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EPOXY-ACID CORROSION CAST METHOD FOR MACRO ANATOMICAL ASPECTS OF THE CORONARY ARTERIES IN DONKEYS (*EQUUS ASINUS* L.)

ASPECTE ANATOMICE ALE ARTERELOR CORONARE LA MĂGAR (*EQUUS ASINUS* L.) EVIDENȚIATE CU RĂȘINĂ EPOXIDICĂ PRIN METODA DE COROZIUNE ACIDĂ

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ABSTRACT | **REZUMAT**

The paper describes a simple, cheap and handy method for the study of the vascular anatomy of the coronary arteries by using a common industrial product-reisin, combined with a corrosion procedure with hydrochloric acid. Using this method, a good evidentiation of the morphology of the coronary arteries was obtained, with some particular minor morphological features not described by the speciality literature.

Keywords: coronary arteries, donkey, corrosion-cast, epoxidic

Lucrarea propune o metodă simplă, ieftină și ușor aplicabilă de studiu a anatomiei arterelor coronare prin utilizarea unei rășini de uz industrial, disponibilă în comerț,combinată cu o procedură de coroziune cu acid clorhidric. Prin utilizarea acestei metode s-a reușit o bună evidențiere a arterelor coronare la specimenele studiate, fiind întâlnite chiar cateva aspect morfologice mai puțin abordate de literatura de specialitate pentru această specie.

Cuvinte cheie: artere coronare, măgar, coroziune, epoxi

Corrosion casting is a technique used in anatomical studies that use a mix of liquid chemicals (polymers) that have, under certain conditions, the chemical feature that allows solidification. Enclosed hollow anatomical entities may be filled with these liquids, solidify and create the negative of the cavity, while the surrounding tissue is removed employing different methods (e.g., mechanical, chemical, and enzymatic) (1, 2, 27). First attempts for casting corrosion techniques were made by Leonardo Davinci for the brain ventricles using bee wax (10). Methods evolved in time, different chemicals or substances being used in more or less conventional ways (e.g., different melted metals and pine resin) culminating with the new generation casting media based on the polymerization of natural or synthetic resins (1, 2).

Studies of the blood vessels were initiated by Alessandra Giliani in the human anatomy that used this new method for the visualization of the blood vessels using a colored mixture that once injected into the vascular elements was hardening, allowing a good preparation and highlighting of the vascular pathways and distribution (13). With the significant development of the chemical industry, nowadays the corrosion cast method was widely improved, new materials, such as different gelatins, epoxy resins, latex, polyester raisins, methyl meta acrylate or polyurethane derivates being used in different recipes in various fields, including anatomical studies (10).

An important element in the choice of the method should be represented by a good balance between the complexity of the method (choice of medium), the scope of the study, size of the specimen, and, one of the most important aspects, the financial one (10).

The corrosion cast technique in the case of coronary arteries was frequently used for anatomical studies in humans (1, 19, 27) and in animals in goat (6), rat (24), or dog (8, 9). The coronary arteries are the vessels that, through their collateral branches, distribute the nutrients and oxygenated blood to the myocardium (26).The embryological development of these arteries follows carefully regulated temporal events, such as vasculogenesis, angiogenesis, arteriogenesis, and remodeling, with a large number of regulatory molecules that conduct the processes of transformations of preliminary cells to establishing the coronary vascular tree (28). The post-partum architecture of vessels is widely discussed in specialty literature, a kind of a general pattern of distribution of the coronary arteries stating the existence of three general distribution models: species where the left coronary distribution is prevailing (as in dog and ruminants), a bilateral one (humans, horse, swine, and rabbit) and an intermediary one (mentioned in cats) (12, 13).

The present study aims to highlight the distribution

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of the coronary arteries in donkey utilizing a simple, cheap method of corrosion casting using commercial epoxy resin and a common red dye, with a chemical hydrochloric acid 10% digestion.

MATERIALS AND METHODS

The hearts were belonging to five adult donkeys (*Equus asinus*) of both sexes, originating from individuals. The material was harvested in 9 months during the years 2019-2020.

The hearts were carefully collected after the thorax opening, to protect the base of the heart with the main vessels being sectioned in such a manner to prevent rupture of the main arteries and veins so they are available for further anatomical investigation.

