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Anatomical and three-dimensional study of the abdominal vascular system in female rabbit using dissections and ultrasound

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Abstract

Our aim is to provide reference data for the arterial diameter of abdominal arteries using ultrasound in healthy rabbits, which can be used to compare changes in arterial diameter in diseased rabbits or when performing experiments and creating a 3D model of abdominal arteries using casting material. The current study was conducted on ten domestic rabbits aged between 2-3 years and weighing 1-2 kg. Ultrasound examination of the abdominal wall was performed in the supine position. The results showed that the mean hepatic artery diameter for 1-1.5 kg was 0.25±0.04 cm while for 1.5-2 kg it was 0.37±0.07, and the mean renal artery diameter for 1-1.5 kg was 0.20±0.03 cm while for 1.5-2 kg it was 0.25±0.04. The ultrasound results indicated that the diameter of the blood vessels increases with weight gain. Anatomically, the rabbits were divided into two groups: the first group was injected with latex arteries, and the second group was injected with resin casting material. The results showed that the arteries of the first group were flexible. In contrast, the arteries of the second group were rigid and fragile and required more care in handling than the first group. The results indicated that latex injections help in accurate anatomical studies. In contrast, resin injections create a three-dimensional model of the arteries. We conclude that color Doppler ultrasound with a linear probe provides clear and accurate images of blood vessels, allowing doctors to accurately measure their diameter and assess blood flow within the vessels, thus helping to diagnose conditions such as atherosclerosis and arterial disease.

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Introduction

The abdominal vascular system in animals has become the focus of many researchers because it plays a vital role in all physiological functions related to blood supply, nutrition, and hormone delivery. Additionally, it is essential to ensure healthy organ transplantations and vasculature repair in animal models (1), so a thorough understanding of the anatomy and three-dimensional configuration of the vasculature network in experimental animals is necessary (2). The rabbit is one of the most widely used animal models in different research fields, including anatomy, toxicology, pharmaceutical, and biomedical research (3-5). An anatomical study of the vascular system in the abdominal cavity is essential and complex for ultrasound surgical procedures in humans and animals for research that requires an understanding of vascular hemodynamic principles (6,7). Therefore, it is necessary to study the abdominal vascular system in rabbits using dissections and three-dimensional ultrasound (8). Moreover, the three-dimensional configuration of the abdominal vascular system in rabbits has not been reported before (9). A vast abdominal vascular network is formed by the connections made by its veins, arteries, and capillaries to nearby reproductive, urinary, and digestive systems (10). Ultrasonography is a precise, dependable, non-invasive imaging technique without adverse effects (11-13). Therefore, it can be used in Doppler mode to non-invasively assess the shape and geometry of

arterial walls of the abdominal (14) and detect and treat vascular disorders (15). Three-dimensional (3D) architecture, neovascularization (the creation of new blood vessels), and arterial wall elasticity may provide helpful information for risk stratification and early and accurate recognition of vascular disease (16). The latest developments in ultrasound technology include shear wave imaging, voltage-gated ultrasound, contrast-enhanced ultrasound, and 3D ultrasound imaging, which allow for assessing these vascular tissue features (15). Recently, simple and cost-effective techniques have been developed to facilitate practical and in-depth experiments on vascular anatomy (17). This allows researchers to perform comprehensive 3D studies more efficiently and effortlessly (18). Notably, the 3D digital experiment can be successfully performed using a basic optical microscope, making it more accessible to educational and research institutions (19).

The aims of the present study were to conduct a detailed anatomical investigation of the abdominal vascular system in female rabbits using ultrasound and anatomical dissection with colored latex injection and to create a 3D anatomical model using resin injection to identify the differences between arterial injections of the two materials so that we can provide good details of the abdominal arteries and recommendations depending on the users' accessibility to this device. In addition, the methodology and approach used in this study can be easily used to study the vascular system in other animal models.

