VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 79, No. 2, pp. 255–264
DOI: 10.5603/FM.a2019.0070

ISSN 0015–5659
journals.viamedica.pl

Diversity of coronary arterial tree in laboratory mice

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[Received: 4 April 2019; Accepted: 24 May 2019]

Background: *Research on the development and topography of mouse coronary arteries has been conducted for many years. Patterns of the course of these vessels have been described in various mouse strains. Our research focused on hearts of MIZZ mice.*

Materials and methods: *We visualised the coronary artery system by means of latex dye perfusion via the aorta. The injected latex did not reach the capillary vessel system.*

Results: *The heart of MIZZ mice is supplied with blood by two main coronary arteries: the right and the left one. They deliver blood to the right and left part of the heart, respectively. The right coronary artery arises from the right sinus of the aorta and the left coronary artery from the left sinus. The interventricular septum is usually supplied by the septal artery, which is the main branch of the right coronary artery. All arteries of the coronary system run intramurally. The number of branches and the location of their ostia differed among the examined individuals.*

Conclusions: *Detailed information about the normal topography of coronary arteries, the number and course of their branches, as well as the area of the heart which is vascularised by these vessels constitutes the basic knowledge necessary to conduct further experiments.* (Folia Morphol 2020; 79, 2: 255–264)

Key words: coronary artery, heart, septal artery, right coronary artery, mouse

INTRODUCTION

Research on the development of the heart and its elements has been conducted for more than a century [25]. Over the last several years the work has intensified. A number of scientists undertook investigation into the successive stages of the development of coronary vessels and their morphology as well as clarification of the mechanisms which regulate this process. The research seems to be focused on coronary arteries [18]: nevertheless, the vein network has also been analysed [10]. Over the recent years the lymphatic system has been a subject of comprehensive study [17, 23, 31].

Ischaemic heart disease is now a major problem. A sudden occlusion of a coronary artery, for instance by a thrombus, which leads to myocardial infarction, might result in heart failure — it is one of the main medical conditions and causes of death. Application of stem cell therapy for the treatment of heart after infarction, as well as in acute and chronic ischaemic conditions is a very promising method [35]. Research on cardiosphere-derived cells has been conducted

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for a few years and it keeps bringing information and increasing possibilities to exploit the cells for the treatment of the cardiac muscle [9, 34], which could restore cardiomyocyte function in the area of post-infarction fibrosis [29]. In these attempts, which are preliminary experiments before pre-clinical research for the application of cell therapy, it is essential to possess the knowledge of the topography and distribution of coronary artery branches in normal heart as targeted administration of cells/medications has a direct influence on the effectiveness of the therapy.

Detailed understanding of normal topography of coronary arteries, the number and course of their branches, as well as the area of the heart which is vascularised by them, constitutes the basic knowledge necessary for further research in this field. It is also essential for identification and characterisation of emerging abnormalities of the course of coronary arteries since it is a reference point in normal conditions. The knowledge of the topography of mouse coronary vessels, which are now one of the most frequently used animal models before further investigation into human disease, makes it possible to induce experimental myocardial infarctions by occlusion of a selected coronary artery [2]. It serves as a tool for the measurement of the area of ischaemia and as a result, provides grounds for development of methods which would assist the rebuilding of the cardiac muscle and coronary vessels of the area. The knowledge of the course of the coronary arteries and the existing diversion from the normal state makes it possible to locate the target where stem cells should be administered/transplanted to rebuild the myocardium. Our team has conducted an analysis of the coronary arterial system of MIZZ mice to provide grounds for continuation of experiments on this strain.

The objective of the study was to analyse the location (course) of coronary arteries in MIZZ mice of both sexes and identify the number and location of their branches.

MATERIALS AND METHODS

The material consisted of 28 hearts of adult MIZZ mice (9 males, 18 females and one individual of unidentified sex). The animals were kept in cages in a specially adapted room with access to food and water *ad libitum*. All animals used in the experiment were treated according to the guidelines of the National Ethics Committee for Animal Research. The mice were euthanised with (inhaled) Narcotan and then injected intraperitoneally with chloral hydrate (100 mg/kg). Then the heart and right caudal vein were exposed by means of thoracotomy and rinsed with physiological saline containing 1% papaverine. Blue latex dye was administered via the vein and red latex dye via the aorta. Next the animals were immersed in 4% formalin for 3 weeks. In order to analyse the coronary arteries, which were located intramurally in the mice, it was necessary to remove the external layer of the myocardium. The topography of the arteries in the examined hearts was analysed under a stereomicroscope connected to a camera and photographic documentation was systematically collected. The arteries were isolated with microsurgical instruments.

