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Microscopic studies on the arterial walls of main arteries supplying the mammary glands of guinea pig *(Cavia porcellus)* at different reproductive stages

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ABSTRACT

A microscopic studies on the arterial wall of main arteries supplying the mammary glands of guinea pig at different reproductive stages was carried out by using a light microscope. The arterial segments from the ascending aorta to the mammary glands were classified into elastic, transitional and muscular types. The ascending aorta, aortic arch, brachiocephalic trunk, the subclavian, the thoracic, and abdominal aorta were elastic type. The subendothelial layer was present. The tunica media contained of well- defined elastic lamellae. The maximum numbers of elastic lamellae were recorded as 25-30, in the ascending aorta. The common iliac, the proximal and distal parts of the external iliac, and the proximal part of the femoral arteries were transitional type. The transitional type had the characteristics feature of both elastic and muscular types. The deep femoral, pudendoepigastric trunk, the external pudendal, the caudal superficial epigastric, and the caudal and cranial mammary arteries were muscular type. The tunica media was completely devoid of elastic lamellae. The reproductive stages did not affect the general histology of the large and medium size arteries. But the internal elastic membrane of the external pudendal, caudal superficial epigastric, and mammary arteries were wavy in the virgin stage. This wavy character was lost following pregnancy to the on the onset of lactation. It may be

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assumed that, during lactating period increased volume and force flow of blood through these arteries gives pressure to the inner arterial wall resulting straightening of the internal elastic membrane. The existence of elastic lamellae in the elastic arteries, probably due to resist comparatively high arterial pressure compared to that of the peripheral muscular arteries.

Key words: microscopic study, arterial wall, mammary gland, reproductive stages, guinea pig

Introduction

Histological studies on the arterial wall of miniature swine (TANIGAWA et al., 1985), Japanese dog (AWAL et al., 1998), and black Bengal goats (AWAL et al., 1999) are available. Effects of different reproductive stages on the arterial wall of Wistar rats have been previously reported (AWAL et al., 1995). A review of available literature reveals no infomation regarding the microscopic study of the arterial walls of main arteries supplying the mammary glands of guinea pig at different reproductive stages.

Therefore, the present study was undertaken to investigate the general histology of arterial walls in order to clarify whether or not the various reproductive stages affect the microanatomy of the arterial walls of guinea pig. In addition, this study will be helpful to compare the histology of the arterial walls of guinea pig with that of other domestic and laboratory animals if any.

Materials and methods

A total of 16 adult female apparently healthy guinea pig *(Cavia porcellus)* were used during the study (Fig. 1). The animals were grouped into 4 stages, each group consisting of 4 animals (4 at 60-day-old virgin, 4 at 35 days of pregnancy, 4 at 5 days of lactation, and 4 at 25 days of post-weaning). They were maintained in the laboratory of Anatomy and Histology, Bangladesh Agricultural University, and provided food and water *ad libitum*. Day 1 of pregnancy was confirmed by observing the vaginal plug in the following morning after coitus (LAWIAH, 1930). The animals were anesthetized with Nembutal® (Sodium pentobarbital 30mg/kg body mass) and bled to death by giving incision on the common carotid artery. The thoracic and abdominal cavity was opened. Whole the vascular system was flashed with physiological saline. Sixteen arterial segments from ascending aorta to the mammary glands were dissected out (Fig. 2), and

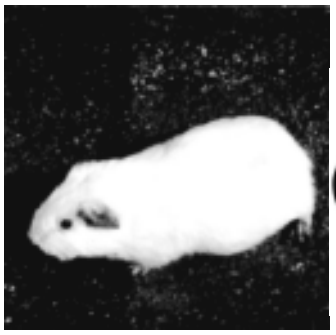

Fig. 1. Guinea pig (*Cavia porcellus*) in Bangladesh

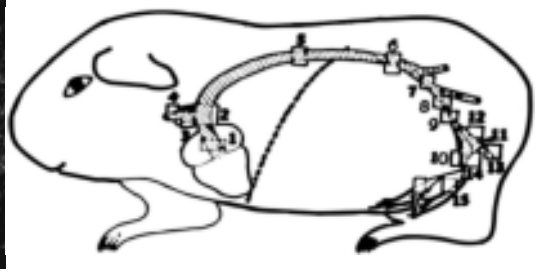

Fig. 2. Location of the arterial segments removed from the guinea pig and type of artery. Elastic type: ascending aorta (1), aortic arch (2), brachiocephalic trunk (3), subclavian artery (4), thoracic aorta (5), abdominal aorta (6). Transitional type: common iliac artery (7), external iliac artery - proximal part (8), external iliac artery - distal part (9), femoral artery (10). Muscular type: deep femoral artery - proximal part (11), pudendoepigastric trunk (12), external pudendal artery (13), caudal superficial epigastric artery (14), caudal mammary artery (15), cranial mammary artery (16).

