstrating lymphatic capillaries in the parenchyma (i.e., the corpus luteum of the second generation), besides venules there were numerous lymphatic capillaries with a lumen wider than that in the periphery of the parenchyma (Fig. 6). These lymphatic networks interconnected as shown with some difficulty using serial, semithin sections. The endothelial cells of these central and peripheral lymphatic capillaries were paler than those of the peripheral lymphatic capillaries in the previous stage. Whereas staining heterogeneity was less visible on light microscopy, heterogeneity of electron density remained.

8 days after the first ovulation

New corpora lutea similar to those after the first ovulation appeared and presumably were derived from the third ovulation. The remaining mature corpora lutea failed to display lymphatic capillaries and thus it was impossible to differentiate between the corpora lutea from the first and the second ovulation.

DISCUSSION

According to Morris & Sass (2), mature follicles and the corpora lutea of the ewe ovary have a profuse network of lymphatics. Graafian follicles display a basket-like lymphatic network in the theca interna and externa surrounding the stratum granulosa, but lymphatics do not enter the granulosa layer. Within 8 hours of ovulation the lymphatics at the margin of the corpus luteum penetrated its substance. In a mature corpus luteum the bulk of lymphatics are detected in the trabeculae of the connective tissue which radiate from the periphery to the center of the corpus luteum where the follicular cavity contains blood vessels and lymphatics among the connective tissue elements. In contrast to the ewe, however, distinct trabeculae in the rat as described in this study were not seen to develop, and lymphatic vessels in the corpus luteum parenchyma were less common. The presence of lymphatics in the central connective

tissue core of the mature corpus luteum, however, is shared by both species.

The most detailed study previously done on luteal lymphatics is that of Anderson (4). He examined sow's ovary using injections of various dyes. In the mature but unruptured sow follicles, he failed to observe blood vessels entering the granulosa layer. Corner (8), however, has noted capillaries entering the granulosa at the base of the cumulus oophorus. In the sow (4) a complete wreath of lymphatics develops between the theca interna and the granulosa of the mature follicles. One or two days after ovulation, tiny blood vessels bud from capillaries of the interna and extend into the granulosa. On the second or third day after ovulation periarterial lymphatics grow out from the theca externa along the strip of newly developed tissue of the theca interna, and form a narrow single-layered sub-granulosa network of non-valved lymphatic capillaries which extend around the surface of the interna cells and enter the granulosa

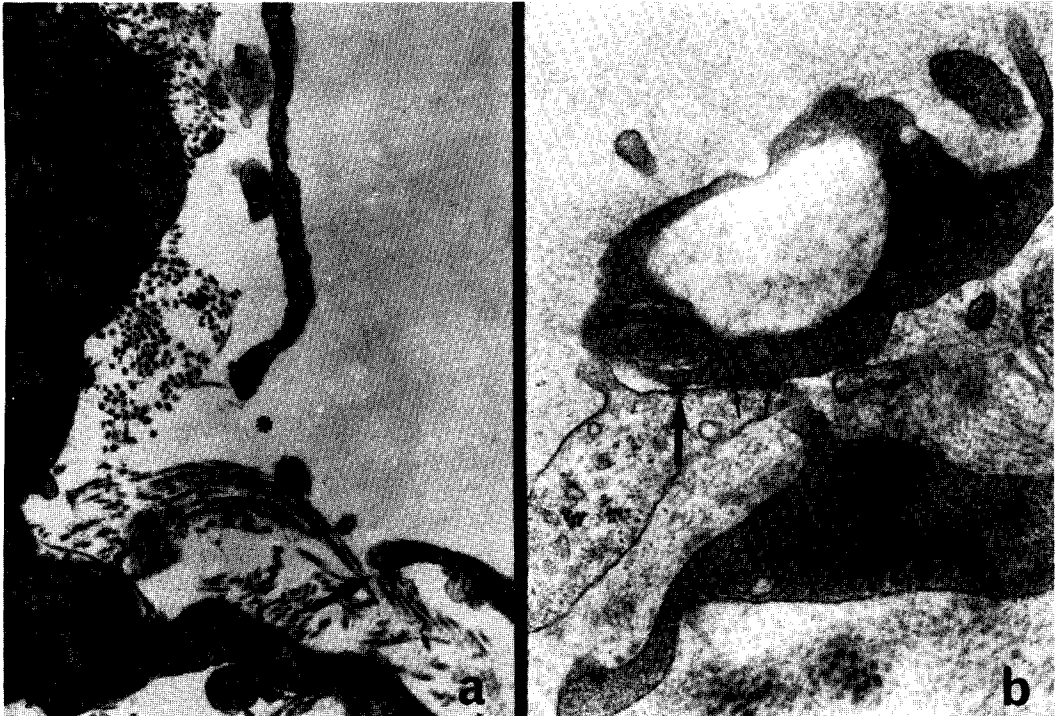


Fig. 8. Electron micrographs of lymphatic endothelial cells. *a*: Wide gap (asterisk) in the endothelial lining and the anchoring filaments (arrow) (x6,000). *b*: Zonula adherens (arrow) between endothelial cells (x3,000).