Materials and methods

Ethical approve

The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study issued at July 2024, numbered VM181. Ten female rabbits were selected at the age of 2-3 years and weighed 1.5-2.0 kg. All rabbits were housed in a sterile animal room, including air conditioning, maintained at a temperature of 23±2ºC and humidity of 65%±5% (20,21).

Ultrasound equipment

Rabbits have one disadvantage: they are difficult to handle during ultrasound imaging, which results in low examination accuracy. Therefore, before the examination, animals were anaesthetized by intramuscular (IM) injection of Xyl-M2 Vmd-Hoge Mauw 900-2370 Arendonk-Belgium (0.10 mg/kg) and ketamine (Sir Aldawa Co. Baghdad, 0.5 mg/kg) (22). The abdominal hair was then carefully shaved, and complete ultrasound examinations were performed on the rabbits using the same ventral approach with the animal lying supine and then examined dorsally, ventrally and laterally by a veterinary sonographer. All images were taken using a Z50Vet ultrasound machine and a linear probe manufactured by Mindray, China in color Doppler mode with frequency: (3.7-7) MHz and (depth: 4.5-6.5), which was maintained and checked regularly. Multiple scanning planes were used to evaluate the significant blood vessels in the abdomen. Image magnification was used continuously and applied in all scans to provide the most accurate measurements (13).

Anatomical examination

The study included two groups. The initial group of five rabbits received Hx-Injection Medium Latex Made in the USA (latex). In comparison, the second group of five rabbits (Self-Curing Powder with Self-Curin) Shanghai New Century Dental Materials (Resin) was injected. All rabbits were killed by injection with a lethal dose of xylazine (0.30 mg/kg) and ketamine (0.15 mg/kg) IM (22). The skin and pectoral muscle were removed, and the sternum was cut with a bone cutter to expose the heart, then the left ventricle was opened. A catheter was inserted to drain the blood. Then 30 cc of Rotting's carmine-stained latex was injected with a syringe to stain the arteries. After 24 h, the sample was preserved in 10% formalin solution, after which the body cavity was opened, and the samples were carefully dissected to study the arteries supplying the abdomen. The results were photographed using a 5-megapixel Sony digital camera (23). The second group, consisting of five samples (rabbits), was injected with 30 cc of red-stained resin via a syringe through the left ventricle and left for 24 hours (24). Then, they were soaked in a hydrochloric acid 30- 33% corrosive substance for 3 days to destroy the tissues and organs surrounding the arteries. After soaking, they were washed with tap water, carefully cleaned, and photographed to obtain the abdominal arterial tree (25).

Statistical analysis

The Statistical Packages of Social Sciences-SPSS program was used to detect the effect of different groups in study parameters. A T-test was used to significantly compare between means in this study (26).

Results

Ultrasonography

In color Doppler ultrasound, we were able to identify the blood vessels. When the pulse was activated, the arteries were confirmed. When the color Doppler was activated, the vein was blue due to the blood flow in the veins towards the linear probe, which caused a decrease in the frequency of the reflected sound waves. At the same time, the artery was red due to the blood flow away from the linear probe, which caused an increase in the frequency of the reflected sound waves. This color distinction helps identify blood flow and diagnose vascular diseases. Our results showed that the blood arteries feeding the abdomen include the portal vein (PV) and hepatic artery (HA) which supply the liver, and renal vein (RV) and renal artery (RA), which provide the kidneys. However, the aorta and some branches were

difficult to detect because they were obscured by the abdominal organs, such as the stomach, intestines and liver, which lie above them. Ultrasound imaging was performed in the upper abdomen in the dorsal position, and the HA and PV were identified. The diameter of HA was also identified where HA was found with a frequency of 6.0MH and a depth of 4.6 (Figure 1 and Table 1). When imaging was performed on both sides of body, in the ventral, dorsal, and lateral positions in the kidney region, the (RA) and (RV) were identified, in addition to that, the arterial diameter of the kidney were measured frequency 6.0MH and depth 3.7 (Figure 2).

