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Characterization of the fish ovarian stroma during the spawning season: Cytochemical, immunohistochemical and ultrastructural studies

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ABSTRACT

The changes in the ovarian stroma of the fish during their spawning season become it an excellent biological model for studies on cellular and vascular elements due to the intense tissue remodeling in fish occur naturally throughout this critical period. The present study aims to investigate the cellular and vascular components of the ovarian stroma of Redbelly tilapia during the spawning season by conventional, immunohistochemical stains as well as to detect the ultrastructural characteristics for each stromal component. The histological examinations revealed a series of blood vessels with special structures, include throttle artery, glomus, spirally oriented arterioles, modified arteries, and veins as well as arteriovenous anastomosis. Various types of cells were detected in the stroma include; telocytes, rodlet cells, mast cells, eosinophils, neutrophils, lymphocytes, fibroblasts, macrophages, melanocytes, adipocytes, dendritic cells, and endocrine (steroidogenic, interstitial) cells. Moreover, these stromal cells showed a broad range of staining affinity against c-kit, desmin, and s100-protein. Bundles of nerve fibers were detected between the follicles. This study exposed various cellular and vascular components with distinct functions in the ovary of Redbelly tilapia during the spawning season.

1. Introduction

The stroma is viewed as the supportive framework of the ovary, comprising mostly of connective tissue, blood vessels, and nerves. These stroma undergoing changes during both the spawning and the non-spawning season, being more intense in the sex reversal species [1].

The blood vessels of the ovary showed a series of histological changes, which arise as a result of reproductive activity. These changes were described in the uterine and ovarian arteries of the buffalos [2] and ovary of camel [3]. Most investigations about the systematic blood vessels have been especially shown in some fishes [4]. However, structural organizations of such blood vessels in the ovary of the Redbelly tilapia was not reported.

Telocytes are interstitial cells characterized by unique long cytoplasmic processes called telopodes [5]. Their presence was proven in a few fish tissues; include the liver [6], and gonads [7]. Rodlet cells were observed in a wide range of fish species and tissue types, particularly epithelial layers in direct contact with the external environment such as olfactory epithelium [8], as well as those of the gut, pancreas [9], kidney [10], gills and intestine [11] and vascular system [12]. The presence of rodlet cells in the fishes' gonads has been reported as infrequent or occasional [13].

The Redbelly tilapia (*Coptodon zillii*) is a species of fish in the cichlid family. It is found widely in Africa but has also been introduced outside its native range. It is an important food fish in Egypt. It was formerly included in the genus Tilapia as *Tilapia zillii*. The length at first maturity is about 10 cm and the spawning season ranged from March to August [14].

However, little is known about the ultrastructure and cytochemistry of the ovarian stroma of *Coptodon zillii*. Therefore, the present study aims to investigate the characteristics of cellular and vascular stromal components and their pattern of distribution in the ovary of Redbelly tilapia using light-, electron-microscopy, enzyme histochemistry, immunohistochemistry and fluorescent stain in correlation with the reproductive activity and physiology.

2. Materials and methods

2.1. Samples collection

The study was approved by the Ethics Committee of Assiut University, Egypt. The materials employed in this study were consisted of randomly obtained twenty-four specimens of the ovary of mature Redbelly tilapia (*Coptodon zillii*) collected from the Nile River in Assuit city during the spawning season (from March to August). All methods

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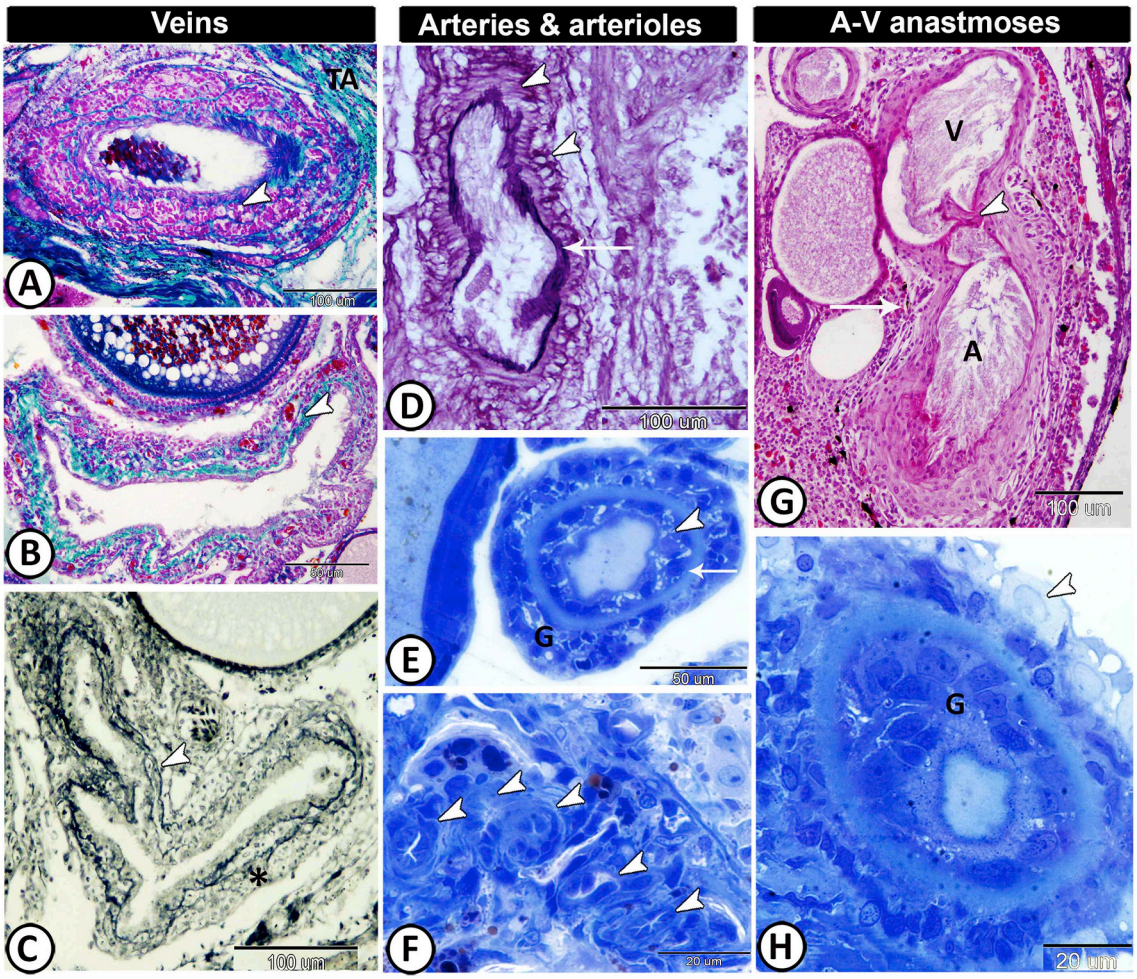

Fig. 1. Light microscopy of special types of blood vessels. **A:** Special vein stained with Crossmon's trichrome showed longitudinally arranged smooth muscle fibers in the tunica media (arrowhead) followed by thick tunica adventitia (TA). **B:** A vein stained with Crossmon's trichrome showed regressive changes (arrowhead). **C:** A vein stained with Verhoeff's stain showed double internal elastic lamina (arrowhead) followed by longitudinally oriented smooth muscle fibers intermingled with elastic fibers (asterisk). **D:** An artery with double tunica media (arrowheads) and elastic interna (arrow) stained by Weigert's Elastica. **E:** Throttle artery stained with toluidine blue (TB) showed longitudinal muscular intimal bolsters (arrowhead), demarcated from the glomus cell media (G) by a fibrous membrane (arrow). **F:** Spirally oriented arterioles stained with toluidine blue showing extreme thickening of their walls (arrowheads). **G:** In bridge anastomoses, the artery (A) connected with the vein (V) and surrounded by one tunica adventitia (arrow). Note, a ring of smooth muscle cells (arrowhead) at the origin of the anastomosis. HE stain. **H:** Glomus stained with toluidine blue showed glomus cell layers (G) surrounded by connective tissue rich in blood capillaries (arrowhead). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

were performed in strict accordance with the relevant guidelines and ethical regulations (please see the supplementary information).

2.2. Histological examination

All specimens were dissected as soon as possible and the ovaries were exposed through a longitudinal incision in the abdominal wall. Samples for histological technique were dissected at 1x1x0.5 cm and were immediately fixed in Bouin's fluid for 22 h. The fixed samples were dehydrated in ethanol, cleared in methyl benzoate and embedded in paraffin wax. Serial longitudinal and transverse paraffin sections (5 μm thick) were cut and stained by Harris hematoxylin and Eosin [15], Crossmon's trichrome [16] for collagenous fibers, Grimelius's silver method for demonstration of endocrine cells [17]. Verhoeff's stain [18] and Weigert elastica [19] for elastic fibers.

2.3. Histochemical analysis and enzyme histochemistry

For carbohydrate histochemistry, sections from the ovary were stained by Periodic Acid-Schiff (PAS) technique [20]. Bromophenol blue was used for basic proteins [21], and Sudan black B for lipid detection [22]. Furthermore, the sections were stained with Safranin O to demonstrate the mast cells' granules [23]. ATPase activity was demonstrated by Bancroft and Gamble [23] and acid phosphatase by the Gomori lead nitrate method [24].