A thorough washing procedure was performed initially, so a good clearance of the aorta and coronary arteries was obtained. Saline solution and a small quantity of formaldehyde were used to prevent further clothing in the smaller arteries (coronary arteries). A calibrated catheter (18G) was used for the correct identification and approach of the coronary arteries at the level of the Valsalva's sinus.

The substance used for the anatomical study that was injected into the coronary arteries consisted of a commonly-used mixture for hobby and construction work, available in specialized shops. It comprised of a low-viscosity epoxy resin and the corresponding hardener/catalyst (Megapoxy H resin, Vivacity Engineering PTY LTD, Australia) that was mixed before the injection. A red dye (red acrylic paint) was also used, so the mixture becomes highly visible. The proportions for resin: hardener were, as suggested by the producers to a 2:1 ratio. The mixture was slowly prepared at room temperature (limiting the air mixing with the substance) to ensure a standard pot-time of few minutes until the debut of the hardening process.

The colored liquid was slowly and carefully injected into the coronary arteries to prevent possible rupture of the smaller arteries because of the pressure. An approximate quantity of 20 ccm was injected into each of the arteries, followed by a ligature made on the end part of each coronary artery origin. The organ was left suspended until the complete hardening of the material. Once sure that the curing and hardening process was finalized, after few hours (usually from one day to the next day), the specimens were furthermore processed for the second phase of the preparation (corrosion). A brief mechanical dissection was performed at the level of the base of the heart, followed by the preparation of the corrosive mixture consisting of 15-20% hydrochloric acid.

The pieces were immersed in this acid solution in laboratory glassware adapted to the size and volume of the specimens. Generally, we used 3-5 l wide recipients that fitted well the anatomic specimen and the surrounding corrosive agent. The pieces were left for several days, with a careful check on the degree of desolvation of the organic material. The remnants of material were washed away with fine water jets (tap water) until a satisfactory level of cleanliness. The cast was then dried at room temperature and then photographed and used for the anatomical description.

Overall, the process of maceration lasted about 2 weeks.

RESULTS AND DISCUSSION

In all studied cases, the presence of both the left coronary artery (*A. coronaria sinistra*) and right coronary (*A. coronaria dextra*) artery was noted. These two main branches originate at the level of the aortic bulb (*Sinus aortae*), a little above the semilunar cusps.

Both right and left coronary arteries may be considered as collateral branches of the ascending aorta (*A. aorta ascendens*). These branches divide terminally into an interventricular branch and a circumflex one.

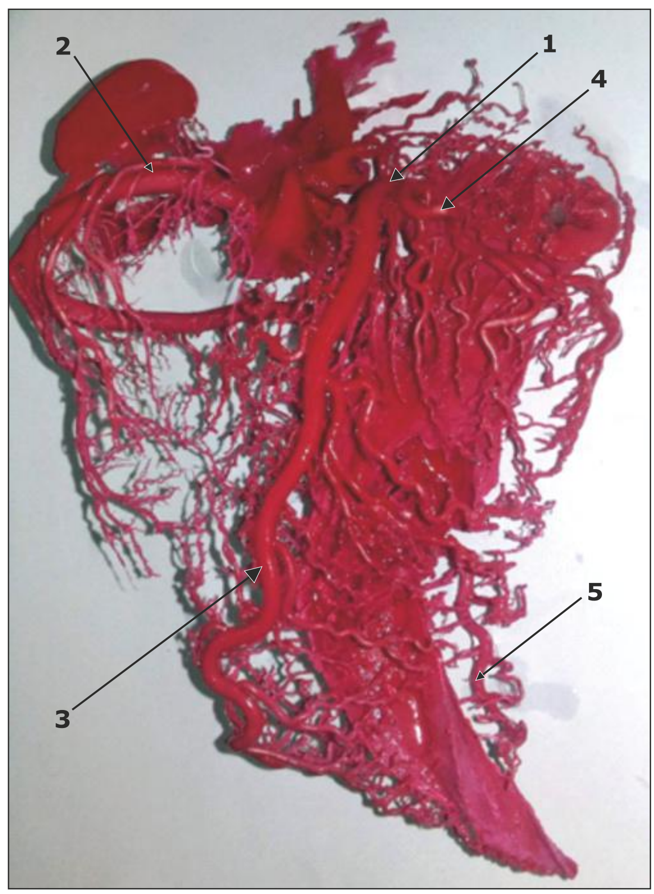

Fig. 1. Coronary arteries in donkey
1. Artera coronaria sinistra 2. Artera coronaria dextra; 3.Ramus interventricularis paraconalis ;
4. Ramus circumflexus sinister;
5. Ramus interventricularis dextra

