# MORPHOMETRIC STUDY OF TRABECULA SEPTOMARGINALIS **IN DOGS**

# ESTUDO MORFOMÉTRICO DA TRABÉCULA SEPTOMARGINAL EM CÃES

# P. P. BOMBONATO<sup>1</sup>\*, A. N. B. MARIANA<sup>1</sup>, V. BORELLI<sup>1</sup>, F. AGRESTE<sup>1</sup>, L. G. NASCIMENTO<sup>2</sup>, A. S. LEONARDO<sup>2</sup>.

## **SUMMARY**

The trabecula septomarginalis of 30 dog hearts, aged between two and six years old, with no history of heart conditions were studied, aiming to characterize the number of insertions, dimensions, constitution and tissue arrangement of trabeculae. The knowledge about this structure should be used as a tool in morphophysiology studies and cardiac clinicsurgical procedures. Trabecula fragments underwent dehydration with ethanol, diaphanization in xylene, impregnation and were embedded in paraffin. Subsequently, prepared tissue fragments were cut into 5-µm-thick slices using a manual microtome (Leica RM 2125RT) and stained with modified Mallory trichrome. Photomicrographs were obtained using a digital optical microscope Zeiss Axioscópio<sup>®</sup> and the images were analyzed by the image analysis software Zeiss KS-400<sup>®</sup>. The data were subjected to Mann-Whitney U test. Macroscopically, the trabecula septomarginalis presented three types of insertion: simple (40%), double (33.33%) and branched (26.67%). Microscopically, the trabeculae septomarginalis consisted of striated cardiac muscle, connective tissue and cardiac conduction myofibers. The mean ratios plus standard deviations of connective tissue, striated cardiac muscle and cardiac conduction myofibers were  $27.1\% \pm 6.92, 67.3\% \pm 6.79$  and  $5.6\% \pm 2.47$ , respectively.

KEY-WORDS: Canis familiaris. Heart. Morphophysiology.

#### **RESUMO**

Foram estudados 30 corações de cães, com idade entre dois e seis anos e sem histórico de afecções cardíacas as trabéculas septomarginais com o objetivo de caracterizar as relações quanto ao número de inserções, dimensões, constituição e arranjo tecidual, visando fornecer subsídios para estudos da morfofisiologia e entendimento clínicocirúrgico de problemas relacionados com essa estrutura. Os fragmentos das trabéculas foram submetidos a desidratação crescente em álcool etílico, diafanização em xilol, impregnação e inclusão em parafina. Posteriormente com uso do micrótomo manual (Leica RM 2125RT), cortados em espessura de 5µm e corados com Tricrômio de Mallory modificado. Fotomicrografias foram obtidas com auxílio de um microscópio óptico Axioscópio Zeiss<sup>®</sup> e as imagens então analisadas através do programa específico de morfometria KS-400 Zeiss®. Os resultados obtidos foram submetidos ao teste "U" de Mann-Whitney (p≤0,05) comparando-se os tecidos constituintes destas trabéculas. As trabéculas septomarginais, macroscopicamente variaram em três tipos de inserção, sendo do tipo simples (40%), dupla (33,33%) e ramificadas (26,67%). Microscopicamente as trabéculas septomarginais mostraram-se constituídas por tecido muscular, conjuntivo, de condução e vascular; A proporção média de tecido muscular estriado cardíaco foi 67,3%±6,79, tecido conjuntivo 27,1%±6,92 e a de miofibras de condução cardíaca 5,6%±2,77.

PALAVRAS-CHAVE: Canis familiaris. Coração. Morfofisiologia.

<sup>&</sup>lt;sup>1</sup> Departamento de Cirurgia, Setor de Anatomia dos Animais Domésticos e Silvestres, Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade de São Paulo (USP). Av. Prof. Dr. Orlando Marques de Paiva 87, São Paulo, SP, ZIP 05508-000, Brasil. \* Corresponding author: bombonat@usp.br <sup>2</sup> Universidade de Brasília.