Haematoxylin and eosin (H&E) stained sections of tissue were also analysed. The examined hearts were isolated from adult individuals of MIZZ strain. The material fixed in 4% formalin was subsequently processed for paraffin embedding. Paraffin sections were cut, deparaffinised and stained in Mayer's H&E. The stained sections were scanned with a Hamamatsu scanner and images were processed to select pictures and take coronary artery diameter measurements. The measurements were taken at various distances from the ostia of both coronary arteries in the upper half of the hearts.

RESULTS

The location and course of the coronary arteries were comparable in the hearts of all examined mice (of MIZZ strain), regardless of sex. Two main arteries were observed in the examined hearts: the right coronary artery (RCA; *arteria coronaria dextra*) and the left coronary artery (LCA; *arteria coronaria sinistra*). Each examined heart had the same number of the main arteries. RCA delivers blood to the wall of the right ventricle (RV) while LCA vascularises the wall of the left ventricle (LV). In almost all examined hearts blood was delivered to the interventricular septum by a branch of the RCA. It can be concluded from the location of the branches of the main coronary arteries that they supplied blood to the same area of the heart in all examined samples. The common trait observed in all the hearts was an intramural course of these vessels with some exceptions, in which the parts of the septum branch arising from the RCA were situated near the endocardium. The differences were connected with the location of their ostia, number of branches and the locations where these

Table 1. Division into three groups depending on the location of the main bifurcation of right coronary artery (RCA)

Location of the main bifurcation of RCA	1st group — next to the ostium	2nd group — at mid-height of right ventricle	3rd group — 1/3 of the lower height right ventricle
Number of examined hearts	2 hearts	13 hearts	13 hearts

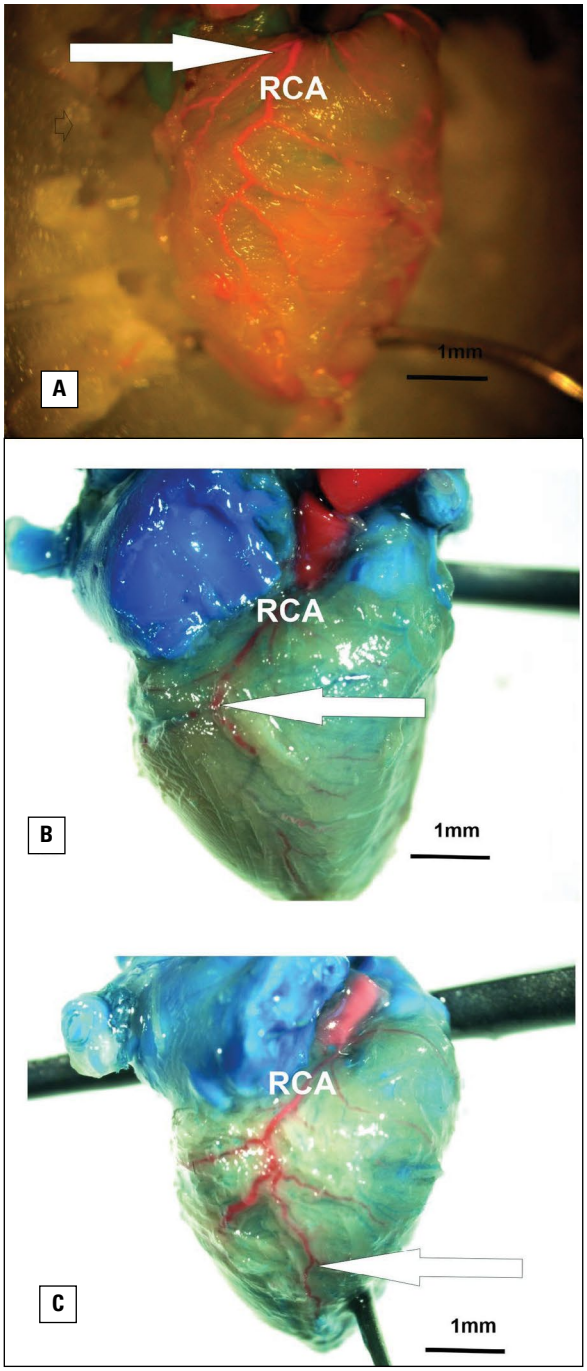

Figure 1. Bifurcation of the right coronary artery (RCA) into two main branches: **A.** 1st group — next to the ostium in the aorta; **B.** 2nd group — at the mid-height of right ventricle; **C.** 3rd group — at the 2/3 of the distance from the ostium; scale bar 1 mm.

branches formed, i.e. the distance from the ostium. In some hearts, the artery of the interventricular septum, which originated from the LCA, was observed. The main differences observed in the course of the study were connected with the ostia of coronary arteries, location of their main branches, as well as emergence of an additional septal artery in a few cases.