Table 1. Name of the arteries and their types

Name of the arteries	Type
1. Ascending aorta	Elastic type
2. Aortic arch	Elastic type
3. Brachiocephalic trunk	Elastic type
4. Subclavian artery	Elastic type
5. Thoracic aorta	Elastic type
6. Abdominal aorta	Elastic type
7. Common iliac artery	Transitional
8. External iliac artery (proximal part)	Transitional
9. External iliac artery (distal part)	Transitional
10. Femoral artery	Transitional
11. Deep femoral artery	Muscular type
12. Pudendoepigastric trunk	Muscular type
13. External pudendal artery	Muscular type
14. Caudal superficial epigastric artery	Muscular type
15. Caudal mammary artery	Muscular type
15. Cranial mammary artery	Muscular type

immediately preserved in 10% buffered neutral formalin. The tissues were dehydrated with a series of graded ethanol, cleared in xylene and routinely embedded in paraffin wax. They were sectioned at 5 μm thickness. Four stains were used during the study. Hematoxylin and eosin for general histological study, Weigert's elastica Van Gieson for elastic fibers, Weigert's resorcin fuchsin for smooth muscle cells and Azan for collagen fibers (GRIDLEY, 1960). All the tissues were studied with a light microscope (Olympus, Japan). Elastic lamellae were counted (30 slides for each arterial segment) by using higher magnification and from the enlarged microphotographs (AWAL et al., 1995). The photographs were produced to illustrate the results.

Results

The arterial segment from the ascending aorta to the mammary glands were consisted of three distinct tunics; the tunica intima, the inner most layer; tunica media, the middle layer, and the tunica externa, the outer most layer of the arterial wall. The ascending aorta, aortic arch, brachiocephalic trunk, the subclavian, the thoracic, and the abdominal aorta were elastic type (Fig. 2 and Table 1). The subendothelial layer was present, but well-distinct in large caliber arteries, particularly in the ascending aorta, aortic arch, brachiocephalic trunk, the thoracic, and the abdominal aorta (Figs. 3-5). The subendothelial layer was composed of predominantly branched elastic fibers, collagen fibers and reticular fibers. The external and internal elastic laminae were absent. The tunica intima consisted of a single layer of flattened endothelial cells. The endothelium made a continuous sheet on the internal elastic membrane. The tunica media was the thickest layer of the three tunics, and contained mainly of well-defined elastic lamellae (Figs. 3-5). The number of elastic lamellae in the tunica media were ranged from 20-30, in the ascending aorta, 20-24, in the aortic arch, 18-22, in the brachiocephalic trunk, 8-10, in the subclavian artery, 1 0-15, in the thoracic aorta and 6-8, in the abdominal aorta, respectively. The tunica externa was thin in the ascending aorta, but well- developed in the thoracic and abdominal aorta, and composed of collagen fibers, elastic fibers, smooth muscle cells and sections of blood vessels and nerve fibers (Fig. 4).

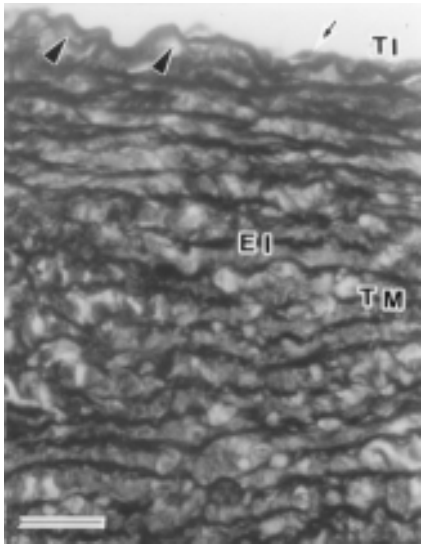

Fig. 3. Ascending aorta. Elastic type. The tunica intima (TI) consists of flattend endothelium (arrow). Subendo-thelial layer is present (arrowheads). Well-developed elastic lamellae (El) are seen in the tunica media (TM). Elastica Van Gieson stain; ×347; scale bar =100 μm.