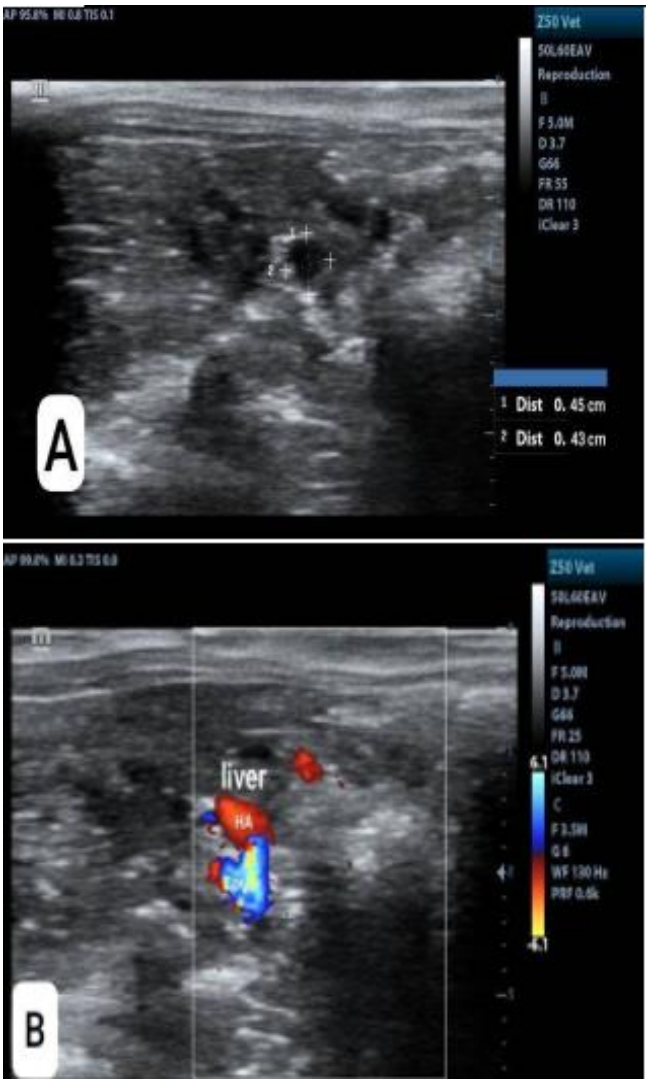

Figure 1: Ultrasound using color Doppler modes of the liver in female rabbits. A- Measurements of the Hepatic artery. B- Color imaging of the HA (red) and the PV (blue).

Table: Comparison between weight with hepatic artery and renal artery diameter

Weight	Hepatic artery diameter (cm)	Renal artery diameter (cm)
1 -1.5 kg	0.25±0.04	0.20±0.03
1.5-2kg	0.37±0.07	0.25±0.04
T-test	0.0739	0.0644
(P-value)	(0.0352) *	(0.0817) ^{NS}

* (P≤0.05), NS: Non-Significant.