Right coronary artery (RCA; *arteria coronaria dextra*)

The RCA is a big blood vessel of a large diameter (93.4 μm on average) and with a few branches. The vessel arose directly from the right sinus of the ascending aorta in all examined hearts. Right next to the ostium, its course was parallel to the right atrio-ventricular sulcus, then it crossed to the lateral portion of the RV and headed for the apex. The RCA runs in the myocardium below big veins — the right conal vein (*vena dextra coni arteriosi*), the right coronary vein (*vena cordis dextra*), caudal coronary veins (*venae cordis caudales*), which can be seen on the heart surface. Along its course, RCA first drains its branch to the interventricular septum and then diverges into two main branches and many smaller ones. Depending on the place of the main bifurcation of RCA, three groups were distinguished (Table 1). In two examined hearts the main branching of the artery was observed in close proximity of the ostium in the right sinus of the aorta (1st group) (Fig. 1A). In the second group (13 hearts) the main divergence was located at the mid-height of the RV (Fig. 1B). In the 3rd group (13 hearts) the RCA divided into two branches at 2/3 of the distance from the ostium of RCA (Fig. 1C).

The branch of the RCA could be described as the septal artery (SA; *arteria septalis*). It branches off into the interventricular septum and is a big vessel of a large diameter (78.02 μm on average). It has a low number of small branches. This vessel originated directly from the RCA next to the coronary ostium in the right sinus of the ascending aorta (Fig. 2A) in most examined hearts. In four cases, the ostium of the interventricular branch overlapped with the ostium of RCA in the right sinus of the aorta (Fig. 2B). In two hearts, the analysed artery originated from the aorta completely separately, in the vicinity of the ostium of the RCA (Table 2).

The SA runs along the septal surface subendocardially and then penetrates its myocardium (Fig. 3). The location and the course of the SA indicate that the vessel is responsible for vascularization of the interventricular septum (Fig. 4).

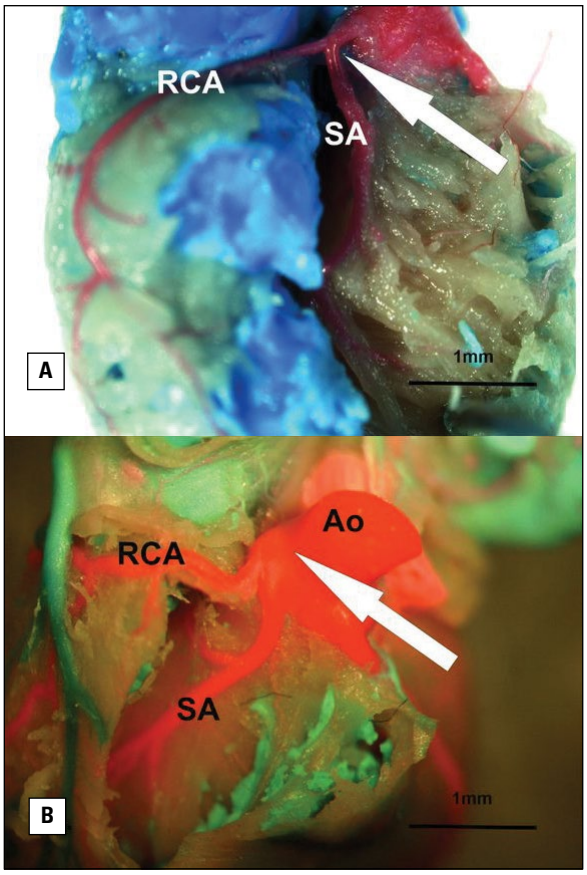

Figure 2. Origin of the septal artery (SA): **A.** Arising directly from right coronary artery (RCA); **B.** Arising together with RCA from the right sinus of the aorta (Ao); scale bar 1 mm.

Table 2. Location of the ostium of the interventricular branch originating from the right coronary artery (RCA)

Ostium of the septal branch	Directly from RCA arising from the aorta	Arising together with RCA from the right sinus of the aorta	Arising directly from the right sinus of the aorta
Number of examined hearts	22 hearts	4 hearts	2 hearts

Apart from the main bifurcation and the SA, the RCA had many smaller branches, which covered the whole surface of the RV. The topography of these branches implies that RCA, together with smaller arteries which arise from it, is responsible for supplying blood (vascularisation, blood delivery) to the whole area of the RV. Among the numerous branches of RCA, fine atrial branches of small diameters, which originated from RCA and ran towards the auricle of the right atrium, drew particular attention (Fig. 5).

