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Functional morphology of arteriovenous anastomoses on rat ovary

Morfología funcional de las anastomosis arteriovenosas en ovario de rata

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ABSTRACT

This study aims to show the morphology of the structure involved in the function of arteriovenous anastomoses (AVA) under electron microscope. The study used 20 adult females of *Rattus norvegicus*. Ovarian tissue samples were fixed in 3% Glutaraldehyde in phosphate buffer, and then post-fixed in increasing concentrations of Ethanol, tissues were embedded in Epon resin. Semi-thin tissue sections were double stained with Uranyl acetate saturated in 70% Ethanol and lead Citrate. The ultrathin sections were examined in a JEOL 100 C electron microscope. In the opened AVA section, tunica intima, tunica media, and adventitia layers were observed in the vessel wall structure. The endothelial cell was present in the tunica intima, and the lumen was open. Thick layered smooth muscle cells were found in the tunica media. The muscles were arranged inner longitudinally and outer circularly. The internal elastic membrane lies between the circular and longitudinal smooth muscle. In another section taken from the tunica adventitia, fibroblasts were observed between dense elastic and collagen fibrils. The longitudinal smooth muscle was contracted in the closed AVA section, and the lumen appeared in the typical asterisks shape. This study showed the functional morphology of the AVA's and detailed vessel wall structures in the rat ovary. Lumen structure with open and closed AVAs is also shown. With observations from this study, the functional properties of the formations in the AVA wall structure are explained in the rat ovary.

Key words: Arteriovenous anastomoses; rat ovary; electron microscopy; functional morphology

RESUMEN

Este estudio tiene como objetivo mostrar la morfología de las estructuras que juegan un papel en la función de las anastomosis arteriovenosas (AVA) bajo el microscopio electrónico. El estudio utilizó 20 hembras adultas de *Rattus norvegicus*. Las muestras de tejido ovárico se fijaron en glutaraldehído al 3 % en tampón de fosfato y luego se fijaron posteriormente en concentraciones crecientes de etanol, los tejidos se incluyeron en resina Epon. Las secciones de tejido semi-delgado se tiñeron dos veces con acetato de uranilo saturado en etanol al 70 % y citrato de plomo. Las secciones ultrafinas se examinaron en un microscopio electrónico JEOL 100 C. En las AVAs seccionadas, se observaron capas de túnica íntima, túnica media y adventicia en la estructura de la pared del vaso. La célula endotelial estaba presente en la túnica íntima y la luz estaba abierta. Se encontraron células musculares lisas en capas gruesas en la túnica media. Los músculos estaban dispuestos internamente longitudinalmente y externamente circularmente. La membrana elástica interna se encuentra entre el músculo liso circular y longitudinal. En otro corte tomado de la túnica adventicia se observaron fibroblastos entre densas fibrillas elásticas y colágenas. El músculo liso longitudinal se contrajo en la sección AVA cerrada y la luz apareció en la forma típica de asteriscos. Este estudio mostró la morfología funcional de los AVA y las estructuras detalladas de la pared vascular en el ovario de rata. También se muestra la estructura del lumen con AVA abiertos y cerrados. Con las observaciones de este estudio, las propiedades funcionales de las formaciones en la estructura de la pared de AVA se explican en el ovario de rata.

Palabras clave: Anastomosis arteriovenosas; ovario de rata; microscopía electrónica; morfología funcional

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INTRODUCTION

Arteriovenous anastomoses (AVAs) are vessels that directly connect arterioles and venules. Thus, blood passes from arterioles to venules without passing through capillaries. In this way, AVAs provide a high level of blood flow in the area without overloading the capillary network. Because AVAs have wider lumens than capillaries [1, 2].

AVAs have been tested in human and many animal organs [3, 4, 5, 6, 7]. AVAs have been observed to have thermoregulatory functions in human skin [8, 9]. Since AVAs are found in the skin, their importance in fingertip transplantations is considered in plastic surgery and used for the blood supply of the transplanted tissue [10].

Lipa *et al.* stated that AVAs are seen in mono chorionic human twins with a rate of 75.4%. They also stated that, in some instances, AVAs may cause specific complications; however, in general, they regulate inter-twin blood exchange and may compensate for unequal placental territory [11]. Grinsell *et al.* stated that AVAs can be congenital or acquired, asymptomatic or symptomatic, and microvascular or macrovascular in the human body [12].