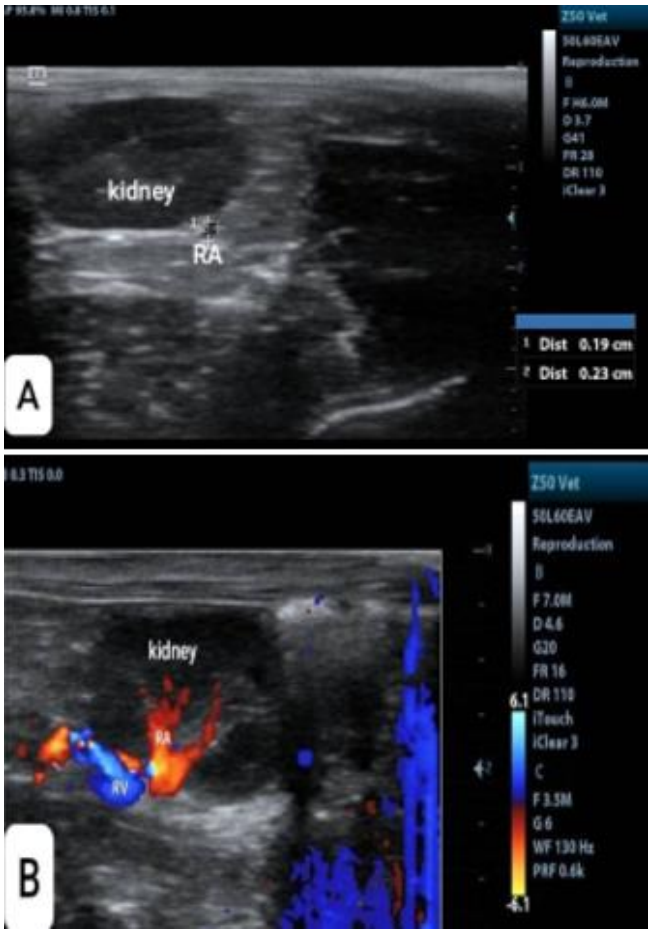

Figure 2: Ultrasound using color Doppler modes of the kidney in female rabbits. A- Measurements of the renal artery. B- Color imaging of the RA (red) and RV (blue).

Gross morphological results

The result showed that the rabbits injected with latex only showed the main arteries. They were flexible, while the rabbits whose arteries were injected with resin showed the main and small arteries. They were rigid, making them in the form of a three-dimensional model. After injecting the arteries with latex and cold resin and while tracing the arteries, it was found that the main artery that supplies the abdomen is the abdominal aorta AA, which is located from

the dorsal of the abdomen. It is branched into the unpaired arteries, including celiac and superior mesenteric arteries. Secondly, there are paired arteries, which include the adrenal, renal, and lumber arteries; all these branches were observed (Figure 3).

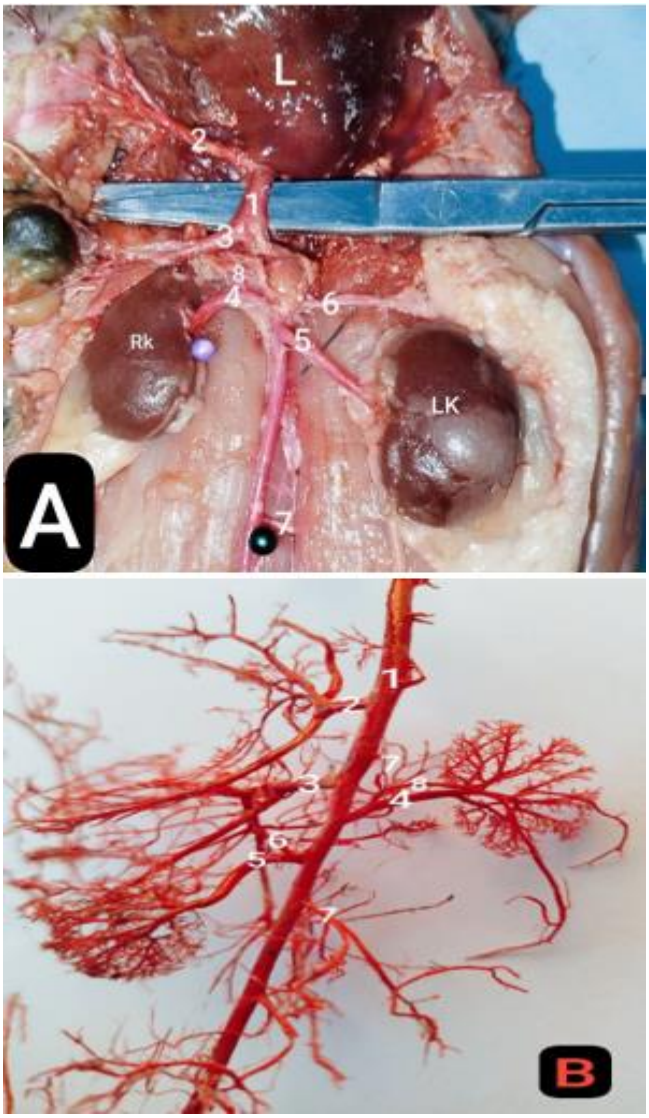

Figure 3: The distribution of arteries in the abdomen of a rabbit injected with (A-latex & B- resin) is shown as follows: 1- Abdominal aorta. 2- Celiac artery. 4- Right-Renal Artery. 5- Left renal artery. 6- Left suprarenal artery. 7- Lumber arteries. 8- Right suprarenal artery. L: Liver, LK: Left kidney, RK: Right kidney.