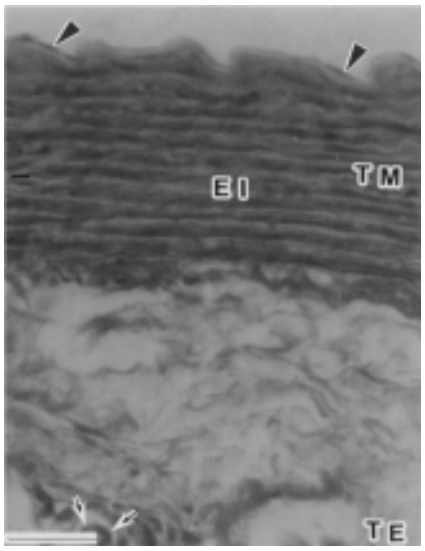

Fig. 4. Thoracic aorta. Elastic type. Endothelium are seen in the tunica intima (arrowheads). Well-defined elastic lamellae (El) in the tunica media (TM). Tunica externa (TE) is thick. Small blood vessel is present (arrows). Elastica Van Gieson stain; × 347; scale bar =100 μm.

The common iliac, proximal and distal part of the external iliac, and the femoral arteries were transitional type (Fig. 2 and Table 1). Likewise elastic arteries, the tunica intima was consisted of a single layer of flattened endothelium. The subendothelial layer was poorly observed in the common iliac artery, but absent in the proximal and distal parts of the external iliac, and the femoral arteries. The tunica media contained comparatively thin elastic lamellae. The number of elastic lamellae in the tunica media were 4-5, in the common iliac, 3-4, in the external iliac, and 2-3, in the femoral arteries, respectively. The interlaminal spaces were comparatively broad and occupied by the bundles of smooth muscle cells. The bundles of smooth muscles in the interlaminal spaces interrupted the continuity of the normal coarseness of the elastic lamellae in the tunica media (Fig. 6). The tunica externa was well developed and composed of predominantly collagen

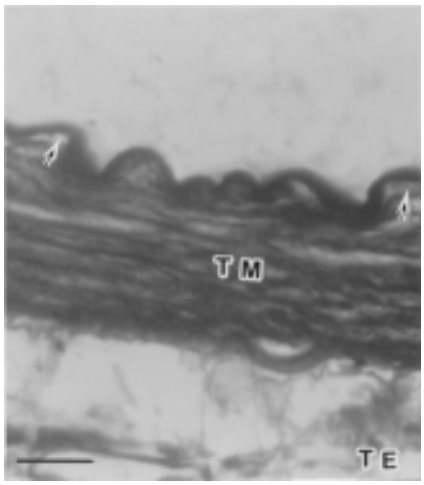

Fig. 5. Abdominal aorta. Elastic type. Subendothelial layer is present (arrows). Elastic lamellae are thin in the tunica media (TM). Tunica externa (TE) is thick. Elastica Van Gieson stain; ×347; scale bar = 100 μm.

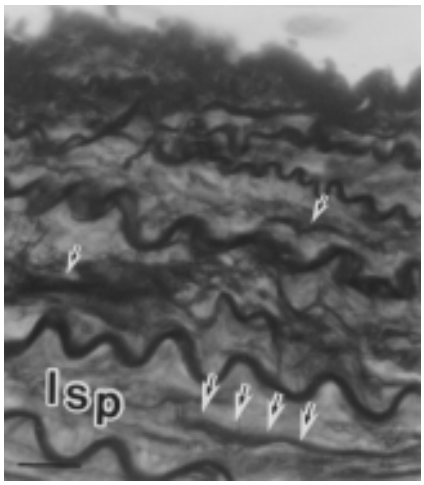

Fig. 6. Common iliac artery. Trans-itional type. The interlaminal spaces (Isp) are broad in the tunica media (TM). Bundles of smooth muscle fibers are seen in the interlaminal spaces (arrows). Elastica Van Gieson stain; ×347; scale bar = 300 μm.

fibers, smooth muscle cells, sections of blood vessels and nerve fibers.