In animal morphology, Krmpotic *et al.* [13] stated that AVAs were found in the skin of weddell (*Leptonychotes weddellii*), leopard (*Hydrurga leptonyx*), and southern elephant seals (*Mirounga leonina*) species and that AVAs had thermoregulatory functions. Additionally, other tissues where the presence of AVAs has been demonstrated are in rabbit (New Zealand White) peripheral pulmonary circulation [14], in late-pregnant ewes' uterus [15], in Holstein cows' corpus luteum [16]. Mokhtar [17] demonstrated the presence of AVA in the ovary of mature Redbelly tilapia (*Coptodon zillii*) using a light microscope, and Mokhtar & Abd-Elhafez [18] demonstrated the presence of AVA in the peripheral circulation of the ovary in one-humped camel (*Camelus dromedarius*).

Yet, the effects and roles of AVAs on tissue have not been fully elucidated. Their morphology must be known to determine these functions because there is a relationship between the structure and functions of AVAs [19]. Lijima *et al.* [19] stated that it is necessary to fully understand the vessel wall structure to understand the function of AVA. However, it is difficult to analyze the AVA wall structure under transmission electron microscopy thoroughly [19]. And also it is known, rat ovaries have not been subjected to AVA's morphological functions.

This study aims to show the morphology of the structures that play a role in the function of AVAs under the transmission electron microscope from unpublished depository data.

MATERIALS AND METHODS

The study used 20 adult females of rats (*Rattus norvegicus*), obtained from the Experimental Animals Laboratory, Faculty of Medicine, Sivas Cumhuriyet University, Türkiye. Ethical approval is also obtained from the institution.

At least two samples were taken from a total of forty rats ovaries from each ovary without distinguishing a specific region. The samples were cut into small pieces of 1 mm^3 in phosphate buffer. By applying double fixation to the pieces, the first fixation of the tissues was made in Glutaraldehyde prepared with Milloning phosphate buffer (pH: 7.4) for 1 h. The second fixation was made with isotonic osmium tetroxide (OsO_4 1%, pH: 7.3). The fixated tissues were dehydrated by passing through the ethyl alcohol series. Dehydrated tissues were embedded in Epoxy resin (Araldite CY-212). The blocks were polymerized in an oven at 60°C for 48 h. Ultra-thin (1 µm) sections were taken from the prepared blocks with the LKB-5 ultra-tome (LKB Co., Biel, Switzerland). The sections were stained with toluidine blue to identify suitable areas. Thin sections of 300–500 angstroms were taken from selected areas. Double staining with Uranyl acetate and lead Citrate was applied to the thin sections. Sections ready to be examined by contrast staining were evaluated under the JEOL-100 C (JEOL, USA Inc., Maryland, USA) electron microscope and photographed at different magnifications.

RESULTS AND DISCUSSION

In this study, simple arteriovenous anastomoses are observed in all eighty specimens. There is no specific way to sectional follow AVAs. The AVA's can be separable from other micro vessels by their unique wall proportion and structures. The obtained observations about AVAs in rat ovary and the morphology of vessels with different contraction positions are as follow:

In a section in which the AVA was open, tunica intima, tunica media, and tunica adventitia layers were observed in the vessel wall structure. The endothelial cell was present in the tunica intima, and the lumen was open. Thick layered smooth muscle cells were found in the tunica media. The muscles were arranged inner longitudinally and outer circularly—the internal elastic membrane lying between the circular and longitudinal smooth muscle (FIG.1).