The renal artery (RA) is a pair of arteries with two arterial branches (right and left) extending from the AA. They arise just below the CMA. The left renal artery (LRA) was longer than the right one. Before each RA reaches the renal apex, it divides into four to five branches. To serve the adrenal gland, ureter, and other surrounding tissues, each artery is also preceded by a few small inferior suprarenal branches. The adrenal gland is supplied by the paired inferior suprarenal arteries. Although numerous variations exist, it typically begins at the renal artery's trunk before its terminal division (Figure 3). The abdominal aorta's (AA) first notable visceral branch is the celiac artery (CA) (Figure 3). The Cranial mesenteric artery (CMA) is an artery that emerges anteriorly from the aorta in the abdominal region, just below the origin of the celiac trunk (Figure 3). The four paired arteries emanating from the dorsal surface of the aorta are lumber arteries (LA) that nutrient the lumber reign of the spinal cord muscle of the back (Figure 4). It's located below the aortic hiatus in the diaphragm, and is an unpaired artery, which splits into two major branches: splenic artery and common trunk. The common trunk gives a common hepatic artery (CHA) with the left gastric artery (LGA). The splenic artery (SA) originates from the greater-pancreatic artery, left gastro-omental artery, short-gastric artery and the dorsal-pancreatic artery. The (CHA) gives Proper hepatic artery, supra-duodenal artery and gastroduodenal artery. The proper hepatic artery appeared in figure 4B but was not shown in figure 4A because it was below the stomach. The pancreaticoduodenal branch is the first to emerge from the CMA. Additionally, the left side of the CMA is provided by the coiled artery and the jejunal artery. The mesenteric colon, right colon, and ileum are the three branches that emerge from the right side of the artery. These branches supply the communicating colon, cecum, terminal ileum, and transverse colon (Figures 5 and 6).

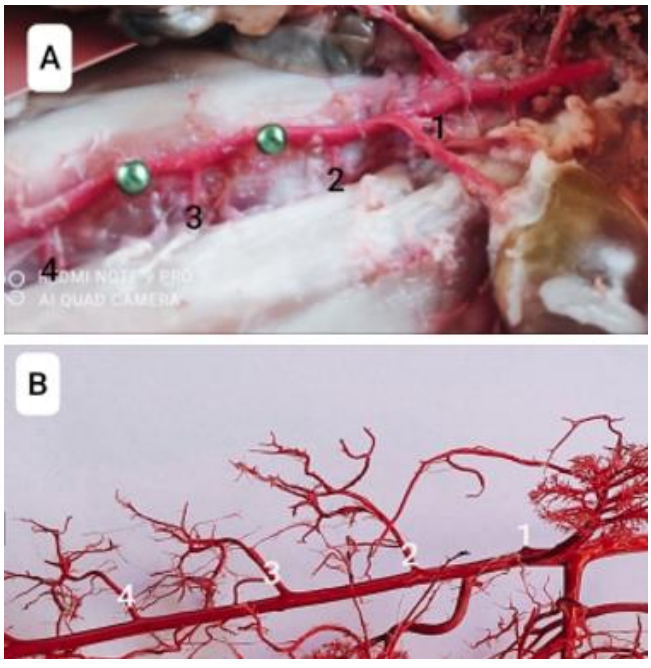

Figure 4: 1-4 Four pairs of lumbar arteries supply the lumbar vertebrae. A: Injected with latex. B: Injected with resin.