### **INTRODUCTION**

The trabecula septomarginalis is an important muscle formation which extends from the septum wall to the papillary muscle base, and helps to prevent excessive distension of the heart during diastole. Its structure also enables the passage of conduction cardiomyocytes that cross the ventricular lumen, thus forming part of the conduction system (EVANS, 1994). Bojsen-Moller & Tranun-Jensen (1971) reported a bundle of conductive tissue in the right branch of the trabecula septomarginalis of pig hearts located partially in the interventricular septum and in the papillary muscle basis while studying the conduction nervous system and its terminations. Lorenzi & Guski (1935) stated that the trabeculae septomarginalis of cattle consisted of conduction cardiomyocytes drivers and connective component variations, both quantitatively and qualitatively. They also state that the cardiac conduction myofibers increase their volume continuously even after somatic development is complete.

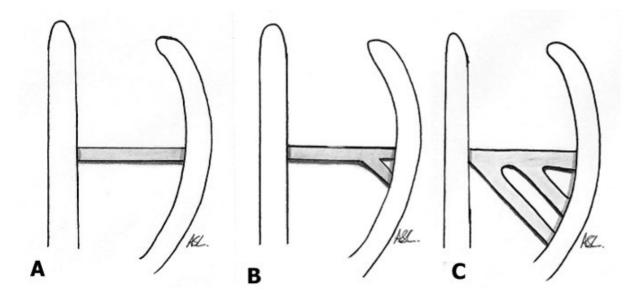
Faced with the conviction that morphological studies are essential to development of applied areas, especially with regard to the clinical and surgical areas, the aim of the this study was to determine the different types of insertion of trabecula septomarginalis and to establish morphologically their constitution and arrangement tissue in dogs.

# MATERIAL AND METHODS

In this study, 30 dog hearts were collected during autopsy classes at the Laboratório de Patologia Veterinária (Laboratory of Veterinary Pathology) for the past five years. These dogs were mixed breed, aged between two and six years old, both male and female with no history of heart disease. During the autopsy, an incision parallel to the eighth intercoastal space in the left antimere was performed by sectioning the skin, muscles, and endothoracic fascia in order to access the thoracic cavity by pushing away the ribs. Subsequently, the pericardium was cut, allowing the visualization of the heart apex, so that the great vessels that connect the heart to the body were isolated and cut. Longitudinal incisions in the left and right interventricular grooves were also performed to enable visualization and removal of the trabecula septomarginalis. The type of insertion of each trabecula septomarginalis was evaluated macroscopically and classified as single, double or branched (Table 1).

In order to analyze quantitatively how the trabeculae are constituted, tissue fragments of about 1 cm<sup>2</sup> were collected, fixed in Bouin solution for 24 hours. Subsequently, the material underwent conventional histological technique preparation, embedded in paraffin, sectioned into 5-µm-thick slices that were stained with modified Mallory trichrome. The optical microscope Axioscópio Zeiss<sup>®</sup> was used to photograph tissue fragments and the images were analyzed by the image analysis software KS-400 Zeiss<sup>®</sup> specific for morphometry.

The tissues that constituted the trabecula septomarginalis, cardiac striated muscle, connective tissue and cardiac conduction myofibers were initially evaluated according to a descriptive analysis, after that, the means and standard deviation were calculated. These data were then submitted to Mann-Whitney U test ( $p \le 0.005$ ) to compare the tissues that make up the trabecula, as shown in Table 2, using the statistical program GraphPad Prism<sup>®</sup>.



**Figure 1** - Schematic illustration of trabecula septomarginalis insertion types in dogs. A) single insertion B) double Insertion and C) branched Insertion.

Tissues	Mean ± standard deviation	Р
Striated cardiac muscle	$67,3 \pm 6,79^{a}$	<0,001
Connective tissue		
Cardiac conduction myofibers	$27,1 \pm 6,92^{b}$	<0,001
	$5,6 \pm 2,47^{\circ}$	<0,001

Table 2 - Tissues present in the trabecula septomarginalis (mean ± standard deviation).

Means followed by the different letters in the column are significantly different according to Mann-Whitney "U" test ( $p \le 0.05$ ).