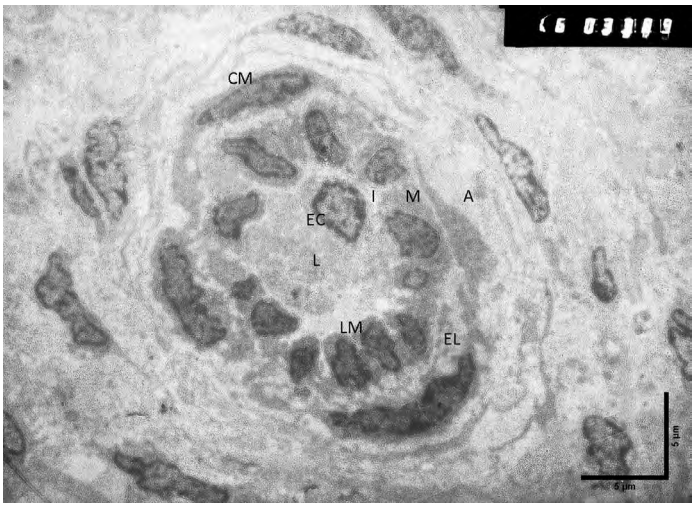

FIGURE 1. Shows an AVA with an open lumen (L). Tunica intima (I), tunica media (M), and tunica adventitia (A) layers can be observed in the vessel wall. The endothelial cell (EC) presents in the tunica intima. Thick layered smooth muscle cells can be observed in the tunica media, which are arranged inner longitudinally (LM) and outer circularly (CM). The internal elastic membrane (EL) lies between the CM and LM

In another section of the tunica adventitia, fibroblasts were observed with dense elastic and collagen fibrils (FIG. 2). The longitudinal smooth muscle was contracted in the section with the AVA closed, and the lumen appeared in the typical asterisks shape (FIG. 3).

In the literature, various researchers have worked to explain the functions of AVAs. These studies focused on the regulatory properties of AVAs on thermoregulation and blood flow.

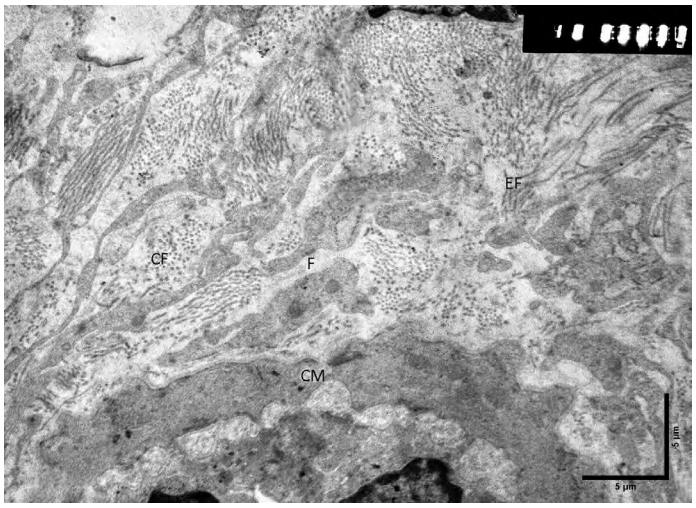

FIGURE 2. Shows the tunica adventitia of the AVAs. Fibroblasts (F) can be observed with dense elastic (EF) and collagen fibrils (CF). The circular muscle cells (CM) can be observed most inner layer.

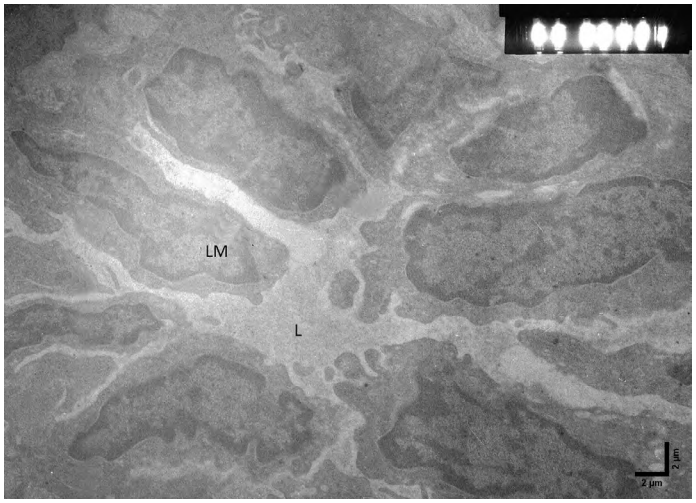

FIGURE 3. Shows an AVA with a closed lumen. The longitudinal smooth muscles (LM) contracted, and the lumen (L) appeared in the typical asterisks shape

Sherman explains the opened and closed AVA functions as follows. When the AVAs are opened, blood is drawn from the capillaries and passes directly into the veins. The flow accelerates, and the pressure in the veins increases—the temperature decreases in the area where the capillaries that receive no or little blood are distributed. When the AVAs close, blood flows back into the capillaries, the temperature rises, the blood flow in the veins slows, and the pressure drops [2]. Conversely, Midgard explains the role of temperature-regulating AVA on the eggs in the departure and arrival of birds (*Larus argentatus)* from the nest as follows. If AVAs are on in brooding birds, their body temperature rises, transferring this heat to the eggs. This process is critical when the bird returns to the cooled eggs after feeding wanders [20]. Conversely, if heat loss from the brood needs to be reduced, as the bird moves away from the nest, the AVAs are likely closed, reducing blood flow to the brood [20].