2.4. Acridine orange (fluorescent stain)

According to the method [25], 4 μm paraffin sections were dewaxed and rehydrated in ethanol, followed by distal water. Then the sections were fixed in methanol and stained with acridine orange (AO) staining solution (0.01%, pH 3). The sections were examined using a Leitz DM

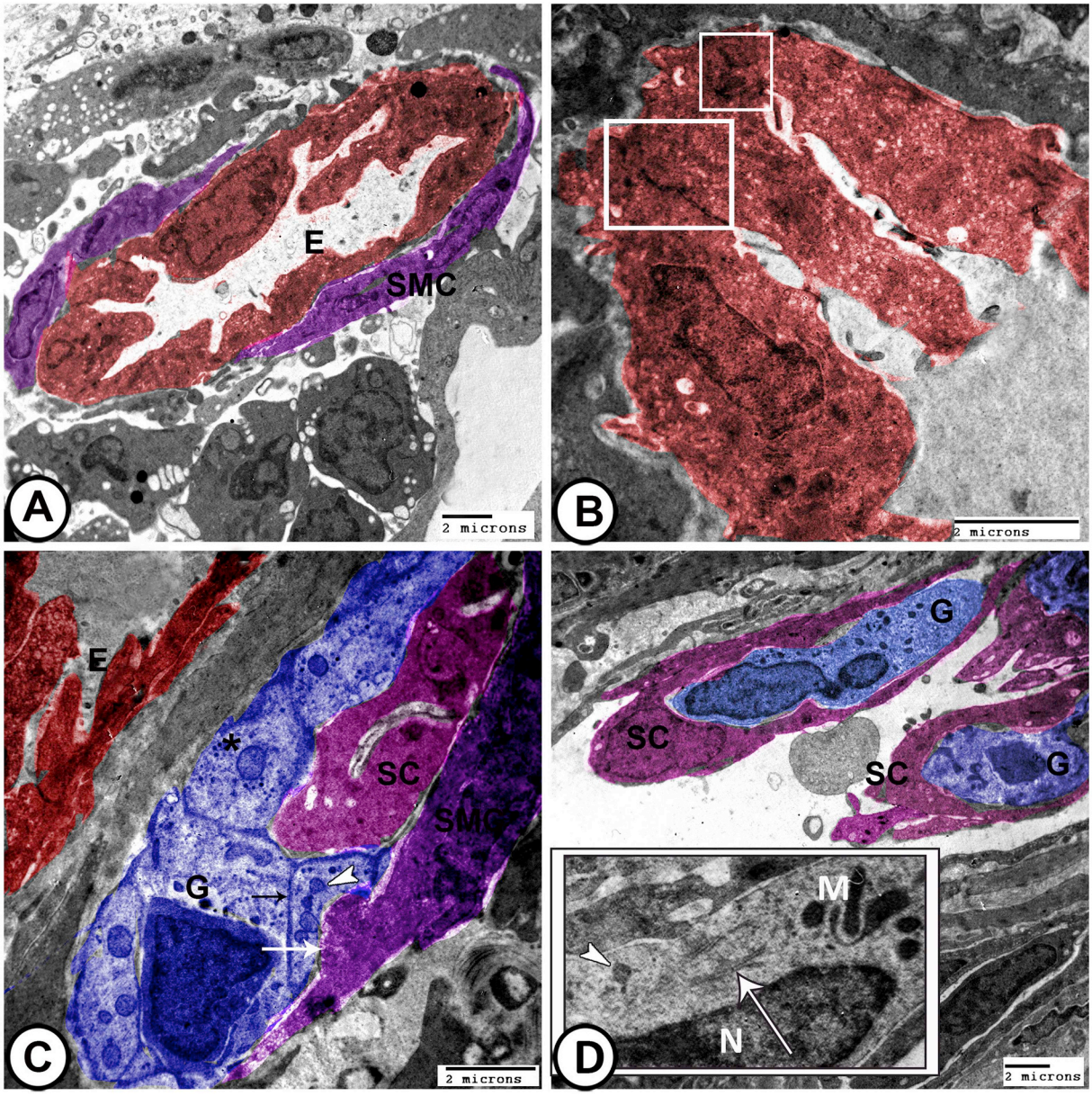

Fig. 2. Digital colored TEM images of the special blood vessels. **A:** The endothelial cells (E, red) of special arteriole contained mitochondria, numerous micropinocytotic pits, and vesicles and surrounded by smooth muscle fibers (SMC, violet). **B:** The endothelial cells were connected by tight junctions and desmosomes (boxed areas). **C:** the wall of the glomus consisted of the endothelium (E, red), tunica media that consisted of glomus cells (G, blue) that were rich by dense vesicles (arrowhead), and few granules (asterisk). Note, desmosomes-like membrane thickening between the adjacent cells (black arrow). Few supporting cells (SC, pink) extended long processes between glomus cells. Tongue-like projection (white arrow) extended from smooth muscle (SMC, violet) to glomus cells. The electron-dense vesicles of glomus cells showed aggregations near this connection (arrowhead). **D:** The lumen of glomus vessel showed protruded expansion of glomus cells (G, blue) that incompletely invested by supporting cells (SC, pink). The glomus cells (inserted box) contained a nucleus (N), mitochondria (M), dense-cored vesicles (arrowhead), and rough endoplasmic reticulum (arrow). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2500 microscope with the external fluorescent unit Leica EL 6000. AO has metachromatic properties that result in the accompanying emission of green and red fluorescence.

2.5. Immunohistochemistry

The immunohistochemistry was performed on formalin-fixed, paraffin-embedded, and 4 μm-thick ovarian tissue sections collected at the spawning season. The sections were treated with 10 ml Mol Tris buffer and 1 ml Mol ethylene-diaminetetra acetic acid (pH 9.0) at 90 °C for 20 min. The endogenous peroxidase was inhibited by immersion the sections in 3% H2O2, then preincubation overnight in 1% bovine serum albumin in PBS at 4 °C. The sections were stained for 30 min at room temperature, using the following antibodies: rabbit polyclonal anti-S100 protein (1:200, Genemed Biotechnologies, South San Francisco, USA) for identifying of cells of epithelial, mesodermal and neuroectodermal origin, rabbit polyclonal anti- CD117 (c-kit) (1:100, Dako, Glostrup, Denmark) for demonstration of hematopoietic stem cells,

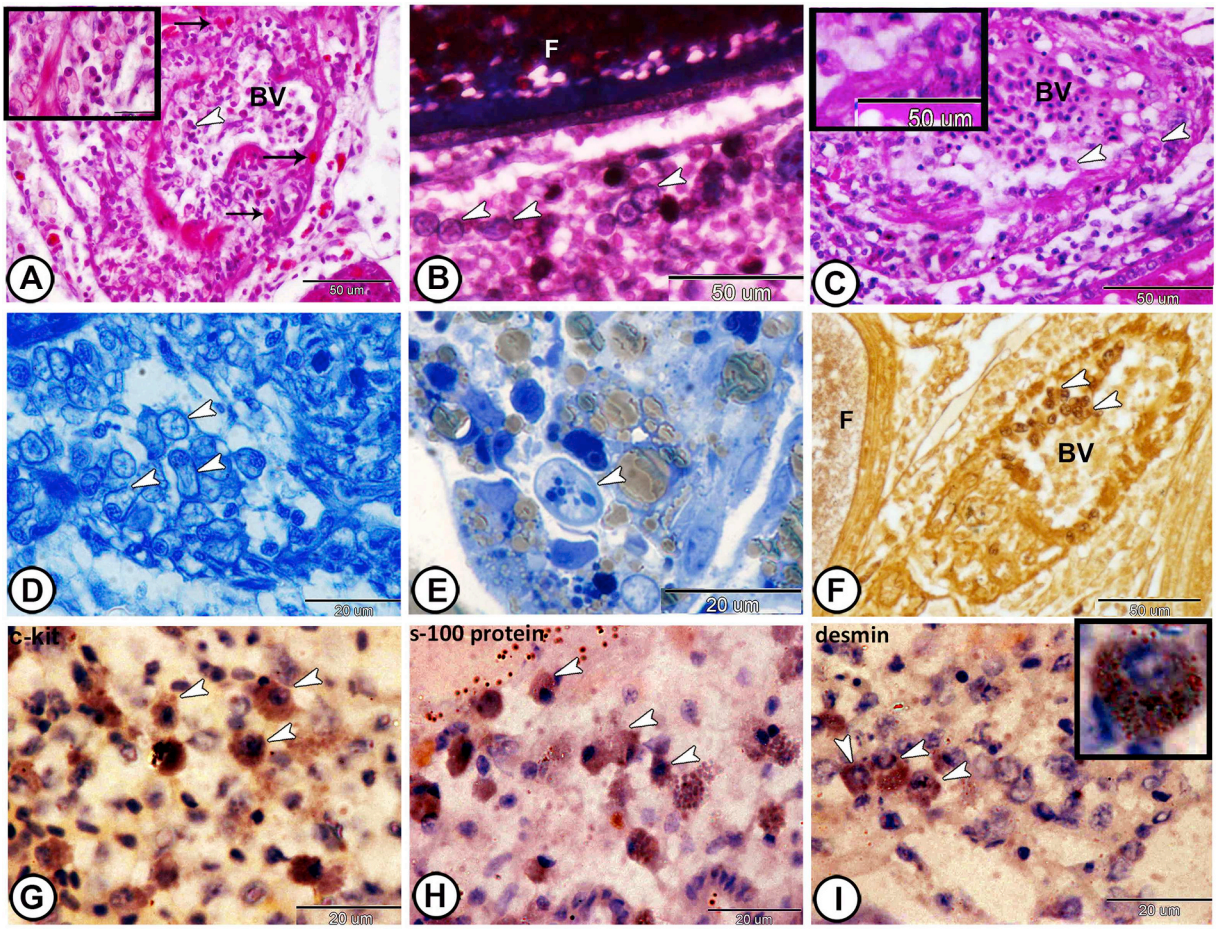

Fig. 3. Histological, histochemical and immunohistochemical identification of rodlet cells. **A:** Rodlet cells are associated with eosinophils (arrows) or found free (arrowhead, inserted box) in the lumen of blood vessels (BV). They enclosed eosinophilic granules or rods by HE. **B:** Rodlet cells (arrowheads) were green structures under the follicles (F) by Crossmon's trichrome. **C:** Rodlet cells (arrowheads, inserted box) attached to the wall of the blood vessel (BV) and showed bright red color by PAS/HE. **D, E:** Rodlet cells (arrowheads) stained blue with bromophenol blue and Toluidine blue respectively. **F:** These cells (arrowheads) form a part of the wall of the special blood vessels (BV) around the follicle (F) and expressed a strong positive reaction with Grimilus silver stains. **G, H, I:** Rodlet cells (arrowheads) expressed c-kit, S-100 protein, and desmin respectively. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

mast cells, telocytes and melanocytes, and mouse polyclonal anti-desmin (1:30, Thermo Fischer Scientific, UK) the intermediate filament protein according to the previously reported method [26]. Sections were counterstained with hematoxylin, examined and photographed under a Leica microscope (Germany).