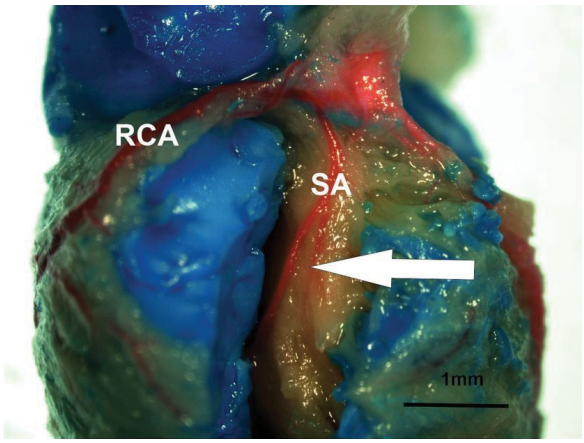

Figure 3. The right septal branch; scale bar 1 mm; RCA — right coronary artery; SA — septal artery.

These small arteries supplying blood to the auricle of the right atrium were observed in 9 hearts.

Left coronary artery (LCA; *arteria coronaria sinistra*)

The LCA, is, like the RCA, a big blood vessel (diameter of 87.8 μm). It has a few branches along its course. LCA arises from the left sinus of the ascending aorta, turns down and initially runs on the left of the arterial conus, then near the interventricular sulcus on the wall of the LV and reaches the apex. The LCA runs intraseptally, under a thin layer of the myocardium. In all the examined hearts, the beginning of LCA and its proximal part ran below the left conal vein (*vena sinistra coni arteriosi*).

The main trunk of the LCA has its ostium in the left sinus of the aorta and runs along the LV as far as the apex. The first branch of the LCA is a vessel which turns right and runs along the upper half of the heart to the edge of the LV. The oblique branch originated from the main artery and ran along the upper surface of the LV. Further on, another branch was a vessel running to the left and obliquely across the LV (Fig. 6A, B). Each of these branches had short vessels which originated from them, usually turned obliquely down in the wall of the LV, and vascularised that particular portion of the myocardium. No vessel equivalent to the course and topography of the human diagonal artery was observed.

In a few examined hearts additional branches were observed, they originated from the initial part of the LCA. In 2 hearts a fine branch arising from LCA and circling the arterial conus was found (Fig. 7). In 1 heart an artery originated from the aorta near the

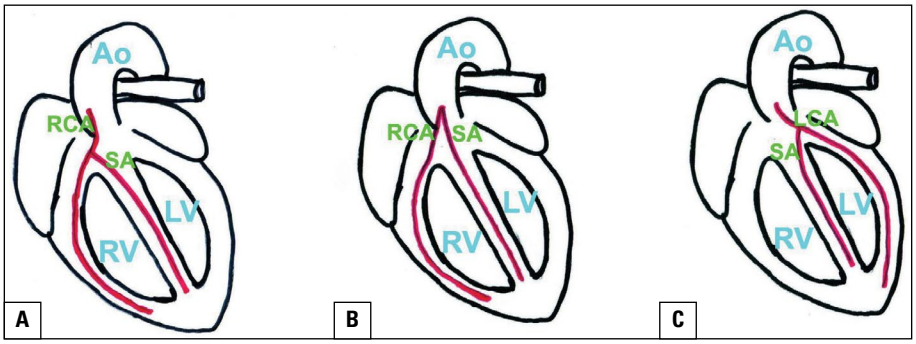

Figure 4. Schematic representation of the ostium and course of the septal artery (SA): **A.** Ostium of SA in the right coronary artery (RCA); **B.** Overlapping ostia of SA and RCA in the ascending aorta; **C.** Ostium of SA in the left coronary artery (LCA); Ao — aorta, RV — right ventricle, LV — left ventricle.