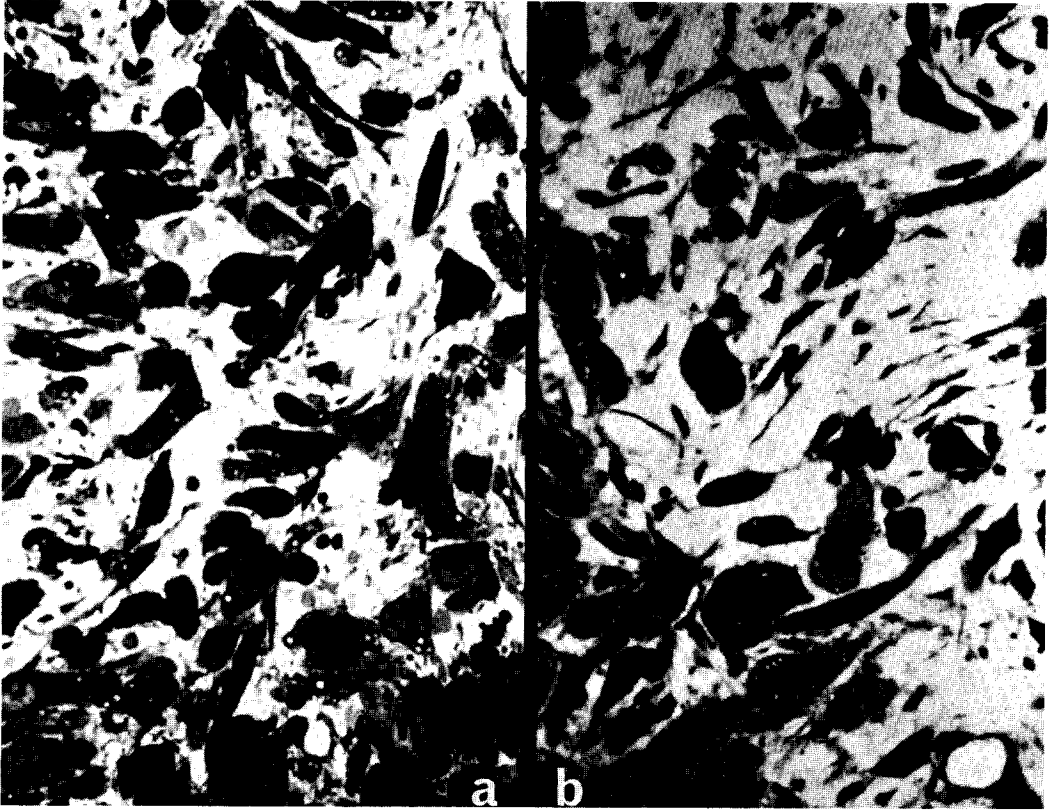


Fig. 9. *a*: densely cellular and *b*: sparsely cellular connective tissue nests in the central part of a corpus luteum 2 to 3 days (*a*) and 6 to 7 days (*b*) after ovulation (x6,000).

the next day. Eventually on the fourth day after ovulation, multitudinous offshoots from the subgranulosa lymphatic network enter the granulosa. With further maturation of the corpus luteum as seen in the sow (4), another set of lymphatics appears (i.e., a capillary network of lymphatic vessels fitted as a single layer between each opposing inner surface corresponding to the former intrafollicular cavity), completely filled in, and only a line remains between the folds of tissue. Fine thread-like lymphatic vessels interconnect this central (internal) network and those emerging with blood vessels from the externa. Whereas, therefore, lymphatics lag behind blood vessels in growing into the corpus luteum, they follow a similar pathway. In the rat, however, the border between the thecal layer and granulosa rapidly becomes indistinct, and thus the sequence as delineated in the sow could not be sub-

stantiated.

Comparison of lymphatic development in the maturing corpus luteum of the ewe and sow with that of the rat shows considerable species differences. These differences may, for example, relate to structural and functional ovarian features such as the size of the follicles, the process of ovulation induction, and the speed of luteal development. Bassett (9) has described in the rat the dynamic blood vascularization in the luteinizing follicles and the dual vascular networks in mature follicles. The inner network or wreath situated at the boundary between the granulosa and theca interna sprouts into the granulosa promptly after ovulation to form a dense network of sinusoidal capillaries. Another outer network wreath begins to sprout inward into the theca 24 hours after ovulation. Thereafter, these two vascular networks interconnect to

form a diffuse sinusoidal meshwork throughout the corpus luteum. Forty-eight hours after ovulation the meshwork constituents differentiate into afferent arterial and efferent venous systems. They intercommunicate at the sinusoidal ring in the central connective tissue core which develops after disappearance of the antrum and contains reticular fibers, degenerated granulosa cells, and connective tissue cells.

The development of the lymphatic system in the rat ovary in the corpus luteum was, however, different from blood vascularization, although the latter process could not be fully corroborated by the limitations of our study. The distinction between lymphatic and blood vascular ingrowth is notably different from findings in the sow where their development in the corpus luteum is similar although lymphatic development lags slightly behind. These species differences may relate to ovarian size difference. Thus in the rat, a much smaller corpus luteum may not require a substantial intraluteal lymphatic network to drain extravasated and secreted interstitial material which may be transported alternatively by simple diffusion toward the medullary portion of the ovary where an abundant lymphatic network exists.

Failure to find lymphatic capillaries of the corpus luteum of the first generation may reflect inadequate luteinization following the first ovulation.

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