2.6. Semithin sections and TEM preparations

Small specimens of the ovaries during the spawning season were preserved by immersion in a mixture of 2.5% paraformaldehyde–glutaraldehyde fixative and left overnight [27]. After fixation, the samples were washed in 0.1 Mol/L phosphate buffer and osmicated with 1% osmium tetroxide in 0.1 Mol/L sodium-cacodylate buffer at pH 7.3. Then, the samples were dehydrated using ethanol followed by propylene oxide and embedded in Araldite. Semithin sections (1 μm thick) were obtained with Richert Ultracuts (Leica, Germany) and stained with toluidine blue. Ultrathin sections were done with Ultrotom VRV (LKB Bromma, Germany). The sections (70 nm) were stained with uranyl acetate and lead citrate [28] and examined by JEOL 100CX II transmission electron microscope at the Electron Microscopy Unit of Assiut University.

2.7. Morphometrical analysis

The mean proportion of the cellular components in the ovarian stroma during the spawning season was performed using Image-J software on randomly selected images of both paraffin and semithin sections using 100 x objective.

3. Results

The ovarian stroma of Redbelly tilapia contained many special vascular and cellular elements.

3.1. Vascular elements

The histological investigation of the ovarian vasculature in Redbelly tilapia revealed several blood vessels bearing special regulatory devices that frequently scattered around the vitellogenic ovarian follicles in the breeding (spawning) season.

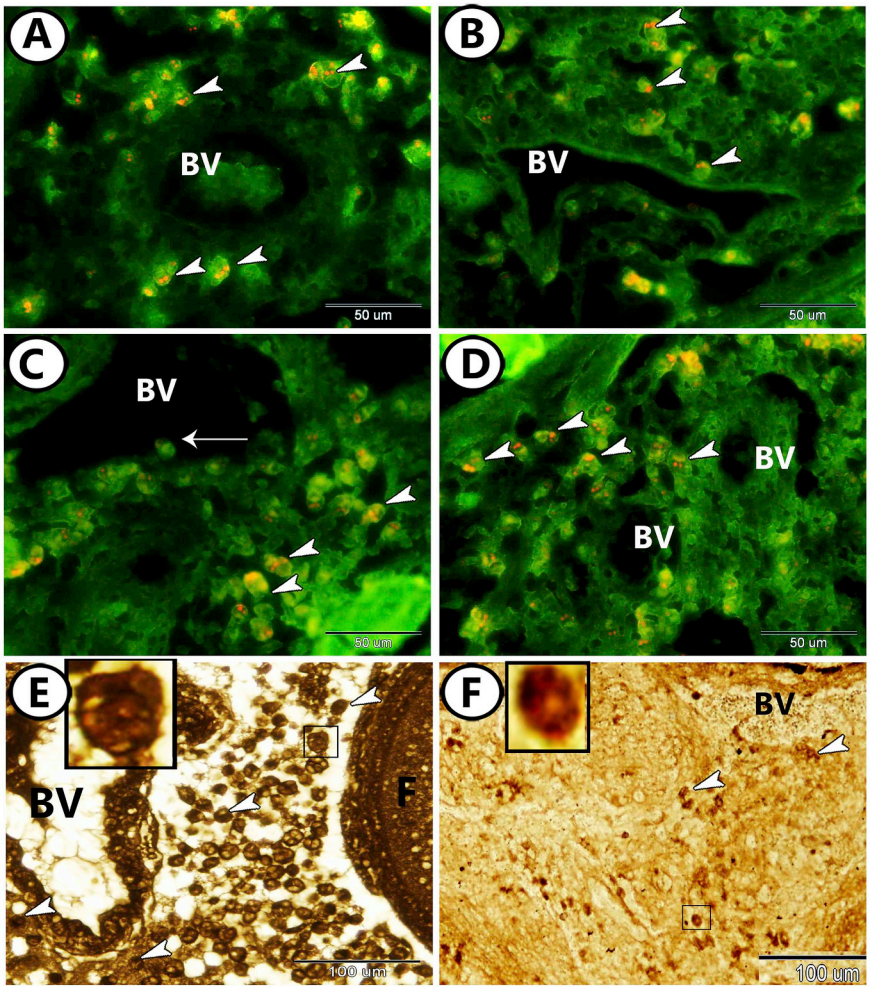

Fig. 4. The fluorescence and enzyme histochemistry of the rodlet cells: **A:** The glomus blood vessel (BV) emit intense green fluorescence after acridine orange stain. The rodlet cells (arrowheads) arranged in tunica media. **B:** Rodlet cells (arrowheads) distributed around the blood vessels (BV). **C, D:** Some rodlet cells (arrow) projecting to the lumen of the blood vessel (BV), the others in tunica media and adventitia (arrowheads) and their cytoplasmic rodlet granules fluoresced brilliant orange after acridine orange stain. **E:** Numerous rodlet cells (arrowheads, inserted box) displayed around blood vessels (BV) and ovarian follicles (F) showed high ATPase activity. **F:** Rodlets cells (arrowheads, inserted box) in the stroma and around the blood vessel (BV) showed acid phosphatase activity. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Some veins represented several layers of longitudinally oriented smooth muscle fibers in the tunica media followed by a thick collagenous tunica adventitia (Fig. 1A). While some veins showed regressive changes in form of thickening of the wall with an increase in collagenous fibrous tissue content (Fig. 1B). Other veins displayed double internal elastic lamina followed by a tunica media made up of longitudinally oriented smooth muscle fibers intermingled with elastic fibers (Fig. 1C). Arteries with double tunica media contained outer longitudinal and inner circular smooth muscle fibers and clear tunica elastic interna were demonstrated (Fig. 1D). Throttle artery was provided with longitudinal muscular intimal bolsters that were made up of longitudinally directed smooth muscle cells and few glomus cells. The bolsters formed a continuous layer surrounding the lumen and were demarcated from the glomus cell media by a distinct fibrous membrane (Fig. 1E). Spirally oriented arterioles were present within the ovarian stroma with extreme thickening of their walls (Fig. 1F). The arteriovenous anastomoses were evident in the ovarian stroma and included simple bridge-like arteriovenous anastomoses and glomus organ. In bridge anastomoses, the thick-walled artery connected directly with a thin-walled vein and they were surrounded by one tunica adventitia, and the origin of the anastomosis was supported by a ring or roll of smooth muscle cells. This transition showed a sharp and abrupt change in the thickness of the tunica media from artery to vein (Fig. 1G). Glomus was frequently observed near the vitellogenic follicles and was composed mainly of glomus cell layers and few smooth muscle cells. The glomus cells were mostly rounded or polyhedral with a large vesicular nucleus. They were surrounded with layers of loose connective tissue rich in blood capillaries (Fig. 1H).

By electron microscopy, in special arteriole, the endothelial cells extended long prolongation to the lumen of the vessel. These endothelial cells were of a fenestrated type and contained euchromatic elongated nucleus, mitochondria, numerous micropinocytotic pits and vesicles. The endothelial cells were connected by tight junctions and desmosomes. They were surrounded by smooth muscle fibers and other stromal cells (Fig. 2A and B). While, the wall of the glomus consisted of endothelium followed by thin subendothelial connective tissue, tunica media was consisted of clusters of large glomus cells that exhibited electron-lucent cytoplasm rich in dense vesicles, and few granules. The nuclei of these cells were large and contained peripherally arranged heterochromatin. Desmosomes-like membrane thickening between the adjacent cells was frequently demonstrated. Few supporting cells were distributed between the glomus cells and their cytoplasm extended long processes and contained mitochondria. Intimate apposition of some parts of glomus cells and intervascular smooth muscle cells were demonstrated. This connection included a tongue-like projection of smooth muscle with mitochondria and free surface of glomus cells. The electron-dense vesicles of glomus cells showed aggregations near this connection (Fig. 2C). While in the glomus vessel, its lumen consisted of the protruded expansion of glomus cells that incompletely invested by supporting cells. The glomus cells characterized by electron-lucent cytoplasm rich in mitochondria, dense-cored vesicles, few granules, and