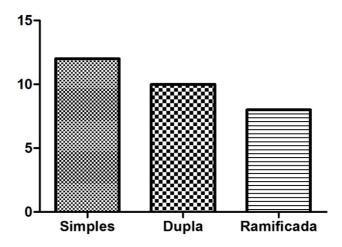
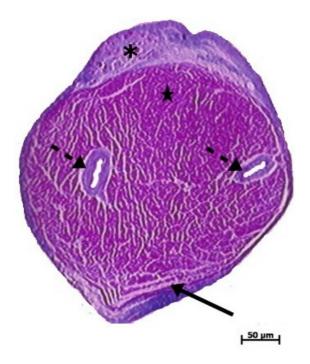


Figure 2- Graph showing the proportions among different insertion types of trabeculae septomarginalis tissue in dogs.



**Figure 3** - Photomicrograph of a cross section of a trabecula septomarginalis of a dog, showing the different proportions of tissues that make up the trabecula. Connective tissue (single arrow), vascular (broken arrows), conductor (asterisk) and cardiac striated muscle (star). Stained by the modified Mallory trichrome.

#### RESULTS

Regarding the type of insertion, the trabecula septomarginalis was classified as single, double or branched/ramified (Figures 1 and 2).

Histomorphometrically, the trabecula septomarginalis was essentially constituted by striated cardiac muscle, connective tissue and cardiac conduction myofibers, conduction tissue, at different proportions according to Table 2 and Figure 3.

# DISCUSSION

During this study, it became clear that in to the interconnection between addition the interventricular septum and the ventricle free walls, nervous stimulus from the atrioventricular fascicle is conducted to the corresponding ventricles by the conduction myofiobers present in the trabecula septomarginalis, which corroborates the findings reported by Leão et al. (2010). However, this finding differs from the report by Depreux et al. (1976), who stated that this arrangement occurred only between the muscle and the stimulation complex branch in the trabeculae septomarginalis that presented in their constitution muscle tissue and not in those of fibromuscular constitution.

In this study, it was observed that the trabeculae septomarginalis occur more often as single (40%), followed by double (33.33%) and ramified (26.67%). It should be noted that in the ramified type, the acute angle is always facing the ventricular septum, implying that the conduction set returns to the interventricular septum. These data are also consistent with those described by Leão et al. (2010), who also reported the highest frequency for single insertions 93.7%, while double was observed in 6.3%, where the trabecula septomarginalis forked and inserted in the papillary muscle and in the part of the right ventricle. Furthermore, in 18.7% it was observed the presence of false tendons connected to the trabecula free part in the lower edge of the septum, while in 68.7% of the tendon chords connected to the septal cusp of the right atrioventricular valve.

The trabecula septomarginalis in dogs is constituted by striated cardiac muscle, conjunctive tissue and conduction tissue, the cardiac conduction myofibers, at varying proportions ( $p \le 0.05$ ). Regarding these proportions, it was verified that for the overall size of the trabecula, the striated cardiac muscle tissue is larger than the conjunctive and conduction tissues together, thus indicating that trabecula thickness is dependent basically on the thickness of the striated cardiac muscle. The data obtained are consistent with studies in ungulates (DEPREUX et al., 1976), sheep (DENIZ et al., 2004; CLELLAND, 1898), goats (DENIZ et al., 2004), and humans (LOTKOWSKI et al. 1997), where different proportions were also reported for the different tissues that make up the trabecula septomarginalis.

Abundant presence of connective tissue surrounding conductor myocytes were seen, as observed in the studies of this structure by Bagalá (1940), the term cardiac conduction myofiber proposed by the ICVGAN (2012) was used. We further observed that the volume occupied by the trabecula in each animal is related to the amount of cardiac muscle tissue disposed along the studied sections, unlike Getty (1975)who concluded that the trabecula septomarginalis consists of muscle and tendon parts that vary according to the animal. On the other hand, Bortolami (1953) divided the trabeculae into three types: fibrous, muscular and cellular fibrous according to the tissue they were made of. Depreux, Mestdagh & Houcke (1976) while studying goats reported such structures made up by fibromuscular tissue with thickness between 1 and 3 mm. The cardiac conduction myofibers were arranged in the peripheral region of the evaluated trabeculae, and remained practically constant in all animals. Likewise, blood vessels were arranged in the center of the trabecula septomarginalis, thus showing the importance of this structure as a means of union and propagation of electrical stimulation.