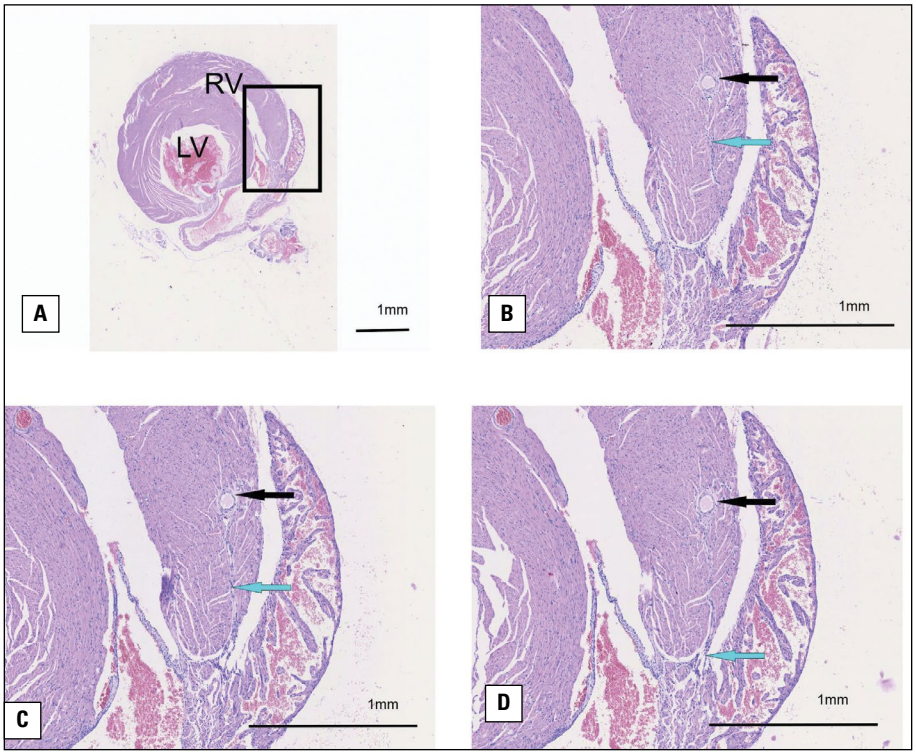

Figure 5. Transverse sections of the heart (H&E stain) with the right coronary artery and its atrial branch; sections cut at the ½ length of the heart between the base and the apex; **A.** Heart: LV — left ventricle, RV — right ventricle; the area marked in the box is enlarged in **B**, **C**, and **D**, which are consecutive sections to demonstrate the atrial branch topography; **B**, **C**, **D.** Transverse section of the right coronary artery (black arrows), longitudinal section of the atrial branch (blue arrows); scale bar 1 mm.

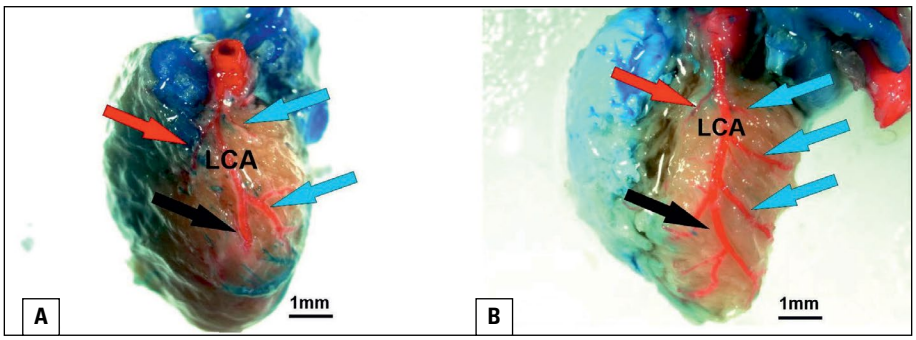

Figure 6. The left coronary artery (LCA) and its main branches; **A, B.** Main trunk (black arrows), right branches (red arrows), oblique branches (blue arrows); scale bar 1 mm.

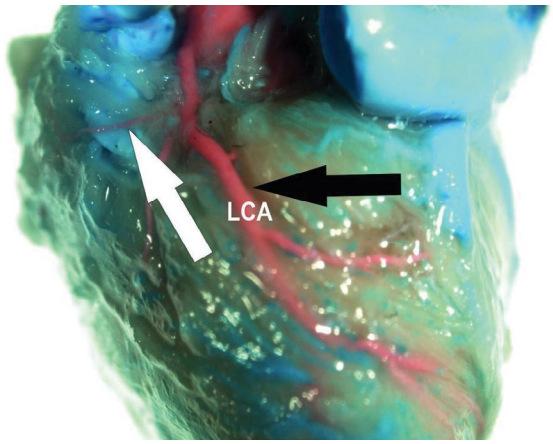

Figure 7. Branch (white arrow) arising from left coronary artery (LCA) (black arrow) and circling the arterial conus; scale bar 1 mm.

