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Intraosseous Arterial Blood Supply of Canine Pelvic Bones

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Abstract: The purpose of this work was to study pelvis intraosseous arterial blood supply in adult dogs. The bones constituting the pelvic girdle have independent blood supply sources-the branches of internal and external iliac arteries. Their nutrient foramina on the surface of iliac, pubic and ischial bone bodies shifted towards the articular cavity and located variably. The branching pattern of I order intraosseous nutrient arteries is magistral or dichotomous, that of II-V order arteries-magistral, dichotomous, or loose. Compact bone has additional source of blood supply-vessels of arteriolar type penetrating from the periosteum. Acetabular zone and peripheral parts of pelvic bone have additional sources of blood supply as well represented by terminal branches of the vessels nourishing the attached muscles and the capsuloligamentous system of pelvic connections. The vessels of arteriolar type included into the intraosseous microcirculatory bed. The analogy detected with the structure of the intraosseous vascular network of tubular bones. The assessment of outside and inside diameter of arterial vessels, the thickness of vascular wall were carried out. The data must be considered in treatment of the dogs with pelvic girdle pathology.

Key words: Dogs · Pelvic Bones · Intraosseous Arterial Vessels · Anatomic Preparation · Roentgen Angiography · Histomorphometric Analysis

INTRODUCTION

Pelvic fractures are one of the complex pathologies of the locomotor system and they account for 11.5-30 % of all skeletal injuries in dogs [1-3]. It is necessary to understand the problem of the blood supply of bones in order to assess the trauma-associated vascular damages, as well as to prevent trauma-associated iatrogenic damages during making surgical approaches and insertion of fixating elements [4-6]. The breakdown of the intraosseous arterial vascular network can cause aseptic necrosis [7]. Experimental and clinical studies have demonstrated a leading role of blood supply in the reparative regeneration of bone tissue [8]. There are some publications that contain information about blood supply of the human and dog pelvis [4, 9-11]. However, so far there is no comprehensive research on studying the topography of nutrient foramina and the morphology of the intraosseous arterial bed of canine pelvic girdle. The purpose of this work was to study the intraosseous arterial blood supply of the iliac, ischial and pubic bones as parts of the pelvic girdle in adult dogs.

MATERIALS AND METHODS

All the experiments were performed in accordance with the Principles of Laboratory Animal Care (NIH Publication no. 85-23, revised 1985). All the manipulations made in the animals were considered and approved by the Ethics Board of RISC "RTO".

The bones of pelvic girdle served as material for this study; they were obtained from 44 normal mongrel dogs of both genders at the age of 1.4±0.09 years with 15±1.2-kg body mass after performing acute experiments on other segments of the locomotor system. Euthanasia was made using a lethal dose of intravenous 5% thiopental sodium (Sintez, Kurgan, Russia) [12].

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All the animals underwent anatomic preparation of the pelvis in order to reveal the sources of blood supply and the localization of the nutrient foramina of the pelvic girdle.

Pelvic girdle was isolated in 30 animals, soft tissues were removed maximally, after it was boiled for 10-12 hours and bleached in 3% hydrogen peroxide. Localization of nutrient foramina was studied by macro-preparations as well.

20,000 units of heparin (Sintez, Kurgan, Russia) were injected in eight animals directly before euthanasia. Catheters were placed postmortem into the abdominal aorta and the caudal vena cava (At LV level) and the vascular bed was washed through these catheters using two liters of 0.9% sodium chloride solution warmed up to 40°C, then two liters of formaldehyde-glutaraldehyde fixative by Karnovsky [13] supplemented with 0.1% picric acid (SERVA GmbH, Germany).

After that the arterial bed of the pelvis and pelvic limbs was injected with radio-opaque Hauch mass (That with red lead) in five animals [14]. Once soft tissues were removed, the macro-preparations of the pelvic girdle placed directly on the X-ray film cassette. Roentgen angiography in dorso-ventral and lateral views was performed using «Premium Vet» x-ray system (Sedecal, Spain) [15].