The left coronary artery (*arteria coronaria sinistra*)

The left coronary artery (Fig 1.) (*arteria coronaria sinistra*) follows a mediolateral course towards the auricular surface (*Facies auricularis*) of the heart into the coronary groove (*Sulcus coronarius*). Small auricular branches –*rami auriculari*- (left auricle) were noted as detaching from this initial trajectory. Shortly after, the trunk divides then into a strong paraconal interventricular branch (*Ramus interventricularis paraconalis*) and a weaker left circumflex branch (*Ramus circumflexus sinister*).

The left interventricular branch (*Ramus interventricularis paraconalis*)

Fig. 2. The left interventricular artery in donkey
1. Ramus interventricularis paraconalis;
2. Ramus coni arteriosus; 3. Cranial ventricular branches; 4. Ramus collateralis sinister proximalis; 5. Dorsal branch; 6. Ventral branch; 7. Middle left collateral ventricular branch; 8.Distal left collateral ventricular branch; 9. Septal branch

On the examined pieces, we noted that the thicker left interventricular branch (*Ramus interventricularis paraconalis*) (Fig. 2.) follows a descending path towards the apical part of the heart (*apex cordis*). In its initial part, the artery has a straighter aspect, to become more and more flexuous in the proximity of the apex of the heart. The artery will give off collaterals in ventro-cranial direction for the wall of the right ventricle and some in dorso-caudal direction for the wall of the left ventricle. In all studied specimens, the first significant branch stems immediatly under the coronary groove in ventro-cranial direction to distribute in the area of the arterial cone (*ramus coni arteriosus*) to the ventricular wall. Other significantly large branches stem similarly, heading towards the auricular surface of the right ventricular wall. The first collateral branch (the largest of them) originates ventrally to the *ramus coni arteriosi* - the left proximal caudal ventricular branch (*ramus collateralis sinister proximalis*) that shortly after splits terminally into a dorsal and ventral branch in caudal direction. The second collateral branch (smaller in size) stems in the middle part of the main trajectory of the main branch, to distribute into the middle area of left ventricular wall- the left middle ventricular collateral branch. Last branch – the left distal collateral ventricular branch (*ramus collateralis sinister distalis*) targets the distal part of the ventricular wall. At the entire extent of the left interventricular branch, the presence of reduced septal branches was noted (*rami septales*).

The left circumphlex branch (*Ramus circumflexus sinister*)

Fig. 3. The left circumflex artery
1. Left circumflex artery; 2. Auricular branches; 3.Ventricular branches; 4. Superficial branches; 5. Deep branches; 6.Branches for the coronary groove

The second terminal branch (half the diamenter of the main paraconal artery), the left circumflex branch (*Ramus circumflexus sinister)* (Fig. 3) follows a medio

-lateral trajectory into the coronary groove (*sulcus coronaris*) towards the dorso-caudal border of the organ (*ramus marginis concavi*). We noticed that this smaller artery emits branches in dorsal direction to the left auricle (*ramus auricula sinistra*) and in ventral direction that extend and terminate into the ventricular wall. The auricular branches seem to be quite diverse in distribution, for the studied specimens, variations of very reduced (small) branches to larger ones, as approaching the terminal branching of the main artery being recorded for different individuals. These branches supply the dorsal auricular wall and the basal part of the heart. The ventricular branches seemed slightly larger when compared to the auricular ones.

After the circumflex artery detachment, the ventricular branches follow in ventro-caudal direction. There is a short trajectory sector that is terminally divided soon, into a deep and a superficial branch. Short, fine branches (reduced as caliber) that detach in the direction of the atrio-ventricular groove tissue were also observed. We noted the presence of other fine branches, situated more deeply, that seem to supply the proximal part of the left ventricular wall (Fig. 3).

The terminal part of the left circumflex artery (*ramus marginis concavi*) follows the dorso-caudal border of the heart, not showing visible anastomotic branches with the right circumflex branch.