The deep femoral, the pudendoepigastric trunk, the external pudendal, the caudal superficial epigastric, the caudal and cranial mammary arteries were muscular type (Fig. 2 and Table 1). As it has been observed in the elastic and transitional types, the tunica intima of muscular artery was also consisted of a single layer of flattened endothelium resting on the internal elastic membrane. The internal elastic lamina was prominent, but the external elastic lamina was indistinct or absent. The internal elastic membrane of the external pudendal, caudal superficial epigastric, the caudal, and cranial mammary arteries was wavy during virgin stage, but their wavy nature gradually disappeared following pregnancy to the onset of lactation (Figs. 7-8). The subendothelial layer was absent. The tunica media completely devoid of elastic lamellae and composed of predominantly smooth muscle cells with a few fine elastic tissue fibers (Figs. 7-8). The tunica externa was well developed and thicker than that of

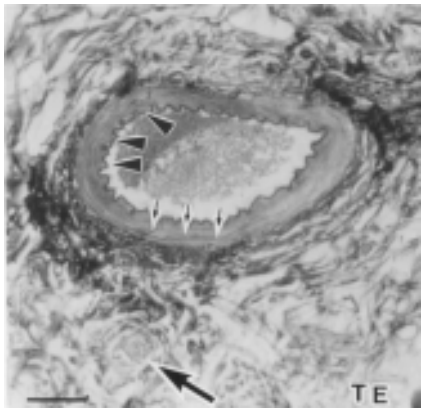

Fig. 7. Caudal superficial epigastric artery. Muscular type. Virgin stage. Tunica media (TM) contains smooth muscle cells. A few elastic fibers are seen in the tunica media (arrows). The internal elastic membrane is wavy (arrowheads). A section of nerve bundle is seen in the tunica externa (TE; large arrow). Elastica resorcin fuchsin stain; ×87; scale bar = 100 μm.

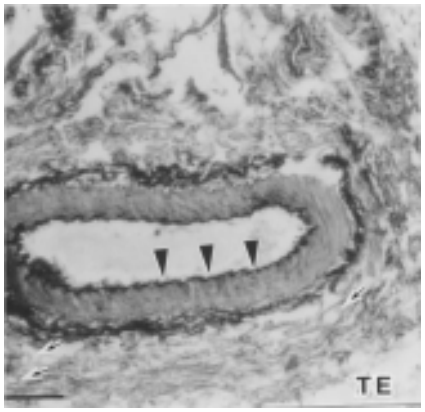

Fig. 8. Caudal superficial epigastric artery. Lactation stage. Wavy character of the internal elastic membrane is lost (arrowheads). Tunica media (TM) consists of smooth muscle cells. Tunica externa (TE) is thick and contains mainly of collagen fibers. Sections of small blood vessels are seen (arrowheads). Elastica resorcin fuchsin stain; ×87; scale bar = 100 μm.

the tunica media. The tunica externa was mainly composed of collagen fibers.

Some elastic fibers were observed in the inner most layer of the tunica externa that surrounded the tunica media in the form of a circular arrangement. Cross sections of small blood vessels and nerve bundles were also observed (Fig. 7).

Discussion

As it has been reported by ARTHUR (1969), ADAM et al. (1970), BUNCE (1974), BLOOM and FAWCETT (1975), and BROWN (1976), the arterial segments of the guinea pig were classified into elastic, transitional and muscular types. This classification was based on the histological characteristics and organization of connective tissue fibers and smooth muscle cells in the

three distinct tunics, namely the tunica intima, tunica media and in the tunica externa. The ascending aorta, aortic arch, brachiocephalic trunk, the subclavian, the thoracic, and the abdominal aorta were elastic type. The arterial segments from the ascending aorta to thoracic aorta were elastic type in miniature swine (TANIGAWA et al., 1985), and black Bengal goats (AWAL et al., 1999). In Wistar rats (AWAL et al., 1995), the arterial segments from ascending aorta to the abdominal aorta were elastic type and were similar to our present study. The subendothelial layer was present in the large caliber arteries, and this space was gradually disappeared when the artery reached to the medium transitional to small muscular arteries. The subendothelial layer gradually becomes thinner and eventually disappears with decreasing vessel size (BUCH, 1979). Tunica media was the thickest layer among the three tunics and consisted of well-defined elastic lamellae. These findings are in well agreement with the reports of Wistar rats (AWAL et al., 1995), Japanese dogs (AWAL et al., 1988), and black Bengal goats (AWAL et al., 1999). The number of elastic lamellae in the tunica media was 20-30, in the ascending aorta, 20-24, in the aortic arch, 18-22, in the brachiocephalic trunk, 8-10, in the subclavian, and 10-15, in the thoracic, and 6-8, in the abdominal aorta, respectively. The number of these elastic lamellae in the tunica media depends on the size and relative distance of the arteries from the heart (AWAL et al., 1995), and also varies among the domestic and laboratory animals (AWAL et al., 1995 and 1999, respectively). The tunica extema was comparatively thin and composed of connective tissue fibers, vasa vasorum, small blood vessels and nerves. Similar histological characteristics were also observed in miniature swine (TANIGAWA et al., 1985), Japanese dogs (AWAL et al., 1998), and black Bengal goats (AWAL et at., 1999).