The vascular network in three animals was injected with the mixture of epoxy resins for electron microscopic study (100 ml Araldite CY-212 (SERVA GmbH, Germany), 90 ml Araldite hardener HY-964 (SERVA GmbH, Germany), 10 ml Methylnadicanhyidride (SERVA GmbH, Germany), 12 ml Dibutyl phthalate (SERVA GmbH, Germany) [16] supplemented with 50 mL 3% solution of Sudan III (SERVA GmbH, Germany) in o-Xylol (Reahim, Russia). Once the abdominal aorta and caudal vena cava were ligated the caudal body part was separated, placed in a container with 1% solution of formalin and resin polymerization was made at 60°C for three days. Then, the pelvic bones were prepared, after that they were sawed into pieces and graduated corrosion of paraosseous tissues was performed with 6% solution of sodium hypochlorite (Merck, Germany) at room temperature under the control of microscope. The samples were dehydrated in ascending concentrations of ethanol, dried in dust-free conditions and coated with a thin layer of electrically conductive material using «IB-6» ion sputter (JEOL, Japan). Vascular replicas were investigated using «JSM-840» scanning electron microscope (JEOL, Japan) in secondary electrons with 20-keV accelerating voltage.

Pelvic girdle was isolated in six animals, as well as released from paraosseous tissues, sawed into pieces and fixed with formalin solution, neutral buffered 10% (Sigma-Aldrich, USA). Decalcification of bone blocks was made in Richman- Gelfand-Hill formic-hydrochloric acid liquid mixture [17]. After complete decalcification the samples were neutralized in 5% solution of potassium alum. A part of the bone blocks was impregnated with celloidin solutions of ascending concentrations in the mixture of absolute ethanol and ethyl ether and after that sections of 10-15 µm thickness were prepared. The other part of the blocks was infiltrated with celloidin and paraffin with subsequent cutting into sections of 5-7- µm thickness and then processed for hematoxylin and eosin B (SERVA GmbH, Germany) staining [18]. The sections were investigated using AxioScope A1 stereo-microscope and AxioCam ICc 5 microscope camera with Zen blue software (Carl Zeiss MicroImaging GmbH, Germany). The inside and outside diameters of vascular cross section profiles, vascular wall thickness were measured on digital images of visual fields using «Video Tes T-Morfologia» software (VideoTesT, Russia). «Microsoft Office Excel 2010» spreadsheet editor (Microsoft Corporation, USA) was used for statistical processing of data. The histomorphometric data are presented as sample mean value (M, µm) and standard deviation (SD, µm).

RESULTS

Blood supply of the iliac, pubic and ischial bones occurred through their own nutrient arteries, as recorded by roentgen angiographic analysis and while studying the boiled macropreparations of the canine pelvic girdle (Table 1).

Internal nutrient artery of the iliac bone (*A. nutricia ilii*) departing from the caudal gluteal artery (*A. glutea caudalis*) carried out blood supply of the iliac bone. The nutrient foramen located on the ventral or lateral surface of the iliac bone body, at the level of its middle or caudal third (Fig. 1). The nutrient artery ran intraosseously in the caudal direction and then it divided into two main branches (*RR. cranialis and caudalis*), which divided into smaller branches. In this case dichotomous type of branching was observed mainly, however, there were arterial areas with magistral and loose type of branching. The cranial branch supplied blood to the middle and cranial parts of the body, as well as the

Table 1: Characteristic of arterial blood supply to canine pelvic bone

	Pelvic bone		
Descriptive parameters	Iliac bone	Pubic bone	Ischial bone
Source of blood supply	*A. glutea caudalis*	*R. obturatoris a. circumflexa femoris medialis*	1. *R. obturatoris a. circumflexa femoris medialis* 2. *A. gluteacaudalis*
Localization of nutrient foramen	On the ventral or lateral surface of the body at the level of its middle or caudal third	On the caudal or dorsal surface of the cranial branch at the level of its middle third	1. On the medial surface of the body at the level of the caudal edge x body articular cavity 2. On the lateral surface of the cranial third of the body
Zone of blood supply	The upper flaring portion and the body of the iliac bone, the cranial third of articular cavity	The body, the cranial and caudal branches of the pubic bone, the ventral part of articular cavity	1. The body and plate of the ischial bone 2. The caudal part of articular cavity
Pattern of branching	Magistral, dichotomous, loose		
Orders of branching	I-V		
Localization of additional nutrient foramina	The gluteal and iliac surfaces of the upper flaring portion of the iliac bone, the lateral platform of *m. rectus femoris*	The ventral and dorsal surfaces of the caudal branch of the pubic bone	The medial surface of the body, the ventral and dorsal surfaces of the caudal part plate, the ventral and dorsal surfaces of the ischial bone branch, tuber of the ischium