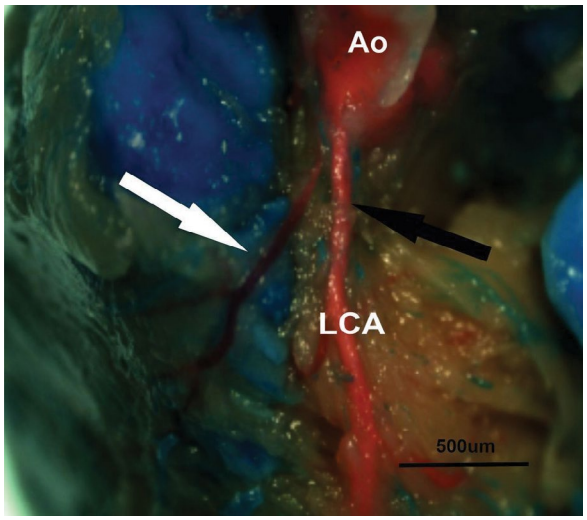

Figure 8. Branch of the left coronary artery (LCA; white arrow), running intramurally near LCA (black arrow); scale bar 500 μm; Ao — aorta.

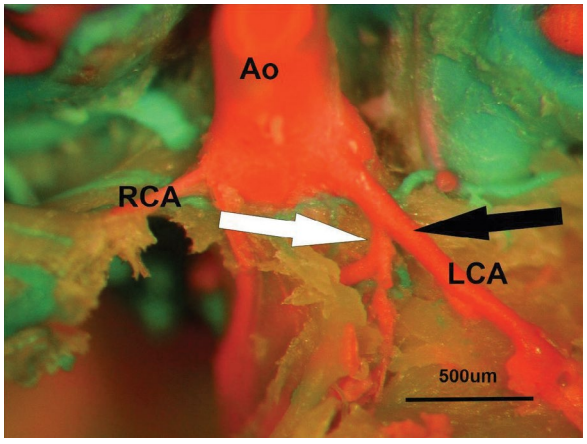

Figure 9. Branch (white arrow) penetrating the interventricular septum, which originates from left coronary artery (LCA) (black arrow); scale bar 500 μm; Ao — aorta; RCA — right coronary artery.

LCA. It ran intraseptally near LCA (Fig. 8). While in the case of the RCA there was always a septal branch or a separate right artery of the interventricular septum, such a SA associated with the LCA was only noticed in 3 out of the 28 examined hearts (Figs. 9, 10). In another 2 cases, a separate artery running close to LCA was observed. It had the ostium in the aorta near LCA on its right and it turned into the interventricular septum, but it did so higher than the branch issuing from RCA. It seems that if the septal branch arising from LCA is present, it supplies the upper part of the interventricular septum. The right SA vascularises the lower half of the septum as it penetrates it at this height.

DISCUSSION

In MIZZ mice the heart is supplied with blood by two main coronary vessels: the right and left coronary arteries. The ostia of these vessels are always located in the right and left sinuses of the aorta, respectively. After arising from the sinuses, the coronary arteries turn towards the apex and each divides into branches which penetrate the myocardium in such a manner that the walls of the ventricles and of the interventricular septum are properly vascularised. The number of branches and the locations where these branches arise differ between the two coronary arteries. The interventricular septum is usually vascularised by a large branch of the RCA, which is described as the SA. The course of the coronary arteries and their branches is intramural. These vessels penetrate the myocardium throughout their lengths. This general topography of coronary vessels is common for both mice and some other rodents [1, 14]. It should be remembered, however, that there is a number of differences between different mouse strains [15, 21, 28]. These differences are connected with the location and shape of the ostia of the main coronary arteries, the number of septal arteries, the number of branches or the area which is supplied by particular arteries. In the case of MIZZ mice, the ostia of the two main arteries, the RCA and the left one, were always located in the sinuses of the aorta. These vessels supplied blood to the RV and LV, respectively. Most differences, both in terms of the location of the ostium and the number of vessels, were associated with the SA. In most examined animals (22 mice), SA branched off the RCA and only in 2 hearts it had a separate ostium in the sinus of the aorta. In the remaining four individuals, SA originated from the right