The right coronary artery (*arteria coronaria dextra*)

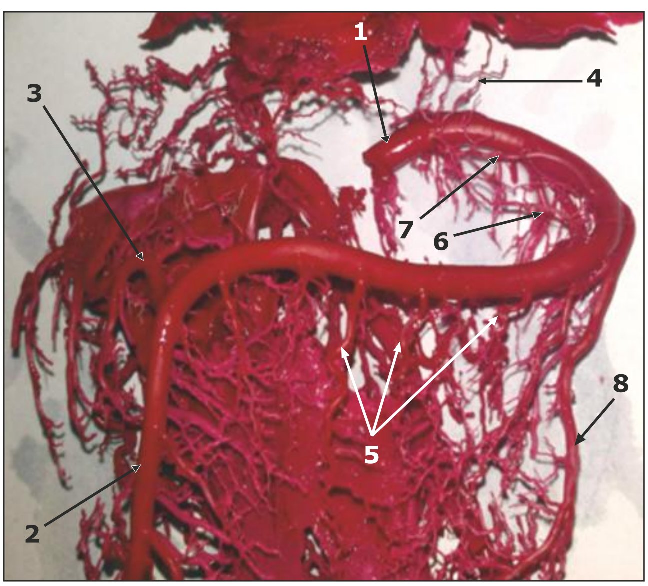

Fig. 4. Right coronary artery
1. Right coronary artery; 2. Right interventricular artery; 3. Right circumphlex artery; 4. Auricular branches; 5. Ventricular branches; 6. Branches for the coronary groove and the atrial wall; 7. Innominate branch; 8. Branch for the arterial cone

The right coronary artery (*a.coronaria dextra*) (Fig. 4) appeared as a much longer branch when compared with the left one. From its origin, at the level of *valvula semilunaris dextra*, it heads in the direction of the coronary groove (*sulcus coronarius*) and follows it to the atrial cardiac surface (*facies atrialis*).

At the level of the atrial cardiac surface, close to the right interventricular groove (*sulcus interventricularis subsinuosus*), the artery divides in all studied specimens, into a significant (as far as caliber is concerned) right interventricular artery (*a. interventricularis subsinuosus*) and a circumflex branch (*ramus circumflexus*) (reduced as caliber).

In its trajectory, one can notice the detachment of branches in three different directions: ascending (targeting the auricular wall), descending (for the proximal ventricular wall) and deep ones (for the atrial wall and the atrio-ventricular groove related areas). The first two sets of branches embrace the projection area of the arterial cone (Fig. 4).

In one of the studied individuals, a reduced innominate branch that detaches close to the origin of the main artery, from the deep part of its wall, passes along the coronary groove structures to rejoin the main trunk of the artery close to the splitting point into the 2 terminals.

The right interventricular artery (Fig 5) (*a. interventricularis subsinuosus*) follows a descending pathway into the right interventricular groove (*Sulcus interventricularis subsinuosus*) reaching the apical part of the heart. Its initial part appears quite straight, but as reaching the apical part, the course becomes more and more flexuous. The artery, in its main course, emits smaller branches that distribute to the ventricular and septal parts of the organ. The first branch to mention is a proximal right cranial ventricular branch (*ramus proximalis ventriculii dextri*) that gets distributed into the atrial surface of the right ventricular mass. Ventrally to this, a slightly larger vessel distributes also into the atrial surface of the ventricle – middle right cranial ventricular branch (*ramus marginis*)**,** followed by another very close branch - the distal right cranial ventricular branch (*ramus distalis ventriculi dextri*)**.** Opposite to these three branches, another set of three branches detach in caudal direction might be noticed. The afoorded mentioned septal branches (*rami septales*), seem denser than the ones from the left interventricular artery.

Terminally, the artery ramifies into the apical part of the heart and also on the ventricular parts of both of the ventricles.

The right circumflex artery (Fig. 6) (*ramus circumflexus*) arises, in all studied specimens, at the level of the right interventricular wall, from the main trunk of the right coronary artery. The artery heads in dorso-medial and caudal direction, following the shape of the coronary groove.