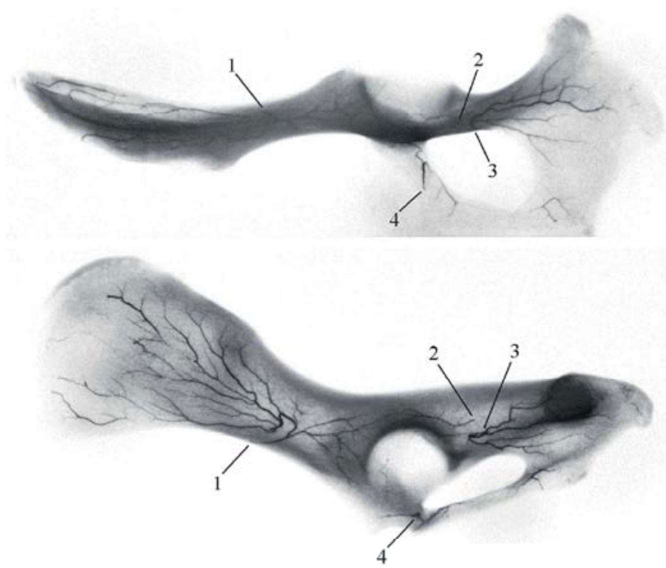

Fig. 1: Localization of nutrient foramina and architectonics of nutrient arteries of canine pelvic bones: 1-internal nutrient artery of the iliac bone (A. nutricia ilii); 2, 3-internal nutrient arteries of the ischial bone (AA. nutricia ischii); 4-internal nutrient artery of the pubic bone (A. nutricia pubis)

upper flaring portion of the iliac bone; the caudal branch supplied the caudal third of the iliac bone body and, respectively, the cranial third of articular cavity.

The internal nutrient artery of the pubic bone (*A. nutricia pubis*) branching out from the obturative branch of the circumflex medial femoral artery carried out the blood supply of the pubic bone. It entered through the nutrient foramen located on the dorsal surface of the pubic bone body in the place of its transition to the cranial branch (Fig. 1). Being branched out in the caudomedial and craniolateral directions (Magistral and dichotomous type of branching), the artery nourished the body, the cranial and caudal branches of the pubic bone, as well as the ventral part of articular cavity.

Two internal nutrient arteries of the same name (*AA. nutricia ischii*) carried out blood supply of the ischial bone. The first of them departed from the obturative branch of the circumflex medial femoral artery (*R. obturatoris a. circumflexa femoris medialis*) and entered through the nutrient foramen located on the

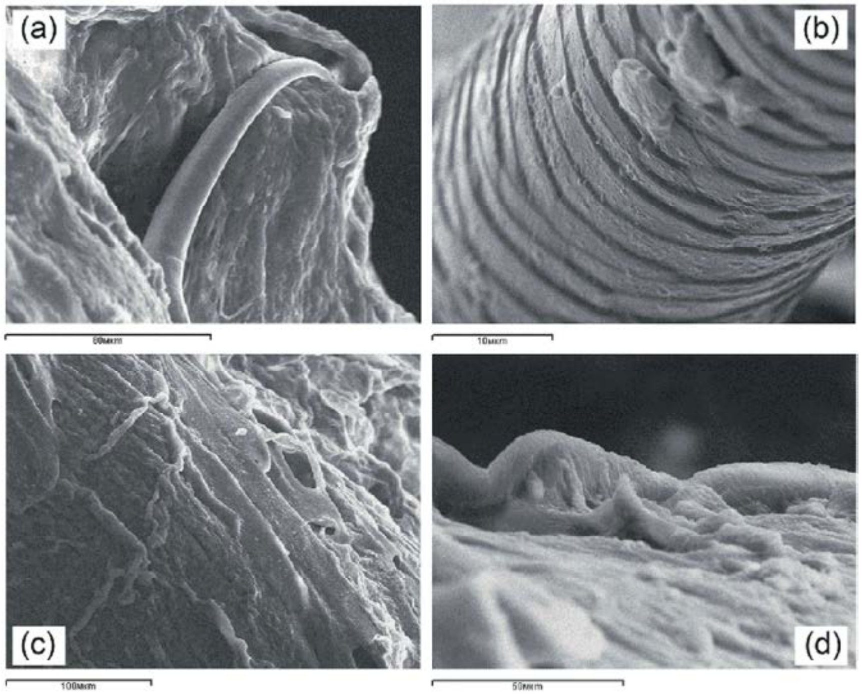

Fig. 2: Periosteal vascular network of pelvic bone. An artery of small caliber, 650× (a); a microrelief cast of the internal surface of artery, 3000× (b); vascular weavings, 370× (c); an arteriole, 950× (d). Electronic scanograms