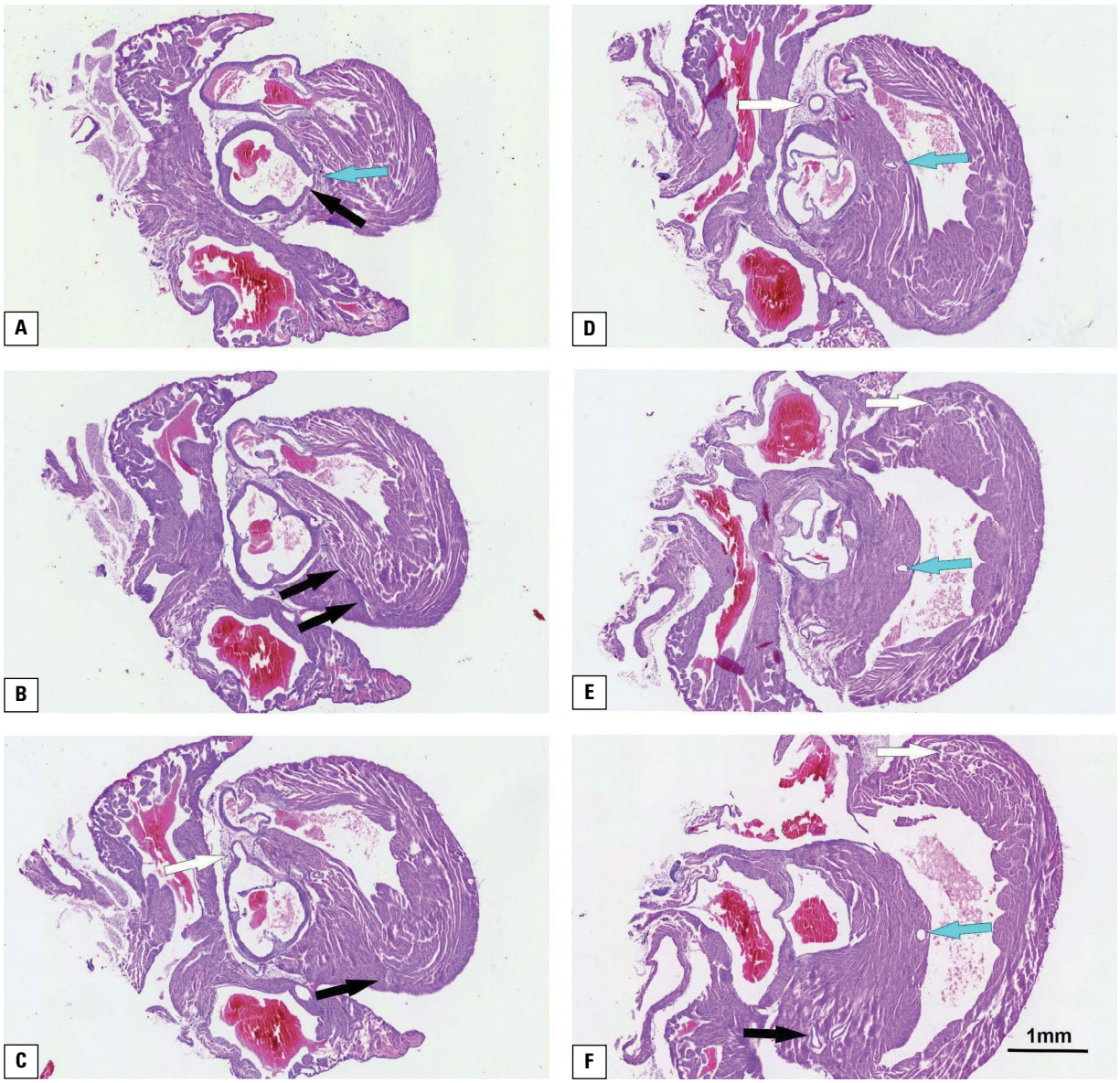

Figure 10. Transverse sections of the heart (H&E stain) with coronary arteries: **A.** The left coronary artery (LCA; black arrow) arising from the ascending aorta and the septal branch (blue arrow) arising from LCA; section cut at the level of the aortic and pulmonary trunk roots; **B.** Longitudinal section of the LCA (marked with black arrows) cut at a small distance from the aortic roots (300–500 μm from the level of cutting section A); **C.** Ostium of the right coronary artery (RCA; white arrow) from the ascending aorta, lumen of RCA (black arrow) visible at a certain distance from the level of cutting section B (300–400 μm); **D, E, F.** Transverse sections of coronary arteries (the white arrow pointing to LCA on section D, E, F, and the black arrow pointing RCA on section F) and septal branch (marked with blue arrows in D, E, and F); scale bar 1 mm.