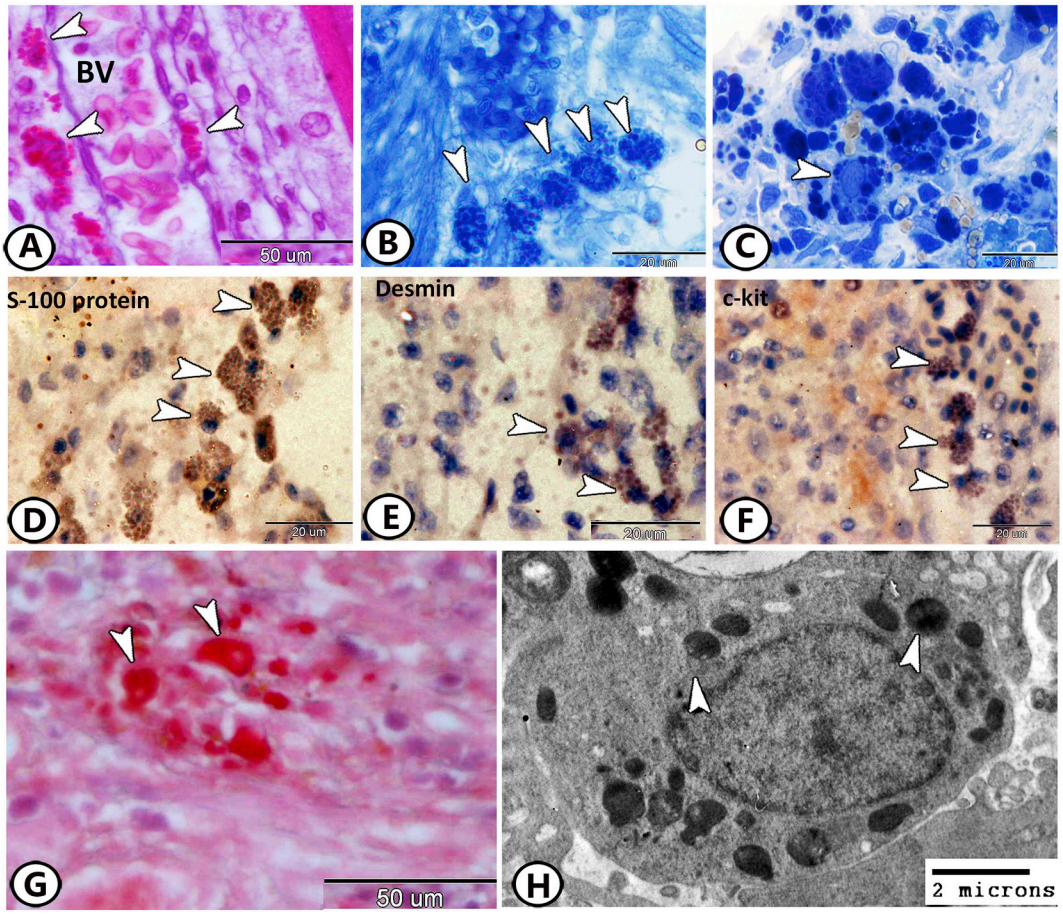

Fig. 5. The mast cells. **A:** Numerous mast cells with eosinophilic granules (arrowheads) recorded in the vicinity to blood vessels (BV) by HE. **B:** These cells were positive to bromophenol blue (arrowheads). **C:** Mast cell granules (arrowhead) showed metachromatic reaction with toluidine blue. **D-F:** Granules of mast cells (arrowheads) expressed staining affinity for s100 protein, desmin and c-kit. **G:** Mast cells (arrowheads) showed a strong positive reaction to Safranin O. **H:** TEM showed that the mast cells contained numerous electron-dense granules (arrowheads). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

rough endoplasmic reticulum. The nuclei of these cells were large and contained peripherally arranged heterochromatin. The supporting cells extended long processes like an octopus that partially enveloped glomus cells. These cells were in close to the blood vessels and contained a large nucleus and thin cytoplasm. The cytoplasm contained vesicles and mitochondria (Fig. 2D).

3.2. Cellular elements

3.2.1. Rodlet cells

The rodlet cells were arranged in groups throughout the ovarian connective tissue stroma with no special orientation, particularly attached to the wall of special types of blood vessels and constituted 10.28% of the cell of ovarian stroma. Rodlet cells may be associated with eosinophils or found free in the lumen of blood vessels. They exhibited oval to rounded-shaped cell bodies, eccentric nuclei with thick capsule enclosed cytoplasmic eosinophilic granules or rods by HE (Fig. 3A). Rodlet cells showed a wide range of staining affinity; they were green by Crossmon's trichrome (Fig. 3B), bright red by PAS (Fig. 3C), while retaining the blue color with bromophenol blue (Fig. 3D) and Toluidine blue (Fig. 3E). Since these cells form a constituent part of the wall of the special blood vessels and could be traversing the endothelium, they expressed a strong positive reaction in form of groups of brown structures with Grimilus silver stains (Fig. 3F). Rodlet cells expressed c-kit, s-100 protein, and desmin (Fig. 3G–I).

The glomus cells emit intense green fluorescence. The rodlet cells were arranged in groups around the blood vessels or in intima layer of some of them or projecting to the vascular lumen and their cytoplasmic rodlet granules fluoresced brilliant orange after acridine orange stain as they showed RNA type histochemical reaction (Fig. 4A–D). Numerous rounded rodlet cells were displayed around the blood vessels and ovarian follicles showed high ATPase and acid phosphatase activity (Fig. 4E and F).

3.2.2. Mast cells

Numerous mast cells were recorded in the ovarian stroma (10.11% of the cellular constituents), particularly in association or in the vicinity to blood vessels. These cells were characterized by rounded cell bodies of various size with an eccentric nucleus and eosinophilic granules with HE (Fig. 5A). The mast cells were positive with bromophenol blue (Fig. 5B) due to the high protein contents in their granules as well as they showed metachromatic reaction with toluidine blue (Fig. 5C). Granules of mast cells expressed the staining affinity for s100 protein, desmin, and c-kit (Fig. 5D–F). Furthermore, these granules showed a strong staining affinity for Safranin O

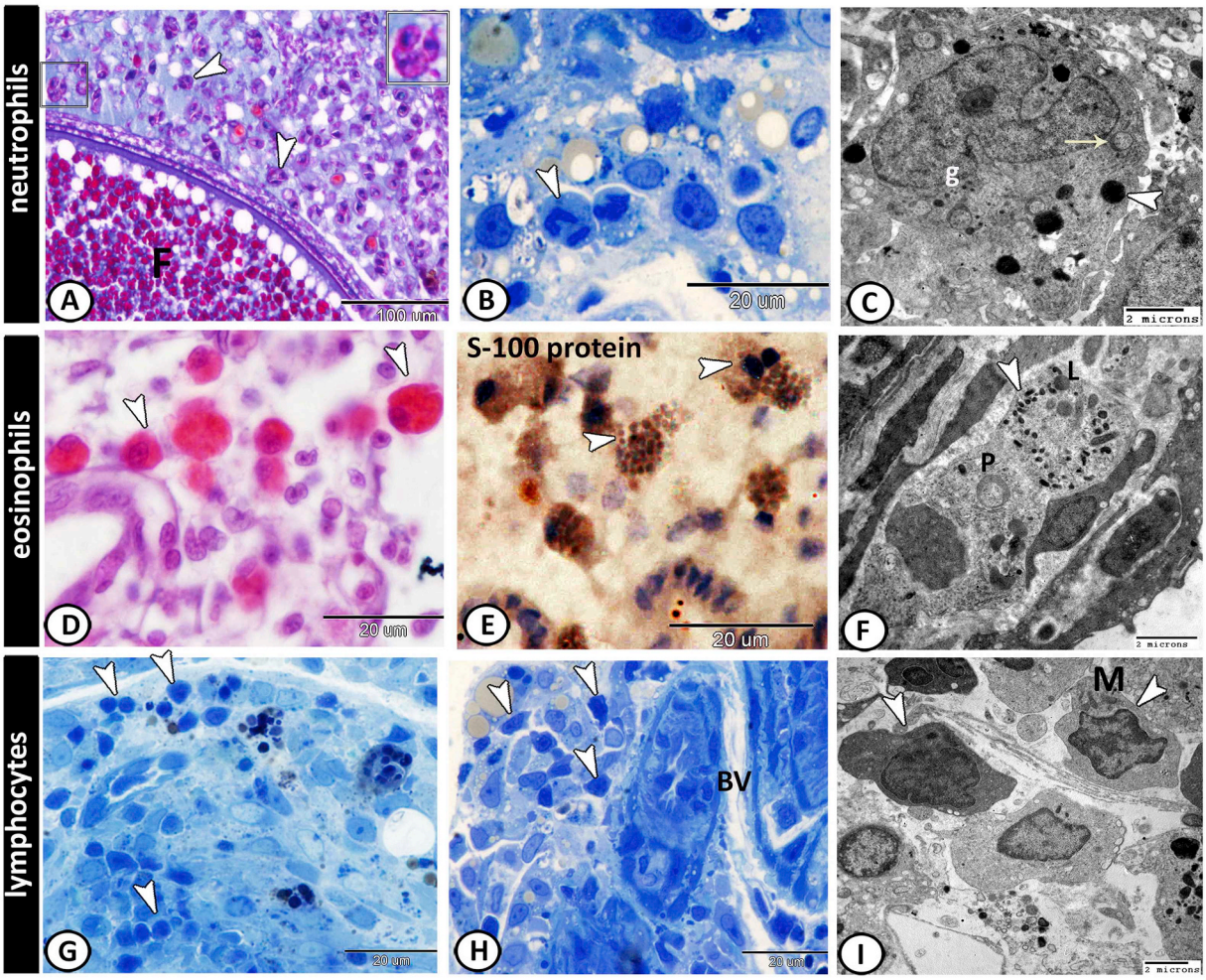

Fig. 6. The leucocytes. **A:** ***Neutrophil*** (arrowheads, boxed area) distributed around the ovarian follicles (F) (Crossmon's trichrome). **B:** Neutrophils (arrowhead) by toluidine blue. **C:** TEM showed that their cytoplasm contained many lysosomes (arrowhead), phagosomes (arrow), and specific granules (g). ***D: Eosinophils*** (arrowheads) characterized by eccentric nucleus and eosinophilic granules with HE. **E:** Eosinophilic granules expressed s-100 protein (arrowheads). **F:** TEM showed the characteristic specific granules of eosinophils (arrowhead), lysosomes (L) and phagosomes (P). ***G, H: Lymphocytes*** (arrowheads) are small rounded cells by toluidine blue distributed in the ovarian stroma and around the blood vessels (BV). **I:** TEM showed that it possessed a heterochromatic nucleus and a cytoplasm contained mitochondria (M). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 5G). By electron microscopy, the mast cells characterized by a large oval euchromatic nucleus surrounded by numerous cytoplasmic electron-dense granules (Fig. 5H).