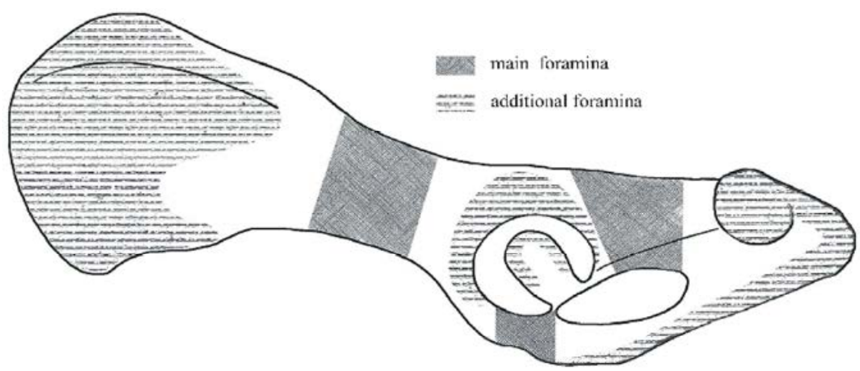

Fig. 3: Zones of localization of the nutrient foramina of canine pelvic bone

medial surface of the ischial bone body at the level of its cranial third. In some observations, its branches entered the ischial bone body through 1-2 additional nutrient foramina located near the main foramen (Fig. 1). Intraosseously, the artery ran in the caudal direction branching out in the body and plate of the ischial bone. The second nutrient artery, branching out from the caudal gluteal artery, entered through the nutrient foramen located on the lateral surface of the cranial third of the ischial bone body and vascularized the caudal part of articular cavity (Fig. 1).

In addition to the above mentioned, there were nutrient foramina located in the zone of attachment of muscle aponeuroses, as well as in that of the capsuloligamentous system of pelvic connections and articular cavity. The highest density of their location was observed on the surfaces of the upper flaring portion of the ilium, tuber of the ischium, the plate and branch of the ischium, the cranial and caudal branches of the pubic bone, in the periacetabular area and the fossa of articular cavity (Tab. 1).

Figure 3 shows the pattern of the zones of localization of the nutrient foramina on the surface of canine pelvic bones.

The study of the histological sections and corrosion preparations of the vascular replicas of pelvic bone

Table 2: Morphometric parameters of pelvic intraosseous arterial blood vessels (M±SD), µm

Type of vessels	Outside diameter	Inside diameter	Wall thickness
Arteries of order I	833 ±84.9	381 ±80.3	238 ±48.1
Arteries of order II	495 ±53.7	217 ±33.3	147 ±12.3
Arteries of order III	317 ±36.1	117 ±19.5	95 ±15.5
Arteries of order IV	196 ±32.5	73 ±6.8	53 ±11.6
Arteries of order V	101 ±12.7	43 ±5.8	25 ±5.4
Arterioles	38±12.6	23 ±6.0	11 ±2.4
Precapillary arterioles	11 ±1.5	6±1.5	3 ±1.5

periosteal surface has demonstrated the intraorganic blood flow in pelvic bones to be realized due to the periosteal and intraosseous vascular networks. The periosteal vessels formed weavings in the periosteum which gave branches to the compact bone. Arteries of small caliber and arterioles were determined as components of the periosteal vascular weavings (Fig. 2).

The light microscopic study of histological sections revealed predominantly parallel, with respect to surface, orientation of Haversian canals in the compact layer of pelvic bone. Intraosseous vascular canals communicated with each other through a few straight or arcuate anastomoses. There was one vessel, at least two ones in the canal lumen. Single capillaries were observed the most often, in some observations-an arteriole and a venule, an arteriole and a capillary. Perivascular spaces were filled with loose connective tissue which bundles of fibers fixed the vascular walls within the canals.

Intraosseous vascular network as a component of spongy bone substance involved blood vessels running in the bone marrow of intertrabecular spaces. Intraosseous arteries had a three-layer wall with pronounced adventitial and muscular layers. The vascular bundles including an artery and two veins-companions were found around the zone of pelvic symphysis and sacroiliac joint. The terminal branches of the arterial bed were represented by arterioles and precapillary arterioles.

The mean values of outside, inside diameter, wall thickness, obtained by morphometry of cross-section profiles of intraosseous arterial vessels are demonstrated in Table 2.

The outside and inside diameter of I-V order arterial branches decreased 1.2-1.5-fold progressively with every branching.

DISCUSSION

We haven't found the information about intraosseous arterial blood supply of the vertebrate pelvic bones in the available literature in order to compare them with our data obtained. Most of the works deals with studying the intraosseous blood supply of tubular bones in mammalians [5,19-23]. However, we have revealed that the organization of intraosseous vascular network of canine pelvic bones has similarities comparing with the structure of the intraosseous vascular network of tubular bones.