### CONCLUSION

The trabeculae septomarginalis in dogs are varied in number and shape. Three types of insertions are observed: single, double and ramified. These trabeculae consist of striated cardiac muscle, connective tissue and cardiac conduction myofibers. These tissues have different sizes and proportions in the trabeculae septomarginalis, thus establishing a characteristic arrangement pattern suitable for dogs.

#### REFERENCES

BAGALÁ G. La trabecola di Leonardo nel cuore umano e di altri mammiferi. **Ricerche di morfologia**. v.18, p.171, 1940.

BOJSEN-MØLLER, F.; TRANUN-JENSEN, J. On nerves and nerve ending in the conducting system of the moderator band (septomarginal trabécula). **Journal of Anatomy.** v.108, n.3, p.387-395, 1971.

BORTOLAMI R. Observazioni sullo svolgimento e sulla struttura del tessuto del fascio moderatore (trabecula arcuata di Lionardo) in Bos Taurus. **Archivio veterinário italiano**. v.4, n.4, p.299-330, 1953

CLELLAND R. J. Note on a moderator band in the left ventricle and perforate septum ovale in the heart of a sheep. **Journal Anatomy and Physiology**. v.32, n.4, p.779, 1898.

DENIZ, M.; KILINE, M.; HATIPLU, E. S. Morphologic study of left ventricular bands. **Surgical** 

and Radiological Anatomy. v.26, n.3, 2004, p.230-234.

DEPREUX, R.; MESTDAGH, H.; HOUCKE, M. Comparative morphology of trabecula septomarginalis in terrestrial mammals. **Anatomischer Anzeiger**. v.139, p.24-35. 1976

EVANS H. E. **Miller's anatomy of the dog**. 3th ed. Philadelphia: W. B. Sanders Company, 1964, p.941.

GETTY, R. Sisson and Grossman's The anatomy of the domestic animals. 5th ed. Philadelphia: W. B. Sanders Company, 1975, p.2130.

KING, T. W. An Essay on the Safety-Valve Function in the Right Ventricle of the Human Heart: and on the Gradations of this Function in the Circulation of Warm-Blooded Animals. **Guy Hosp Rep**. v.2, p.126, 1837.

LEÃO, C. R.; PACHA, D. L.; CYRIACO, T.; SILVA, C.; WAFAE, N.; PEREIRA, H. M. L.; RUIZ, C. R. Anatomy of the septomarginal trabecula in goat hearts. **Italian Journal of Anatomy and Embryology**. v.115, n.3, p.229-234, 2010.

LOMBARDO, S.; CALLEGARI, E. La nuova vetrinaria. Instituto di anatomia comparada degli animali domestico di bologna. v.28, 1952, p.102-3.

LORENZI, G.; GUSKI, H. Histotopographic and morphometric studies of the intra-mural coronary arteries in the trabecula septomarginalis of swine and pigmy

goats. Zentralbl Allg Pathol. v.136, p.87-95,1990

LOTKOWSKI, D.; GRZYBIAK, M.; KOZLOWISKI, D.; BUDZYN, K.; KUTA, W. A microscopic view of false tendons in the left ventricule of the human heart. **Flia Morphol**. v.56, p.31-39, 1997.

INTERNATIONAL COMMITTEE ON VETERINARY GROSS ANATOMICAL NOMENCLATURE(ICVGAN). 2012. Nomina Anatômica Veterinária. 5th ed. ICVGAN, New York. 78p.

PARTO, P.; TADJALLI, M.; GHAZI, S. R. Macroscopic and microscopic studies on moderator bands in the heart of ostrich (*Stuthio camelus*). **Global Veterinaria**. v.4, n.4, p.374-379, 2010.