Arterial segments from the common iliac to the femoral artery were transitional type. The transitional type was between elastic and muscular types, and possesed a mixture of characteristic's features common to both of them. The subendothelial layer was poorly observed in common iliac artery. In other transitional arteries, this layer was indistinct or absent. The internal elastic lamina was present but, the external elastic lamina was not clear or absent. The elastic lamellae in the tunica media were thin and irregular in their course. The interlaminal spaces were broad and

occupied with the bundles of smooth muscle fibers. The present findings are well agreements with the reports of TANIGAWA et al. (1985) in miniature swine, and AWAL et al. (1999) in black Bengal goats. The tunica externa was well-developed. Elastic fibers in the tunica externa of transitional arteries, formed a circular arrangement around the tunica media of Japanese swine (AWAL et al., 1997) and dogs (AWAL et al., 1998). But this finding was absent in the present study, and are agreed well with the report of AWAL et al. (1999) in black Bengal goats. Elastic fibers formed a circular arrangement around the tunica media in dogs (BUNCE, 1974), and Japanese swine (AWAL et at., 1997). But this histological character was absent in the present study. The present study was very similar to that observed in black Bengal goats (AWAL et al., 1999).

The deep femoral, the pudendoepigastric trunk, the external pudendal, the caudal superficial epigastric, the caudal and cranial mammary arteries were muscular type. Subendothelial layer was absent. Both the internal and external lamina was present. The tunica media was consisted of predominantly smooth muscle cells and completely devoid of elastic lamellae. A few fine elastic fibers were observed in the tunica media of Wistar rats (AWAL et al., 1995), and Japanese dogs (AWAL et al., 1998). Usually, the smaller arteries, the peripheral branches of the arterial tree, were identified as muscular type and the media was composed of circularly arranged smooth muscle cells with a few fine elastic fibers (GROSS et al., 1933; AWAL et al., 1999). The similar findings were observed in our present study. The internal elastic membrane of the external pudendal, the caudal superficial epigastric, the caudal and cranial mammary arteries were wavy in virgin stage, but this wavy character was gradually disappeared following the onset of lactation. The wavy character of the internal elastic lamina was observed in the arteries supplying the mammary glands of Wistar rats in virgin stage, and gradually lost their wavy character following pregnancy to the onset of lactation (AWAL et al., 1995). They also reported that during pregnancy through lactation the circulation to the mammary glands dramatically increased for the production of large quantities of milk. The increased volume and force flow of blood gives pressure to the internal elastic membrane of the arteries supplying the mammary glands, resulting

dilatation of the arterial walls and straightening of the internal elastic membranes (AWAL et al., 1995). It was confirmed in our present study.

Conclusions

The histological characteristic's of the arterial wall varies at different regions for proper responding with the physiological conditions of the body. The artery nearest to the heart contained more elastic lamellae was assumed to be resist comparatively high arterial pressure than that of the peripheral arteries. Similarly, the smooth muscle cells in the muscular artery was due to be adopted with functional physiological condition of the body. The internal elastic membranes those were found wavy in the virgin stage, gradually became straight on the onset of lactation. During lactating period, the amount of circulating blood to the mammary glands increased dramatically. The increased volume and force flow of blood through the arteries supplying the mammary glands gives pressure on the internal walls, resulting straightening of the internal elastic membrane.

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References

ADAM, W. S., M. L. CALHOUM, E. M. SMITH, A. W. SISSION (1970): Microscopic Anatomy of the Dog. Charles Thomas Pub. Springfield, Illinois.

ARTHUR, W. H. (1969): Histology. Igaku Shoin Ltd. Tokyo, Japan.