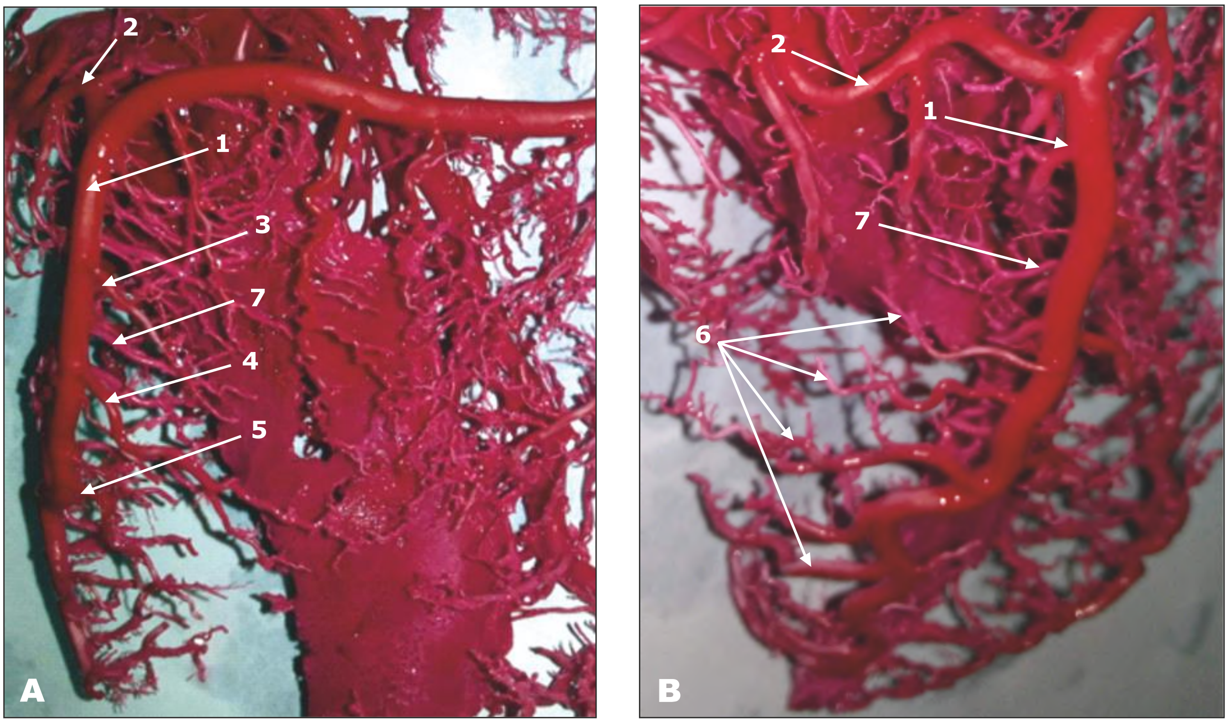

Fig. 5. The right interventricular artery
A. Cranial perspective; B. Caudal perspective.
1. Right interventricular artery; 2. Right circumflex artery;
3. Right cranial proximal ventricular; 4. Right cranial middle ventricular branch;
5. Right cranial distal ventricular branch; 6. Caudal ventricular branches; 7. Septal branches

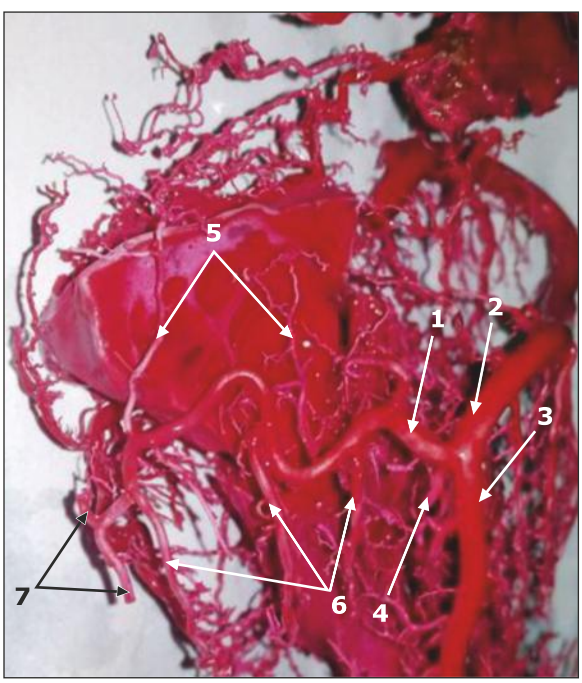

Fig. 6. Right circumflex artery
1. Right circumflex artery; 2. Right coronary artery; 3. Right interventricular artery; 4. Branch for the interventricular septum; 5. Auricular branches; 6. Ventricular branches; 7. Terminal branches of the right circumflex artery

Collateral branches from the right coronary artery stem in the direction of the right auricle - in dorsal direction and for the ventricular wall, in the ventral direction. The first collateral that is emitted at the deep surface of the artery is a branch that targets the interventricular septum. A series of other smaller branches arise in the same area that supplies the entire area of the atrioventricular groove.