3.2.3. Leucocytes

3.2.3.1. Neutrophils. They distributed around the ovarian follicles and characterized by multi-lobed nucleus (Fig. 6A and B). TEM showed that their cytoplasm contained many lysosomes, phagosomes, and specific granules (Fig. 6C).

3.2.3.2. Eosinophils. Massive aggregations of eosinophilic granular cells were recorded in the ovarian stroma that represented 10.96% of the cellular constituents, particularly in association or in the vicinity to the endothelial tissues. These cells were characterized by rounded cell bodies of various size with an eccentric nucleus and eosinophilic granules with HE (Fig. 6D). Eosinophilic granules expressed s-100 protein (Fig. 6E). TEM showed the characteristic specific granules of eosinophils, lysosomes, and phagosomes (Fig. 6F).

3.2.3.3. Lymphocytes. The lymphocytes constituted 14.87% of the cellular constituents of the ovarian stroma. They were small rounded cells with a high nucleus to cytoplasmic ratio distributed randomly in the ovarian stroma and around the blood vessels (Fig. 6G and H). TEM showed that they possessed heterochromatic nuclei with some indentations and their cytoplasm contained mitochondria (Fig. 6 I).

3.2.4. Macrophages

Macrophages were distributed around the atretic follicles. They characterized by an eccentric nucleus and the presence of phagocytosed materials within their cytoplasm (Fig. 7A and B). They showed acid phosphatase activity (Fig. 7C) and expressed s-100 protein and desmin (Fig. 7D and E). TEM showed that the macrophage possessed a kidney-shaped nucleus and its cytoplasm contained heterogeneous vesicles, phagosomes, electron-dense granules, and lysosomes (Fig. 7F).

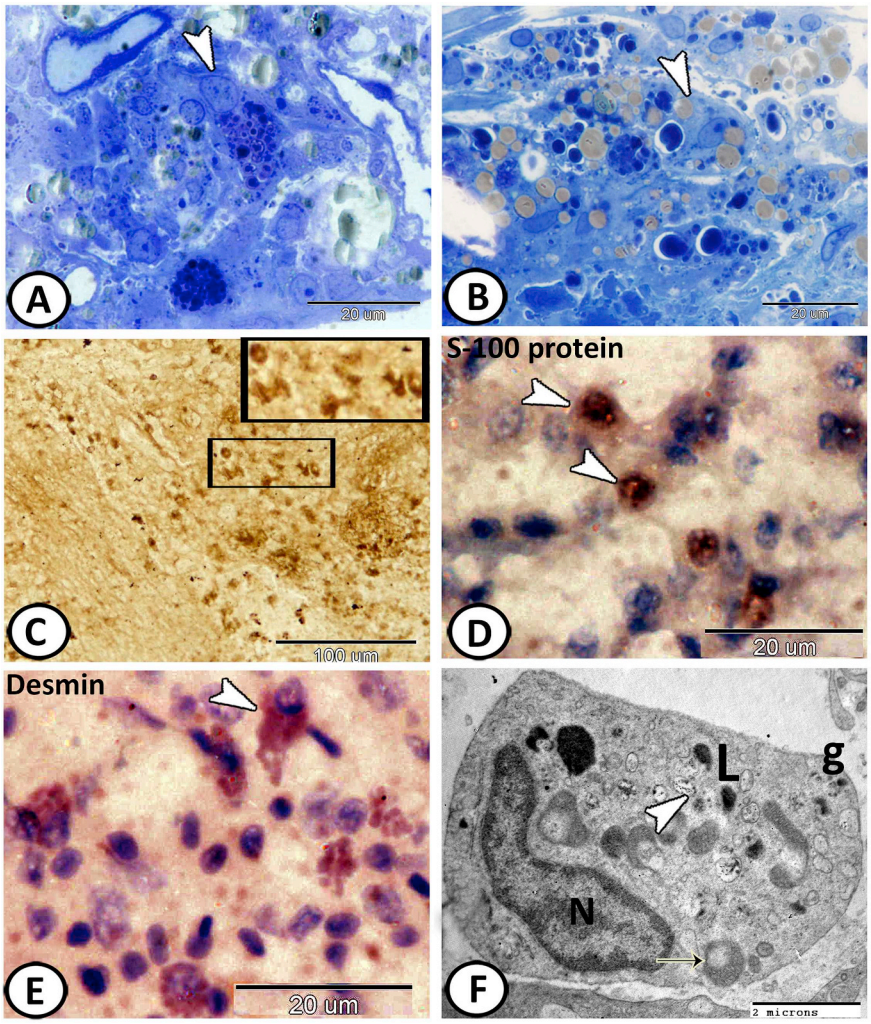

Fig. 7. Macrophages. **A, B:** Macrophages (arrowheads) characterized by the presence of phagocytosed materials within their cytoplasm by toluidine blue. **C:** They showed acid phosphatase activity (boxed areas). **D:** Macrophages (arrowheads) expressed s-100 protein. **E:** They also expressed desmin (arrowhead). **F:** TEM showed that macrophage possessed kidney-shaped nucleus (N), heterogeneous vesicles (arrowhead), phagosomes (arrow), electron-dense granules (g), and lysosomes (L). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2.5. Dendritic cells

Dendritic cells were small dark cells with fine processes that found around theca cells and blood vessels in association with macrophages and constituted 8.14% of the cellular constituents (Fig. 8A). They expressed S-100 protein and c-kit (Fig. 8B and C). TEM showed that dendritic cells characterized by their processes, large indented heterochromatic nucleus, and rER (Fig. 8D and E). The cytoplasm contained lysosomes and sometimes showing phagocytized materials (Fig. 8F). They also displayed macropinocytotic vesicles periphery located, vacuoles of various size, mitochondria, and phagosomes (Fig. 8G).

3.2.6. Endocrine (steroid producing) cells

They are clusters or cords of polyhedral cells observed in the stroma in close apposition to small blood vessels (Fig. 9A) or at the periphery of mature vitellogenic follicles (Fig. 9B). These cells represented 9.44% of the ovarian stroma and showed positive reactions to bromophenol blue (Fig. 9B), silver stain (Fig. 9D), and expressed strong activity to S-100 protein (Fig. 9C). TEM showed that the endocrine cells were dominated by polymorphic secretory granules, tubular mitochondrial cristae, and sER (Fig. 9E and F).

3.2.7. Melanocytes

Melanocytes were demonstrated in the ovarian stroma around the special types of blood vessels and other cellular elements and constituted 5.57% of the ovarian stroma. They were characterized by their irregular-shaped cell bodies and the presence of dark brown to black melanin pigments (Fig. 10A). They expressed a strong positive reaction to s-100 protein and c-kit (Fig. 10B and C).

3.2.8. Adipocytes

Adipocytes or fat cells were main components in the ovarian stroma (7.73%). They gave a positive reaction to Sudan black B (Fig. 10D). Their thin cytoplasm expressed s-100 protein (Fig. 10E) and their fat globules reacted positively to c-kit (Fig. 10F).

Bundles of non-myelinated nerves, presumably autonomic, accompanying the ovarian artery and vein were observed in the ovarian stroma. The bundles consisted of nerve axons and Schwann cells (Fig. 10G and H), and gave a positive reaction to silver stain (Fig. 10 I).

3.2.9. Telocytes (TCs)

TCs were small spindle-shaped cells with two or more long cell processes called telopodes. They were distributed in the ovarian