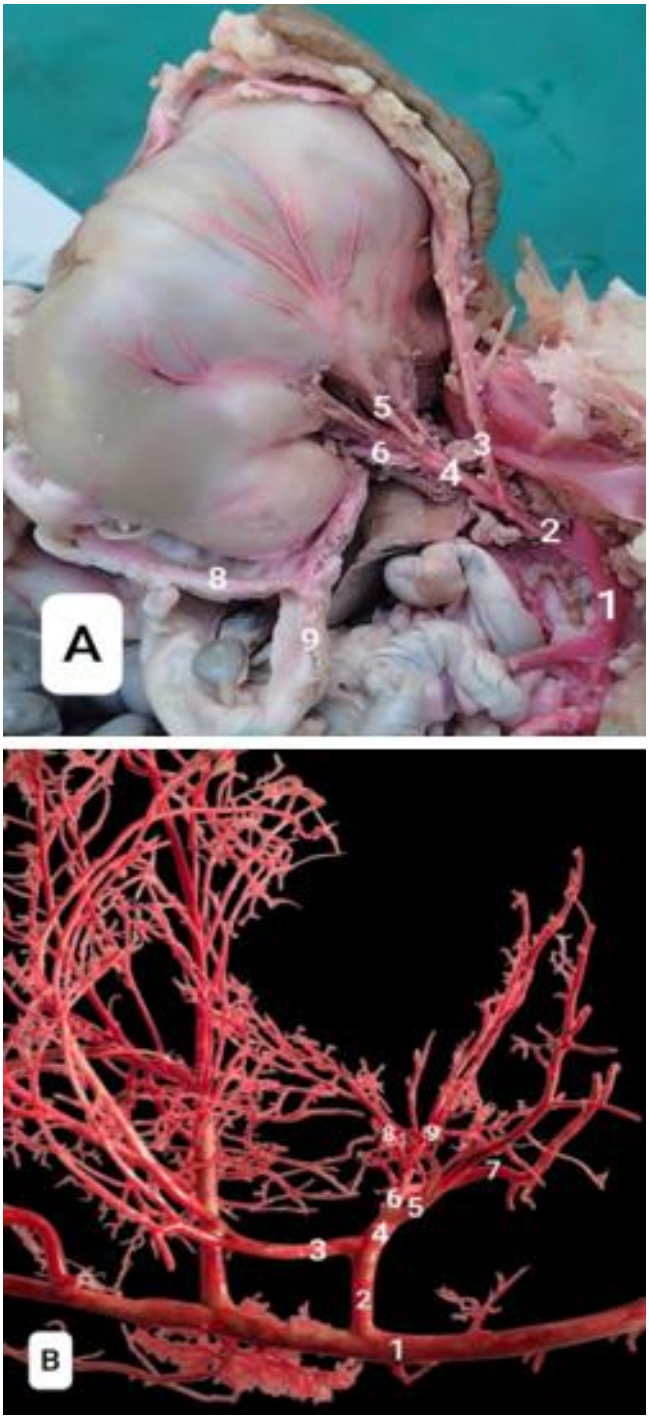

Figure 5: The distribution of CA injected with latex (A) and injected with resin (B) is shown as follows: - 1-AA. 2- Celiac artery. 3- Splenic artery. 4- Common trunk. 5- Left gastric artery. 6- Common hepatic artery. 7- Proper hepatic artery. 8- supra-duodenal artery. 9- Gastroduodenal artery.