The arterial supply of the heart in donkey is ensured using two coronary arteries - the right and left coronary arteries and their collaterals and terminals. Data provided by this study confirm earlier studies made by Ozgel (22).

The left coronary artery detaches from the left Valsalva sinus, above the dorsal border of *valvula semilunaris sinistra*, as a relatively short artery that terminates as the paraconal artery (*ramus interventricularis paraconalis*) and the left circumflex artery (*ramus circumflexus sinister*). A similar situation is cited in the horse (15), bison (16), dog (20), pig (8, 9, 12), goat (6), chinchilla (21), roe deer (14), alpaca (23), and capuchin monkey (17) . A particular situation is cited for the Angora rabbit, where apart from the paraconal and the left circumflex artery, another proximal atrial branch is cited as a third branch of the left coronary artery (6, 23). In dogs, for a reduced number of individuals, the detachment of the left interventricular artery and the left circumflex artery as direct branches from the left Valsalva sinus is cited - the bipartite model (19) but most of the times literature cites the existence of tripartite type of detachment for left coronary arteries (16, 24).

For the paraconal branch, one must notice that the branches for the right ventricle are reduced when compared to the left ones, similar to the fact reported for goat (6), bison (16), and some domestic carnivores (5). The existence of the conal artery is mentioned for donkey (22), Angora rabbit (2), and goat (6). The identification of the three caudal collateral branches for the left ventricular wall (as branches of the paraconal artery) was reported also in the case of bison (16) and goats '–'(6). In the studied species, similarly, with the situation reported for bison (16), the most developed branch was the proximal left caudal collateral while the middle one was the smallest. In the goat, the proximal left caudal collateral is mentioned as a reduced one (16). In regards to the distal trajectory part of the left interventricular artery, the artery showed a flexuous trajectory for all studied individuals, most probably serving as a length reserve as the ventricular volume increases in diastolic time. The septal area vascularisation seems linked to the left interventricular artery, which sends deep septal branches, similar to the perforating arteries described by Sahni (25).

The second terminal branch of the left coronary artery is the left circumflex artery, an artery that has a reduced caliber in comparison with the paraconal artery. Its terminal part shows no anastomotic branches for the right circumflex artery, fact confirmed also in Ozgel's study (22). The same stands for most of the domestic animals listed by classic sources (4) or even the capuchin monkey (17). The artery seems like terminating at the level of the dorso-caudal border of the organ. In the bison, the branch (in 75.5% of the studied individuals) appears like continuing with the interventricular subsinousal artery (16), similarly with 31% of the goat individuals studied (6) capucine monkey (17), dog, and ruminants (4).

The right coronary artery (*a. coronaria dextra*) is longer than the left one. The artery terminates as the right interventricular (*ramus ventricvularis subsinuosus*) and right circumflex arteries (*ramus circumflexus dextra*), as also mentioned in earlier studies (22). In other species like the alpaca, authors mention just the identification of a right interventricular branch, that continues as the single terminal branch of the right coronary artery (23). For the goat, 97% of the individuals show terminal branching of the right coronary artery onto the atrial surface of the heart, with 3% of cases continuing with the right interventricular artery (6). For humans, the homologous artery for the right interventricular branch is called the posterior interventricular artery (25). The artery disperses in a flexuous manner into the apical part of the ventricular wall. From the inner surface of this artery, branches for the ventricular septal part stem out. In this way, we can conclude that the ventricular septum area is supplied by branches that originate into the right and left interventricular branches, similarly with data illustrated in the case of the Swiss albino mice (29), pig (25), horse (4), and dog (19)

CONCLUSIONS

In the case of the studied species (*Equus asinus*), the arterial supply of the heart was easily highlighted by this method and is made through the two coronary arteries- right and left, from which the right one is the most developed. The distribution patterns of the main branches of these arteries are generally similar (with few minor differences) to the data literature available.

Some peculiar morphological aspects were highlighted in the case of the *ramus collateralis sinister proximalis*, the presence of middle collateral detached from *ramus interventricularis paraconalis* and the existence of an innominate branch that connects the initial part of the *a. coronaria dextra*, before splitting terminally.

The used technique highlights these elements of morphology and may serve as a handy, easy, and cheap tool in didactical and scientific approaches.

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