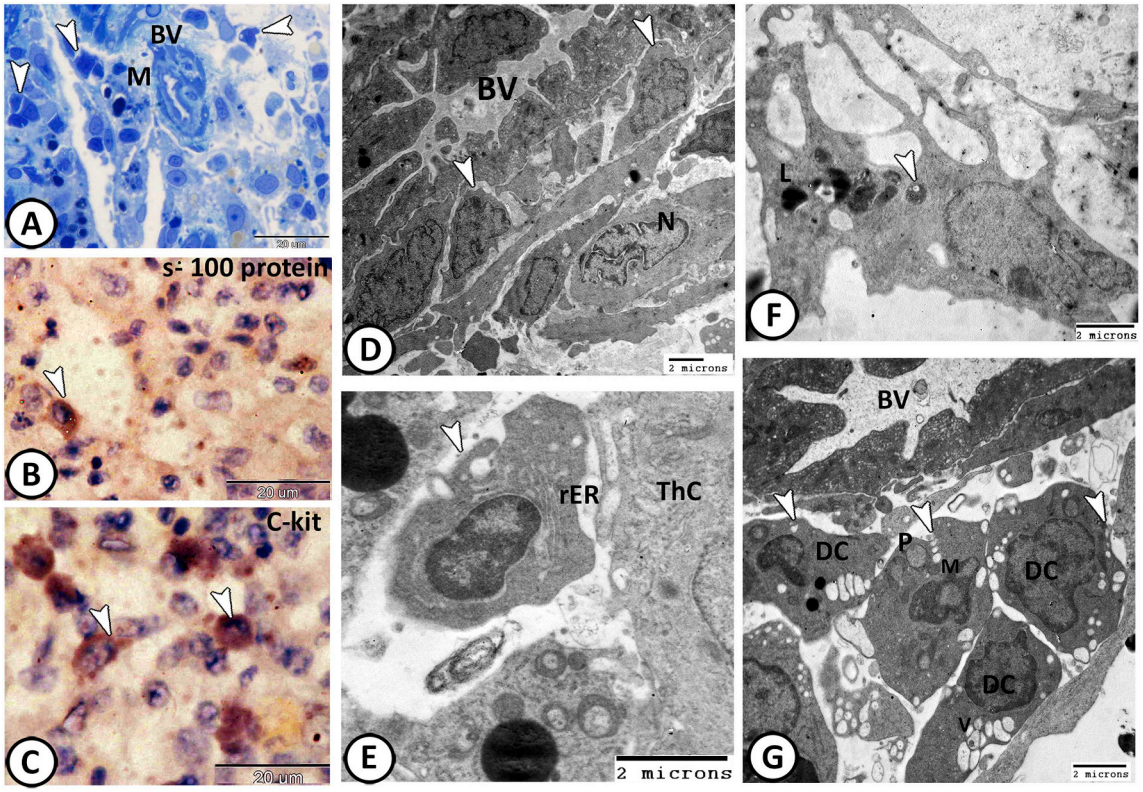

Fig. 8. The dendritic cells. **A:** Dendritic cells (arrowheads) found around blood vessels (BV) in association with macrophages (M). **B:** They expressed S-100 protein (arrowhead). **C:** They expressed c-kit (arrowheads). **D:** TEM showed that dendritic cells (arrowheads) arranged around the blood vessels (BV) in the vicinity to neutrophils (N) and characterized by their processes, large indented heterochromatic nucleus. **E:** Dendritic cell (arrowhead) found around theca cells (ThC) and contained rER. **F:** The cytoplasm contained lysosomes (L), and phagocytized materials (arrowhead). **G:** The dendritic cell cytoplasm (DC) displayed macropinocytotic vesicles (arrowheads), vacuoles (V), mitochondria (M), and phagosomes (P). Note their distribution around the blood vessels (BV).

stroma around the blood capillaries (Fig. 11A), arteriovenous anastomoses (Fig. 11B), small blood vessels (Fig. 11C, E), and atretic follicles (Fig. 11D), or scattered all over the stroma between the other connective tissue cells (Fig. 11F). TCs represented 7.94% of the cellular constituents. They stained positive with silver stain (Fig. 11A) and were darkly stained with toluidine blue (Fig. 11C–F). TCs' bodies and telopodes expressed strong immunoreaction to desmin (Fig. 11G). Moreover, the telopodes expressed positive immunoreactivity to both c-kit (Fig. 11H), and s-100 protein (Fig. 11 I).

By EM, TC consisted of cell body contained euchromatic oval nucleus, and telopodes displayed mitochondria and secretory vesicles (Fig. 12A). Their telopodes form a labyrinth network between the stromal cellular and vascular components. TCs established contact with each other (homocellular contact) (Fig. 12B) and heterocellular contact with immune cells (macrophages and dendritic cells) (Fig. 12C), and endothelium of blood vessels (Fig. 12D). Stromal telocytes shed their secretory vesicles to the blood vessels (Fig. 12C and D). They exert their effect either by direct contact or by paracrine mode.

3.2.10. Fibroblasts

They characterized by triangular-shaped cell bodies with an oval large euchromatic nucleus and cytoplasmic electron-lucent vesicles of variable shapes and sizes and rER (Fig. 12D).

4. Discussion

Oocyte maturation in fish during the spawning season involves major events include the presence of numerous blood vessels, mast cell activation, and the eosinophils appear to be phagocytic [29]. It was possible that the distribution of special types of blood vessels may be related to the ovarian development, which might supply the endocrine activities including vitellogenin uptake and estrogenic control. The present results showed special blood vessels with several blocking devices as longitudinal muscular intimal bolsters. These muscular pads are able to construct or even occlude the lumen of the blood vessel. The latter devices seem to have an active dynamic function in regulating the blood flow and pressure through throttling or occlusion mechanism. This type of special regulatory device was observed in many forms, which correspond to similar findings [3]. Haemodynamic, humeral and thermoregulatory functions are the main roles of the glomus. Glomus was frequently observed near the vitellogenic follicles that may be related to the maturation or rupture of the follicles. The observed regressive changes occurred in the veins is supposed to be developed as the result of increased circulatory demands or due to strain on the dynamics of the peripheral circulation. However, these modifications in blood vessels are considered as physiological processes as they are not uniform in nature, intensity, and distribution. Spirally oriented arteriole and venule with extreme thickening of the walls were observed and may occur as a result of repeated functional activity of the ovary.

The electron microscopy revealed numerous micropinocytotic pits and vesicles in the endothelium that indicate active transcytosis process during the spawning period. The glomus-smooth muscle connection is suggested to participate in vascular regulation. The dense vesicles of glomus cells showed aggregations near this connection that supposed exocytosis of the contents of vesicles to the connection. Moreover,

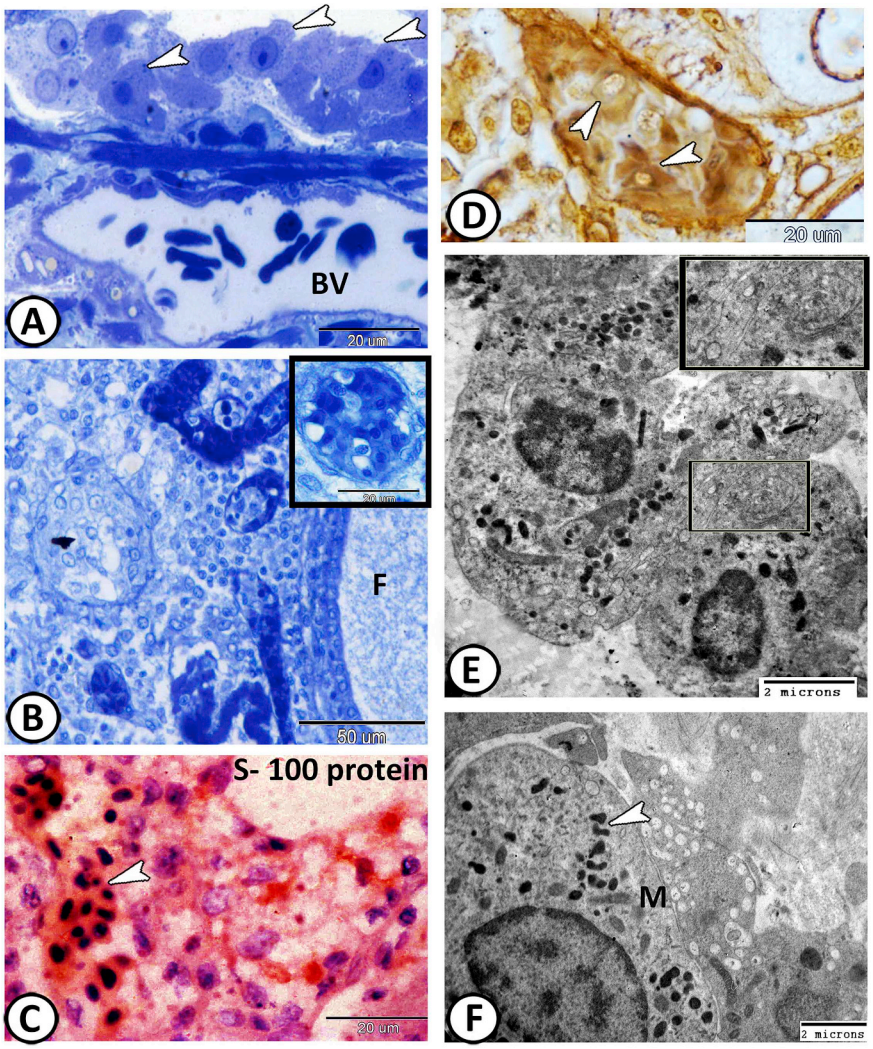

Fig. 9. Endocrine (steroid-producing cell). **A:** Semithin section stained by toluidine blue showing endocrine cells (arrowheads) in apposition to small blood vessels (BV). **B:** Clusters of endocrine cells (boxed area) at the periphery of mature vitellogenic follicles (F) and showed positive reactions to bromophenol blue. **C:** Endocrine cells (arrowhead) express strong activity to S-100 protein. **D:** Endocrine cells were positive with silver stain (arrowheads). **E, F:** TEM showed that endocrine cells were dominated by polymorphic secretory granules (arrowhead), mitochondria (M), and sER (boxed areas in E). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

glomus cells act as local secretory cells affecting smooth muscle [30]. The presence of desmosomes-like junction between adjacent glomus cells indicated communication between them. Since the glomus cells are usually arranged in clusters, excitation of one glomus cell may electronically spread to other cells in the cluster. Consequently, a cluster may behave as a functional unit.

This is the first report of the presence of rodlet cells in ovaries of tilapia. The distribution of rodlet cells in ovarian stroma is probably linked to their potential role as regulatory elements and non-specific immune cells. They are considered migratory cells [31,32]. The current study recorded the presence of rodlet cells lining the vascular endothelium and this finding is previously reported [33,34]. Rodlet granules in mature cells stained positive to bromophenol blue, which indicated the protein nature for these cells. The distribution of rodlet cells in the wall of blood vessels supports the proposal that rodlet cells secrete some enzymatic and proteinous substances to the circulation [35]. Rodlet cells have been implicated in many functions; sensory function [36], the secretory function via holocrine mode [37], they also involved in immune response and considered as a type of the granulocytes [32,38]. Rodlet cells act as ion-transporting cells and have a role in osmoregulation [39,40]. The present study revealed a high proportion of rodlet cells in the ovaries at spawning season that matches with the previous study [34] as the number of rodlet cells showed seasonal variation and the drastic changes in reproductive hormones during the spawning season might have a role in rodlet cells abundance distributing. The acridine orange (AO) is a fluorescent versatile dye that enters the cytoplasmic acidic compartments such as the lysosomes as well as stains DNA and RNA in living cells. Therefore, primary lysosomes, phagosomes, and apoptotic cells could be identified [41]. In the present study, AO is used for the first time to identify the rodlets inclusions within the cytoplasm of the rodlet cells, which stained orange to red color.