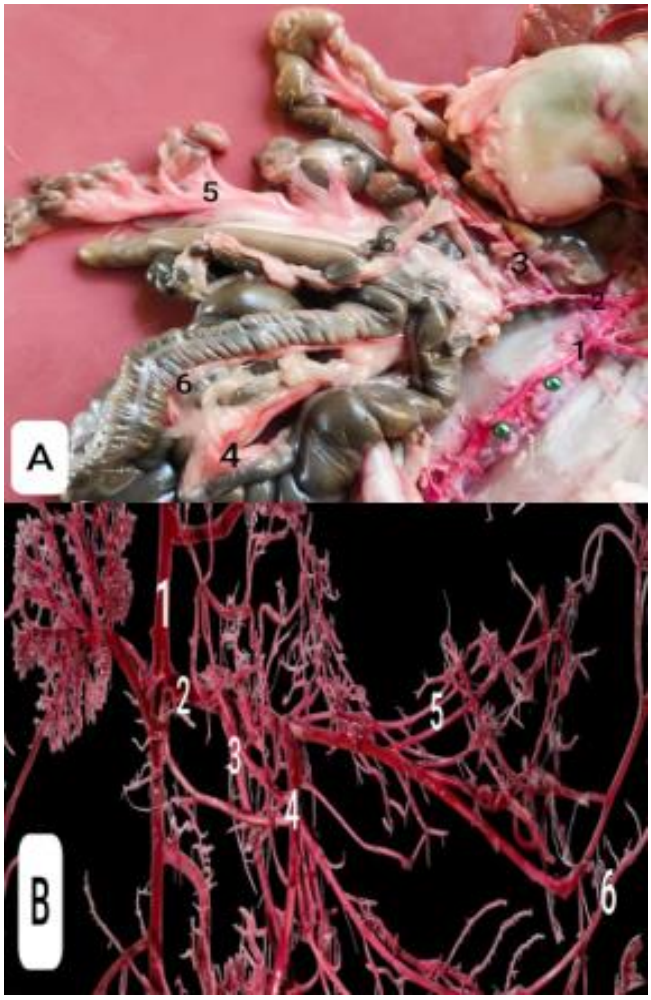

Figure 6: The distribution of CMA injected with latex (A) and injected with resin (B) is shown as follows:- 1-AA. 2-CMA. 3- Inferior pancreato-duodenal a. 4- Colic a. 5-Jejunal. 6- ileal a.

Discussion

According to human medical studies, Doppler ultrasound's great sensitivity has substantially contributed to illness detection and staging. Studies that evaluated blood vessels using color Doppler ultrasound in animals, especially rabbits, are limited. Therefore, the current study dealt with the evaluation and imaging of the thickness of the blood vessels branching from the abdominal aorta in the abdomen of a rabbit using color Doppler ultrasound. In the current study, clear and colored images of the arteries and veins supplying the liver and kidneys were obtained using color Doppler ultrasound, which will contribute to evaluating the effectiveness and thickness of blood vessels. These studies are consistent with the authors (27). The blood vessels in the pancreas were evaluated using Doppler (28). In rats, the thickness of blood vessels was measured during blood flow

using high-resolution ultrasound (29). Vascular evaluation was performed using Doppler ultrasound after liver transplantation to determine the outcome of the operation. Also, renal artery stenosis can be assessed using Doppler ultrasonography (30,31). The current study's findings demonstrate that latex-injected anatomical dissection improves the study's anatomical correctness by identifying the major blood arteries and their primary branches. Our present study is in agreement with other authors who have used latex with a vascular supply to facilitate the examination of blood vessels in abdominal and Pelvic cavities in cats (8) and in rats (32), the arterial supply of brain in rabbits (33,34) and human brain (35). We created a three-dimensional image of the abdominal arteries using the injected resin dissection. These studies are consistent with other authors who used anatomical resin injecting blood vessels in the lumbar arteries in the European rabbit (24), renal venous system in birds (36), hepatic venous system in rabbits (37), and hepatic vascular system in cats (38).