Long tubular bones of mammalians are known to have three sources of blood supply: nutrient artery, periosteal network and epiphyseal vessels. Blood supply of the diaphyseal zone occurs mainly on account of the periosteal and the intraosseous vascular networks [5,19-23].

Studying the vascular bed of canine pelvic girdle, we have established that blood supply of pelvic bones in all the animals, regardless of gender, occurs by the branches of the caudal gluteal and medial circumflex femoral arteries, that are, in turn, the branches of the internal and external iliac arteries. Each of bones making up the pelvic girdle has its own nutrient foramina like tubular bones. The arrangement of pelvic bone nutrient foramina is characterized by some variability in the zone of preferential localization and has no gender differences.

The main branches of intraosseous arteries are divided and follow in the cranial and caudal directions. In this case, one of their branches supplies the zone of articular cavity. The nutrient arteries have magistral or dichotomous type of branching predominantly, however there are areas with loose type of branching in the terminal parts of vascular bed. The peripheral parts of the ilium, the ischium and the acetabular zone have their own multiple nutrient foramina into which the vessels penetrate from the attached structures of the capsuloligamentous system of pelvic connections and from those of muscle aponeuroses. Thus, the analogy can be traced with the structure of the intraosseous vascular network of tubular bones. Their nutrient arteries also have highly branched descending and ascending rames and the epiphyseal parts are blood-supplied by the vessels penetrating through additional nutrient foramina [5, 23].

The injuries of the zone of nutrient foramen localization accompanied by occlusion or rupture of the main nutrient artery are especially dangerous for tubular bone fractures, because they lead to appearing the extensive ischemic foci transforming into bone infarction. The mentioned injuries appeared to be one of the main causes of unsatisfactory treatment outcome [7]. In this regard, on the basis of analyzing the map of pelvic bone nutrient foramina we have defined the zones which traumatic or iatrogenic injuries may have similar effects (Fig. 3).

A major role in supplying the compact layer of tubular bones is allotted to periosteal blood supply which occurs mainly by arterioles penetrating from the periosteum and giving rise to numerous capillaries. The blood supply of bone marrow is mainly carried out by the terminal branches of the magistral and epiphyseal nutrient arteries [19, 22, 23].

We have established that like tubular bones a developed network of arterial vessels branching out to the compact bone layer locates on the periosteal surface of canine pelvic bones. A system of parallel Haversian canals has been found in the compact bone layer. The network of narrow microcirculatory vessels located in them provides an adequate nutrition of bone tissue normally, however, a small number of branchings and anastomoses evidences of a low adaptive capability for injuries. The cancellous bone network is represented by arterial part and multiple vessels of the microcirculatory bed. The vessels of the microcirculatory bed penetrate the entire volume of bone, no avascular zones are found.

The morphometric parameters of the diameter of intraosseous vessels obtained by different researchers while analyzing micro-angiograms and clean sections after their injecting with radio-opaque or stained mixtures allow to judge the order of branching and caliber of micro-vessels, but do not provide full information about their structure [15, 20]. The made assessment of vessels by transverse histological sections allows to simultaneously classify the type of vessels in accordance with the structure of their vascular wall. Pre-perfusion of the vascular bed with histological fixative solution prevents recalibration of vessels due to their stretching and the development of postmortem changes, thereby allowing to avoid the associated measurement errors. The inside diameter of nutrient arteries (Order I branches) ranges within 300-400 μm, that is comparable with the diameter of the nutrient artery of canine tibia shaft [5]. The walls of intraosseous arteries have developed muscular layer. Consequently, in cases of traumatic pelvic injuries bone ischemia may be associated with inflow disorder due to the intensive arterial spasm. The assessment of such parameters as the outside and inside diameter of vessel, the thickness of vascular wall is important as well. The change in parameter values is an adaptation response to blood flow velocity change in arterial vessels and it allows to judge the presence of pathological process [24, 25].

CONCLUSIONS

The iliac, pubic and ischial bones comprising the canine pelvic girdle have their own independent sources of blood supply. The nutrient foramina on the surface of the bone bodies are shifted in the direction of the articular cavity and characterized by their location variability. The zone of acetabulum and peripheral parts of the pelvic bone have additional sources of blood supply represented by terminal offsets of the vessels supplying the attached muscles and the capsuloligamentous system of pelvic connections. The compact bone layer also has an additional source of blood supply-vessels of arteriolar type penetrating from the periosteum. The data should be considered when assessing vascular damages as results of trauma, when performing reconstructive-and-restorative surgeries and correcting osteotomies of pelvic bones, as well as when obtaining bone autografts.

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