Lijima *et al.* [19] investigated the presence and function of AVA in rabbit (*Oryctolagus cuniculus*) ears. Moreover, they describe AVA's functions and morphology as follows. If the rabbit's ear temperature rises above 40°C, the muscle in the AVA wall relax, blood flow increases, and cooling is observed. When the local temperature drops below 15°C, the muscles in the AVA wall relax again, increasing blood flow and helping to raise the local temperature [19]. The contraction and expansion of AVAs in the rabbit ear can be explained as follows. Outer circular and inner longitudinal smooth muscle cells contract simultaneously, causing lumen closure. When the muscle relaxes, the lumen expands. Connective tissue elements can also act as elastic support to aid the expansion and expansion of circular and longitudinal smooth muscle [19].

The internal elastic membrane separates the sub-endothelial structures from the muscular layer of the tunica media and tunica adventitia. The internal elastic membrane sometimes preserved its structure, sometimes regressed, and sometimes could not be seen ultimately. In this study, the internal elastic membrane and the layers in the wall structure of AVA were clearly shown in the rat ovary (FIG. 1).

It has also been observed that the circular smooth muscle cells in the tunica media rotate and end by regressing. Since the lumens of AVAs are broader than those of capillaries, they provide a high level of blood flow in the area without overloading the capillary network. The AVA lumen may appear misshapen, slit, or asterisk. This study showed smooth muscles in the vessel wall and AVA lumen as asterisks in the rat ovary (FIGS. 1, 3).

Similar observations have been noted in studies of different animal species on ovarian tissue. Mokhtar (mature Redbelly tilapia, *Coptodon zillii*) [17] and Mokhtar & Abd-Elhafez (one-humped camel, *Camelus dromedarius*) [18] indicated the following observations in their studies: "The simple AVAs were evident in the ovary. The AVAs were surrounded by one tunica adventitia, and a ring or roll of smooth muscle cells supported the origin of the anastomosis." These observations in the literature are compatible with our observations in this study (FIG 1).

The covering cells consist of 4–6 layers of fibroblasts in the tunica adventitia. They perform three functions: fibrogenesis, phagocytosis, and barrier [19]. In the current study also shows the presence of fibroblasts in the tunica adventitia in the rat ovary (FIG. 2).

There are some differences between the previous studies conducted by the senior investigator in this study on AVA development [21], types [22], and vascular wall structure [23] in rats—the main difference with the previous studies is that these studies were conducted with light microscopy. Regarding AVA types, only simple AVA was observed in ovarian tissue in this study. It was reported that simple and glomus anastomoses were observed together in rat embryos. Circular smooth muscle cells in the vascular wall structure are similar. In addition, in previous studies, the typical asterisk open position of the AVA lumen could not be observed.

In female mammals, the ovaries play a role in both the genital and endocrine systems. The temperature differences during ovulation and bidirectional hormonal transport can cause changes in blood flow rates in the ovary. Open and closed AVA types can prove the ovaries' thermoregulation needs. However, this study is not a physiological function study. This functional morphology study aims to reveal the states of the AVA wall structures during contraction–relaxation and their positions with each other.

CONCLUSIONS

The main conclusions of this study are as follows:

1. All simple type AVAs were observed in the rat ovary (*Rattus norvegicus*).
2. The layers in the vascular wall structure and the structural elements in these layers were tried to be shown using transmssion electron microscopy.
3. It has been observed that the vascular wall structure consists of three layers. Circular and longitudinal muscle cells, connective tissue elements, and fibroblasts were also evident in the vessel wall structures.
4. AVA with its lumen open in the typical asterisk shape are this study's main observations and conclusions.

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No funding was received for conducting this study.

Conflict of interest

The authors declare that there is no conflict of interest

Ethics approval

This study was carried out under the supervision of the Experimental Animals Laboratory of our institution and the ethical principles for using experimental animals in pre-clinical studies. However, there is no protocol number provided as there is no experimental animal ethics committee in our institution at the time of the study.

Authors' contributions

MÇ: Conceptualization, data curation, investigation, supervision, writing – review & editing KÇ: Writing – original draft, review, and editing. All authors approved final version of manuscript.

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