In general, as we have shown by tracing the abdominal arteries injected with latex and resin, the abdominal blood supply of rabbits is similar to the abdominal blood supply of most other animals and humans, with the AA being the primary source of blood flow to the abdomen. Our study showed that the lumbar arteries, which are four bilateral arteries, emanate from the dorsal aspect of the AA. Rabbit and the European Hare, where it was mentioned that the aorta gives off six pairs of lumbar arteries. Our latest investigation indicated that the celiac artery bifurcates into two principal branches: SA and CT (24). The common trunk gives off the CHA and LGA. Our study agrees in result in New Zealand rabbits (39) and in cats (8). In chinchillas the CA gives off four main branches: LGA, HA, SA, and the gastro-celiac artery (39). The SA runs to the left along the gastro-splenic ligament, giving off 3-5 splenic branches in rabbits (33). The SA gave small pancreatic branches, which reached 3-6 branches, and similar to dogs (40) and chinchillas (40). The gastro-duodenal artery gives rise to the cranial-pancreatic artery, as mentioned in the badger (36) and in carnivores (40). Our results are inconsistent with result in chinchilla because he verified that the cranial pancreaticoduodenal artery comes from the HA (39). The CMA originates from the ventral side to the AA near the level for the first lumbar vertebrae as in goat (38) and in buffalo (41). Our results do not agree with result in camels (42), where it was mentioned that it originates near the level of the third lumbar vertebrae. These studies are similar to result in Baladi rabbit (43). The present research on resin and latex corrosion moulds observed that the third branch of the aorta is the RA. These studies agree that the correct branch arises anteriorly and is carnal of the left branch because it is removed by the stomach and viscera on the left side (44). This study disagrees that rabbits the RA almost originated at the same level (45). These studies consistently separate each renal artery into two or three interloper arteries before entering the renal hills (46).

Conclusions

Using colored latex and resin injections in anatomical dissections has enabled the precise depiction of vascular structures in ultrasound images. The images obtained through this study served as a fundamental anatomic reference tool for clinicians, allowing the diagnosis of maladies in these regions and further developing and applying these techniques in rabbit medicine.

Conflict of interest

The authors assert the absence of any conflicts of interest.

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دراسة تشريحية وثلاثية الأبعاد للجهاز الوعائي البطني في أنثى الأرنب باستخدام التشريح والموجات فوق الصوتية

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الخلاصة

هدفنا الى توفير بيانات مرجعية لقطر شرايين البطن باستخدام الموجات فوق الصوتية في الأرانب السليمة، والتي يمكن استخدامها كمقارنة لتقييم التغيرات في قطر الشريان في الأرانب المريضة أو عند إجراء التجارب وإنشاء نموذج ثلاثي الأبعاد لشرايين البطن باستخدام مادة الصب. أجريت الدراسة الحالية على عشرة أرانب محلية تتراوح أعمارها بين ٢-٣ سنوات ووزنها ١-٢ كجم. تم إجراء فحص بالموجات فوق الصوتية لجدار البطن في وضعية الاستلقاء على الظهر وأظهرت النتائج أن متوسط قطر الشريان الكبدي لوزن ١-١,٥ كجم كان ٠,٠٤±٠,٢٥ سم بينما لوزن ١,٥-٢ كجم كان ٠,٠٣±٠,٣٧سم، ومتوسط قطر الشريان الكلوي لوزن ١-١,٥ كجم كان ٠,٠٣±٠,٢٠ سم بينما لوزن ١,٥-٢ كجم كان ٠,٠٤±٠,٢٥سم. وأشارت نتائج الموجات فوق الصوتية إلى أن قطر الأوعية الدموية يزداد مع زيادة الوزن. تشريحيا تم تقسيم الأرانب إلى مجموعتين المجموعة الأولى تم حقن الشرايين باللاتكس والمجموعة الثانية تم حقن الشرايين بمادة الصب الرزن. وأظهرت النتائج أن شرايين المجموعة الأولى مرنة بينما شرايين المجموعة الثانية صلبة وهشة وتحتاج إلى عناية أكبر في التعامل من المجموعة الأولى. وأشارت النتائج إلى أن حقن اللاتكس تساعد في إجراء دراسات تشريحية دقيقة بينما تساهم حقن الرزن في إنشاء نموذج ثلاثي الأبعاد للشرايين. ونستنتج أن الموجات فوق الصوتية دوبلر الملونة باستخدام مسبار خطي توفر صورا واضحة ودقيقة للأوعية الدموية، مما يسمح للأطباء بقياس قطرها بدقة وتقييم تدفق الدم داخل الأوعية، وبالتالي المساعدة في تشخيص حالات مثل تصلب الشرايين وأمراض الشرايين.