The present study revealed the presence of telocytes in the ovarian stroma. While few studies demonstrating telocytes were done in fish and birds. The EM results indicated the presence of heterocellular contact with immunoreactive cells as macrophages. A similar finding was reported by Gherghiceanu, colleagues [42]. Moreover, telocytes possessed receptors for inhibitory and excitatory neurotransmitters [43]. In addition, occasional fibroblasts were demonstrated in the association of blood vessels and telocytes and may be involved in the regulation of the regeneration process in the ovary during the spawning season.

The immune system contributes to the regulation of gonadal function. Few or rare studies demonstrated a pattern of distribution of leucocytes in aquatic species. The presence of leukocytes in the ovary may represent potential in-situ modulators of ovarian function that work through local secretion of regulatory soluble factors. These factors include many cytokines that mainly produced by the action of immune

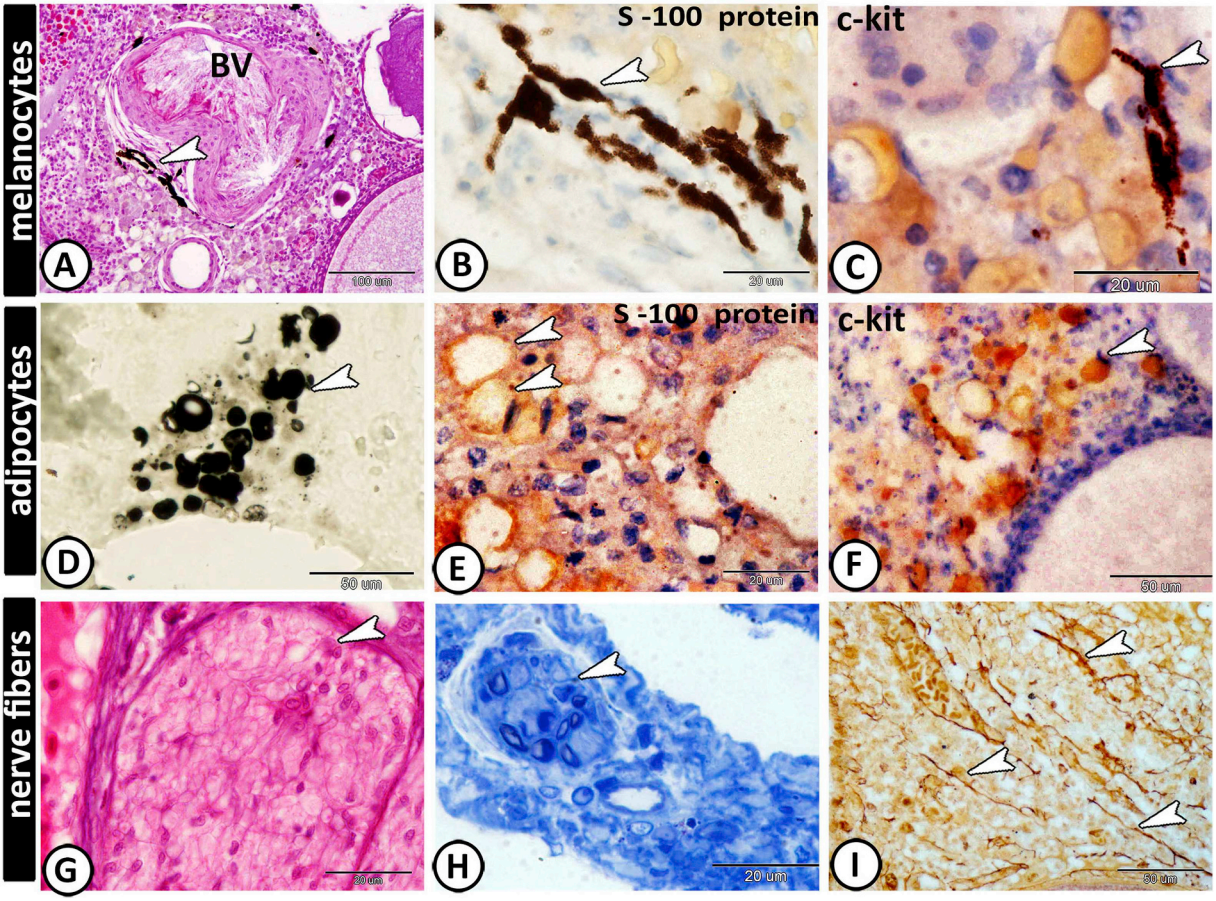

Fig. 10. Melanocytes, adipocytes and nerve fibers. **A:** Melanocytes (arrowhead) stained by HE were demonstrated around the special type of blood vessels (BV). **B:** Melanocytes with characteristic melanin pigments (arrowhead) expressed a strong positive reaction to s-100protein. **C:** Melanocytes expressed c-kit (arrowhead). **D:** Adipocytes (arrowhead) gave a positive reaction to Sudan black B. **E:** Their thin cytoplasm expressed s-100 protein (arrowheads). **F:** Their fat globules reacted positively to c-kit (arrowhead). **G, H:** Bundles of nerve fibers (arrowheads) were observed in the ovarian stroma, stained by HE, and toluidine blue respectively. **I:** Nerve fibers (arrowheads) gave a positive reaction to silver stain. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cells within the ovary [44]. Actual follicle rupture during the ovulation may be dependent on tissue remodeling that is characteristic of an acute inflammatory reaction and includes increased leukocyte migration, the release of various mediators and loosening of connective tissue elements around the follicles.

Macrophages are frequent throughout the female reproductive tissues and could be distinguished by their characteristic morphology. They are multifunctional cells that mainly involved in the immune response including phagocytosis and degradation of foreign antigens, tissue remodeling, and production of cytokines, chemokines and growth factors [45]. Their specific localization and variations in the distribution in the ovary during the spawning season, suggest that macrophages play diverse roles in intra-ovarian events including folliculogenesis, and tissue restructuring after atresia.

The enzyme histochemistry revealed that macrophages and rodlet cells showed high acid phosphatase activity. It is well known that acid phosphatase localized in the lysosomes, the main organelles in the cytoplasm of macrophages, and the acid phosphatase activity increased in the ovaries during maturation and spent stage. In addition, acid phosphatase is involved in the synthesis of essential metabolites in the ovary during the spawning season [46].

In the view of the current work, steroid-producing cells are suggested to be originated from stromal elements. In addition, the steroids produced by steroid-producing cells play a role in the formation of the ovarian lumen and the differentiation of germ cells [47]. It is suggested that these cells may produce estrogens during vitellogenesis [48]. Teleosts haven't typical interstitial cells like mammals.

The current results indicated that the dendritic cells (DCs) were observed in the ovaries of tilapia for the first time. DCs were identified in some teleosts as rainbow trout, salmonids, medaka, zebrafish and Channel catfish in different tissues include; kidney, spleen, and gills [49–52]. They shared many functional and morphological features reported in mammals [53]. DCs are one of antigen-presenting cells with dendritic morphology, phagocytic ability and strong T-cell stimulatory properties [52]. The features of phagocytosis in Fig. 8F, G may signify the role of DCs in immune surveillance in the ovary. Furthermore, the present study recorded the presence of numerous mast cells in the ovarian stroma of tilapia. Reite, Evensen [54] reported that mast cells in teleosts are like those in mammals and could generate many mediators, chemokines, and cytokines.

S100 is a multigenic protein exclusively distributed in the cells of epithelial, mesodermal and neuroectodermal origin [55]. The present study revealed positive immunolocalization of S100-protein in rodlet cells, mast cells, DCs, TCs, macrophages, endocrine cells, adipocytes, and melanocytes. The differential localization of S100- protein in the ovary of the tilapia suggesting its multifunctional role mediated by its