sinus of the aorta together with RCA. A comparable topography of the SA was described in the wild-living house mouse [14] and in the Swiss albino strain of the laboratory mouse [21]. Kolesova's team [24], who worked with Cx40:GFP knock-in mice, showed in their publication of 2018 that the SA in these animals originated exclusively from the RCA. They did not observe any case in which the vessel would arise from another place. In our research, five out of the 28 analysed hearts had additional vascularisation of the interventricular septum in form of a branch of LCA (3 hearts) which penetrated the myocardium of the septum or a separate artery (2 hearts) branching off the aorta and running close to LCA. Such double vascularisation of the interventricular septum and the place of penetration of the septal arteries into the myocardium indicate that the septal branch of RCA supplies blood to the upper part of the septum while the branch issuing from the LCA supplies the lower portion. The existence of the SA originating from the

LCA was described in two models of mutated mice: iv/iv mice [21] and Cx43 knockout mice [11, 26]. The existence of the SA branching off LCA and co-existence of two septal vessels in C57BL/6J mice, reported in 2004 [32] was confirmed in more recent papers [15]. Apart from that, Fernandez and his team described the same topography in hearts of C57BL/6N mice. The existence of two septal arteries is also common in other rodent species, for instance Syrian hamster (Mesocricetus auratus) [14, 33]. Anatomic origin of the SA seems rather changeable and depends on the strain and analysed population of animals. These results are particularly important in the context of experimental research on heart ischaemia in the mouse model. The occlusion of the coronary artery is usually performed by ligation of the proximal part of the LCA [7, 20, 22, 30]. For this reason, the existence of a SA branching off the LCA below the place of ligation might definitely lead to an expansion of the ischaemic area. The changeable number of septal arteries is connected with different locations of the ostia of these vessels. In our experiments, in most cases (22 hearts), the SA originated directly from the RCA. On the other hand, in 4 hearts the ostium of the septal vessel was located in the right sinus of the aorta, overlapping with the ostium of RCA. In 2 cases, the SA originated in the wall of the aorta, near the right sinus. If the interventricular septum was vascularised by 2 arteries, the left septal artery originated from the LCA or directly from the aorta.

The arrangement of coronary arteries in mice differs from the one in humans. Except for the right and left coronary arteries, we do not use the terminology associated with human coronary topography in order to avoid suggestion of particular correspondence between the species. Thus, there are no branches identical to the human circumflex artery, left anterior descending (LAD; anterior interventricular), or right posterior descending (RPD; posterior interventricular). Another difference can be found in the arrangement of the blood supply to the interventricular septum. In humans there are a number of septal branches originating from LAD and RPD while in mice there is one SA, which may be considered an independent third coronary artery due to its diameter and area supplied with blood. The arrangement of coronary arterial system in mice, with an intramyocardial course is characteristic for small animals with a very high heart rate. Nevertheless, it is necessary to pay attention to the number of ostia in the aorta. In humans [6] and other mammals [15, 16] the existence of more than one arterial ostium in the sinus of the aorta is not regarded as an anomaly. Such topography is normal and does not involve a risk of coronary disease. On the other hand, patients with a single ostium in the aorta are exposed to a risk of ischaemic heart disease and sudden death [4, 5, 8, 27]. Ischaemia is caused by constriction of the coronary artery trunk, which might occur when the proximal part of the vessel runs between the aortal and pulmonary trunks. In humans such a condition [12, 19] is very rare (0.04–0.66%) and is a serious pathology. In mice, a single coronary ostium in the sinus of the aorta is more common than in humans, although there are strains, such as the one researched by our team — MIZZ, in which this anomaly has not been observed. In C57BL/6 such topography is found in approx. 6% of examined cases [15, 28]. Such a single ostium in the aorta has been observed in different rodent species, including the house mouse [3, 13]. Although such arterial topography is regarded as an anomaly, it does not affect the functioning of the coronary system and does not cause heart ischaemia in rodents. It probably results from the fact that coronary arteries branching off the aorta do not run between the pulmonary and aortal trunks but penetrate the myocardium of the ventricular walls and interventricular septum. This topography, characterised by intramural course of coronary arteries, is common for all examined rodents, and as a consequence, the single coronary ostium in the aorta does not lead to the development of the disease in these animals or pose a threat to their lives.

CONCLUSIONS

Summing up, it could be concluded that different strains of mice display a number of similarities in the topography of coronary arteries. There are always two main arteries: the right and the left coronary artery. Each of them usually originates from the corresponding sinus of the aorta. The interventricular septum has its own artery, which usually branches off RCA. The course of coronary vessels is intramural. Researchers of the anatomy, development and physiology of coronary arteries usually use mice as an animal model and for this reason they should be aware of these similarities as well as the differences in the topography of the artery system between the

different strains since they might lead to incorrect interpretations of their results.

Acknowledgements

This study was supported by internal funds of Medical University of Warsaw (1M11/N1) and of Warsaw University of Life Sciences (505 20 023300--Q00226-99).

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