AWAL, M. A., M. MATSUMOTO, H. NISHINAKAGAWA (1995): Morphometrical changes arterial walls of main arteries from heart to the abdomino-inguinal mammary glands of Wistar rat from virgin through pregnancy, lactation and post-weaning. J. Vet. Med. Sci. 57, 251-256.

AWAL, M. A., H. NISHINAKAGAWA, M. MATSUMOTO (1997): Histological studies on the arterial walls of main arteries supplying to the mammary glands of Japanese swine (Berkshire). Progress. Agric. 8, 73- 76.

AWAL, M. A., M. MATSUMOTO, H. NISHINAKAGAWA (1998): Histology on the main arteries of mammary glands of Japanese dog. Bang. J. Anim. Sci. 27, 49-55.

AWAL, M. A., M. A. A. PRODAN, M. ASADUZZAMAN, M. KUROHMARU (1999): Histological studies on the arterial walls of main arteries supplying the mammary glands of black Bengal goats *(Capra hircus)* in Bangladesh. Vet. arhiv. 69, 309-318.

BLOOM, W., W. D. FAWCETT (1975): A Textbook of Histology. W. B. Saunders Co. Philadelphia. London.

BUCH, R. C (1979): The longitudinal orientation of structures in subendothelial space of rat. Am. J. Anat. 156, 1-14.

BUNCE, D. F. M. (1974): Atlas of Arterial Histology. Warren H. Green Inc. Mizouri.

BROWN, E. M. (1976): Textbook of Veterinary Histology. Lea & Febiger Inc. Philadelphia. London.

GRIDLEY, M. F. (1960): Manual of Histologic and Special Staining Techniques. McGraw-Hill Book Co. Inc. New York.

GROSS, M. D., Z. E. EPSTEIN, M. A. KUGEL (1933): Histology of the coronary arteries in human heart. Am. J. Pathol. 10, 253-273.

LAWIAH, J. W. (1930): Studies on the physiology of the accessary glands of reproduction of the male guinea pig. Anat. Rec. 45, 163-175.

TANIGAWA, M., J. ADACHI, K. MOCHIZUKI (1985): Histological study on the arterial wall of Gottingen miniature swine. Exp. Anim. 35, 35-45.

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AWAL, M. A., M. A. A. PRODHAN, M. KUROHMARU, M. MATSUMOTO, H. HISHINAKAGAWA: Mikroskopska istraživanja stijenki glavnih arterija za opskrbljivanje mliječnih žlijezda zamorčeta *(Cavia porcellus)* u različitim reprodukcijskim stadijima. Vet. arhiv 71, 19-30, 2001.

SAŽETAK

Obavljeno je mikroskopsko istraživanje stijenki glavnih arterija koje opskrbljuju krvlju mliječnu žlijezdu zamorčeta tijekom različitih faza reprodukcije. Arterijski segmenti uzlaznog dijela aorte klasificirani su kao elastični, prijelazni i mišićni tip. Uzlazni dio aorte, brahiocefalični trunkus, supklavijalna, torakalna i abdominalna aorta elastičnog su tipa s prisutnim subendotelijalnim slojem. Tunika medija sadrži dobro definirane elastične lamele kojih u uzlazne aorte ima 25 do 30. Zajednička ilijačna, proksimalni i distalni dio vanjske ilijačne arterije i proksimalni dio femoralnih arterija prijelaznog su tipa koji ima karakteristike elastičnoga i mišićnoga epitela. Dubinska femoralna arterija, pudendoepigastrični trunkus, vanjska pudendalna arterija, kaudalna superficijalna epigastrična arterija te kaudalne i kranijalne mamarne arterije mišićnoga su tipa. Tunika medija u navedenih arterija u

potpunosti je bez elastičnih lamela. Potrebno je istaknuti da različite faze reprodukcije ne utječu na histološku građu velikih i malih arterija. U razdoblju prije gravidnosti zapažena je valovitost interne elastične membrane vanjske pudendalne arterije, kaudalne superficijalne epigastrične te mamarne arterije koja se gubi nakon nastupa gravidnosti pa sve do razdoblja laktacije. Pretpostavlja se da je to odgovor na povećan protok krvi koji svojim tlakom mijenja ujedno i morfologiju interne elastične membrane.

Ključne riječi: mikroskopska građa, arterijska stijenka, mliječna žlijezda, reprodukcijski stadij, zamorče