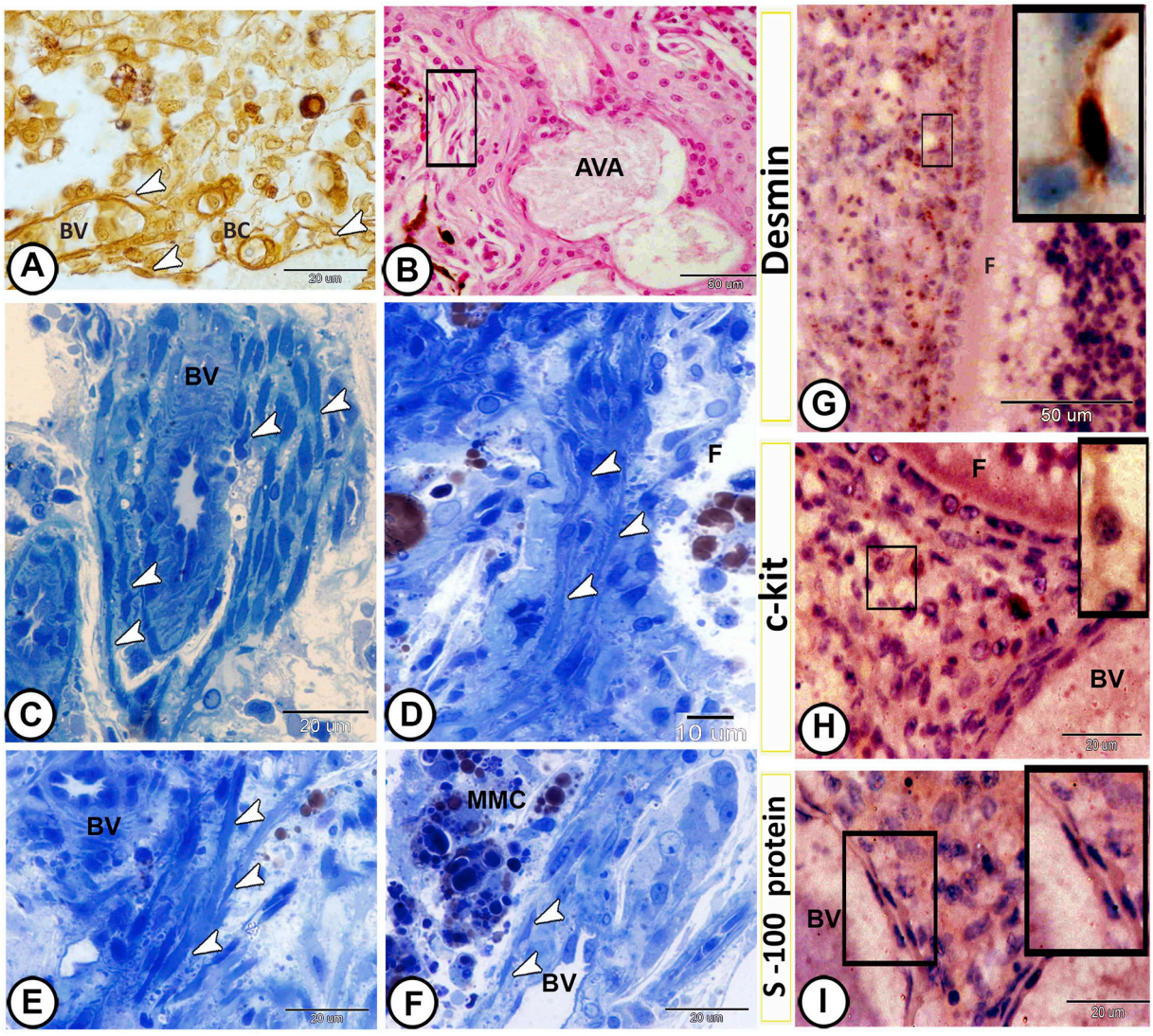

Fig. 11. Histological and immunohistochemical detection of telocytes. **A:** TCs (arrowheads) were positive for silver stain and distributed around the blood capillaries (BC), and small blood vessels (BV). **B:** TCs (boxed area) stained by HE and found in the vicinity to arteriovenous anastomoses (AVA). **C, E:** TCs (arrowheads) stained by TB and arranged around blood vessels (BV). **D:** TCs (arrowheads) were stained by TB and scattered around atretic follicles (F). **F:** TCs (arrowheads) were stained by TB and found near melanomacrophage center (MMC). **G:** TCs' bodies and telopodes (boxed areas) expressed strong immunoreaction for desmin around follicle (F). **H:** TCs (boxed areas) expressed positive immunoreactivity to c-kit around the follicle (F) and blood vessels (BV). **I:** TCs (boxed areas) expressed positive immunoreactivity to s-100protein blood vessels (BV).

calcium modulating activity. It has been involved in the regulation of enzymatic activity, protein phosphorylation, cell proliferation angiogenesis and vascular tone [56].

The current results showed that Rodlet cells, mast cells, dendritic cells, melanocytes, adipocytes, and TCs expressed c-kit. CD117 or c-kit is a cytokine receptor expressed on the surface of hematopoietic cells as well as melanocytes, mast cells and interstitial cells of Cajal [57]. C-kit is involved in many functions such as gametogenesis and hemostasis. Furthermore, signaling through CD117 plays an important role in survival, differentiation, and proliferation of various cells [58]. No available literature concerning the immunohistochemical studies on intermediate filaments like desmin in the teleosts' ovaries. In the ovarian stroma of Redbelly tilapia, desmin was immunolocalized in rodlet cells, TCs, and mast cells. These immunoreactive cells may play a role in the contractile process of the ovaries. Furthermore, desmin is involved in tissue differentiation and provide a structural framework for the ovary [59].

The present results revealed the presence of bundles of non-myelinated nerve fibers in the ovarian stroma. These bundles were previously identified in the ovaries *of Oreochromis niloticus* [60] and suggested to have a critical role in the regulation of the steroid production. Uematsu [61] added that these nerve fibers regulate the ovarian muscular contraction at oviposition during expelling of the oocytes.

The present study showed a wide distribution of melanocytes in the ovarian stroma. Kumar and Joy [62] suggested that gonadal melanin showed seasonal variations with a low level in the resting phase and the peak level in the post-spawning season. Moreover, the current work demonstrated deposition of many adipocytes in the ovarian stroma. This fat storage may be important for vitellogenesis. Tingaud-Sequeira et al. [63] added that vitellogenin, a key player of reproduction, is expressed by adipocytes of teleostean ovaries.

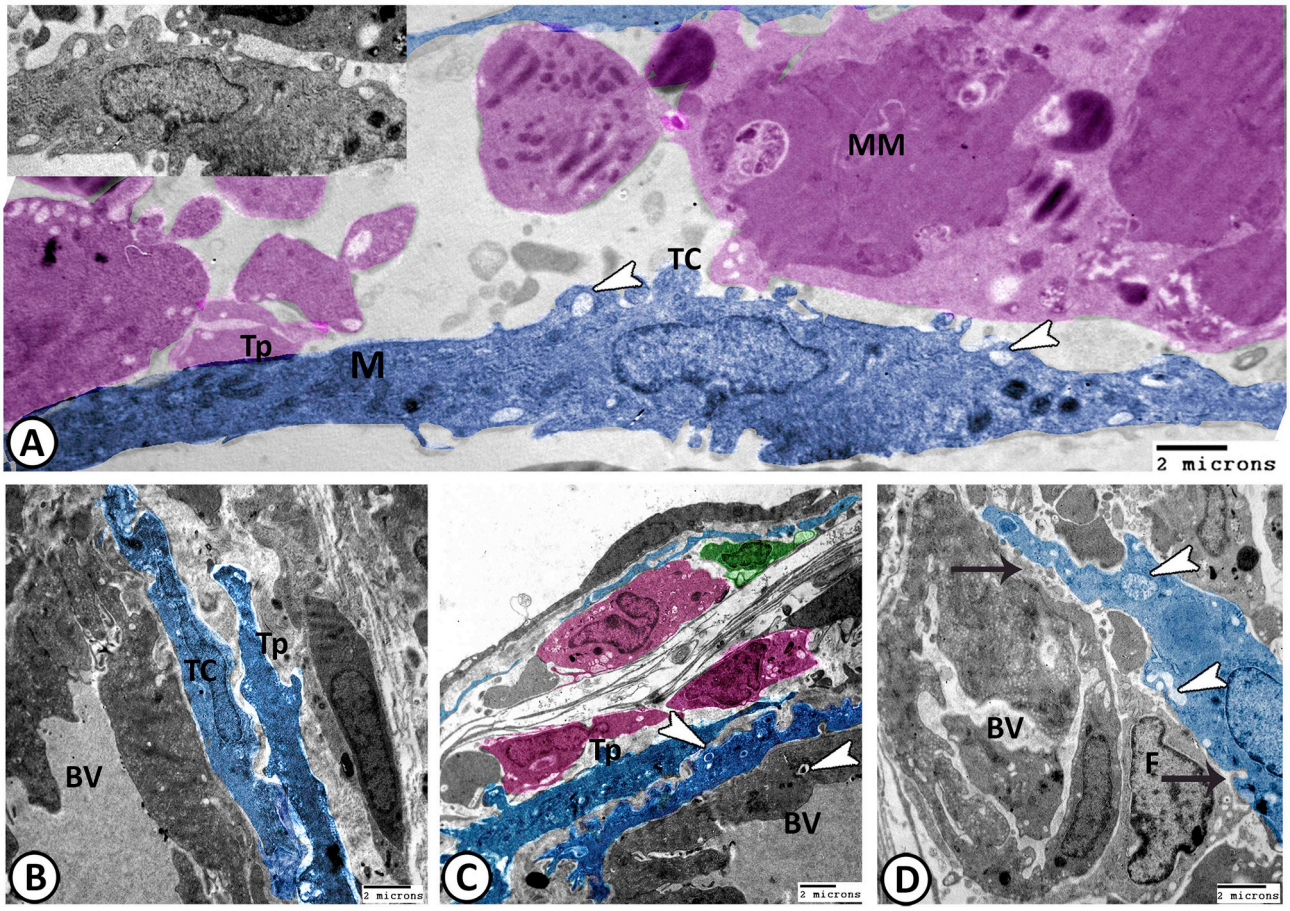

Fig. 12. Digital colored TEM images of TCs (blue): **A:** Telocyte (TC, boxed area) in association with melanomacrophage (MM), telopodes (Tp) displayed mitochondria (M) and secretory vesicles (arrowheads). **B:** TC′ cell body established contact with telopdes (Tp) of another TC around blood vessels (BV). **C:** Heterocellular contact was observed between TCs and macrophages (pink) and dendritic cells (green) and homocellular contact between telopodes (Tp) around blood vessels (BV). Note, TCs shed their secretory vesicles (arrowheads) to circulating blood vessels. **D:** TC showed heterocelluar contact with the endothelium of blood vessels (BV) and fibroblast (F). Note, TCs shed their secretory vesicles (arrowheads) to circulating blood vessels and fibroblast (arrows).. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusion

This work exposed different cell types with distinct function in the ovary based on their morphological features. The telocytes, rodlet cells, and mastocytes were identified in the ovary of tilapia. The present study is the first one to highlight on dendritic cells distribution in the fish ovary. The ovary of tilapia differed from other previously studied fish species, highlighting the importance of making this study to offer specific frameworks that can explain specific physiological processes.

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List of abbreviations

AO	Acridine orange
DCs	Dendritic cells
rER	rough endoplasmic reticulum
TCs	telocytes
TEM	transmission electron microscopy
Tps	telopodes

Ethical approval and consent to participate

The study was approved by the Ethics Committee of Assiut University, Egypt.

Conflicts of interest

The author declares that she has no competing interests.

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Author contributions

D. M Mokhtar collected the samples, performed the light and electron microscopy study, immunohistochemistry, morphometry and writing the paper.

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