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Auricular pavilion arteriography in cattle

Arteriografia do pavilhão auricular bovino

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Abstract

Although bovine auricular pavilion has been considered to have large surface vessels, descriptions of its vascularization are scarce. Notwithstanding this region has been used as an alternative route of administration of drugs. With help of an Anatomical specimen, arteriography and dissection have revealed a vast vascular network in the auricle. In this sense, once considered, this knowledge is capable of minimizing prospective complications from the unsuitable use of this region for therapeutic and anesthetic procedures. This study aimed to standardize and describe the arteriography of bovine auricular pavilion in order to acquire a greater knowledge about the local vasculature. Five steers, about one-year-old and weighing about 200 kg, were used, among which three Holstein x Gir crossbred and two Gir purebred. None had lesions in the ear. Of these, two animals were submitted to the standardization of procedures for arteriographies; two others underwent the examination itself. Another bovine, from necroscopy, was subjected to dissection of the ear. The arteriographic images and anatomic dissection pictures were compared to describe anatomical relationships. Arteriography enabled the visualization of lateral, lateral intermediate, intermediate, medial intermediate auricular branches, as well as the medial auricular branch. Conversely, the dissection of the superficial cervicoauricular muscle, between the auricular pavilion and the cornual process base, indicated a poor subcutaneous vascularization of this region, contrasting the rich vascularization near the auricular pavilion. The auricular pavilion arteriography identified a complex vascular network, which in terms of a practical application precludes the administration of slow-absorption drugs in this area. Nevertheless, the dissection images showed the poor subcutaneous vascularization of superficial cervicoauricular muscle surrounding area, making this site more favorable for the administration of long-acting substances.
Key words: Blood vessel. Bovine. Diagnosis. Ear. Therapy.

Resumo

Apesar de já ter sido considerado que o pavilhão auricular bovino apresenta grandes vasos superficiais, as descrições da vascularização desse local, que tem sido utilizado como via alternativa de administração de medicamentos no campo, são escassas. Nesse sentido, a arteriografia e a dissecação da região da

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concha auricular em peça anatômica demostraram a existência de uma vasta rede vascular local, de modo que, se considerado, esse conhecimento é capaz de minimizar possíveis intercorrências oriundas do uso inadequado da região para procedimentos terapêuticos e anestésicos. Este estudo objetivou padronizar e descrever a arteriografia do pavilhão auricular em bovinos, como base para ampliação dos conhecimentos acerca da vasculatura local. Para tanto, foram utilizados cinco bovinos machos, três mestiços (Holandês x Gir) e dois da raça Gir, com aproximadamente um ano de idade, peso médio de aproximadamente 200 kg e sem alterações na aurícula. Destes, dois foram submetidos à padronização dos procedimentos para confecção das arteriografias, dois à execução efetiva do exame e um bovino, oriundo de exame necroscópico, foi submetido à dissecção da região em questão. As imagens do exame arteriográfico e a documentação fotográfica da dissecação anatômica da concha auricular foram confrontadas e descritas as relações anatômicas. Através da radiografia contrastada, foi possível observar os ramos auriculares lateral, intermédio lateral, intermédio, intermédio medial, bem como o ramo auricular medial. Entretanto, a dissecação da área do músculo cérvicoauricular superficial, entre a base do pavilhão auricular e a base do processo cornual, indicou pequena vascularização subcutânea desta região em contraposição com a rica vascularização junto ao pavilhão auricular. A arteriografia do pavilhão auricular identificou uma malha vascular complexa que, em termos de aplicação prática, tornaria o local desfavorável à administração de fármacos que visam lenta absorção. Por outro lado, a dissecação da região adjacente, do músculo cérvicoauricular superficial, demostrou a existência de pequena vascularização subcutânea, conferindo ao local características favoráveis à administração de substâncias de ação prolongada.
Palavras-chave: Bovinos. Diagnóstico. Orelha. Terapêutica. Vasos sanguíneos.

Introduction

Arteriography and venography have been employed in studies on the vascularity of dermis, subcutaneous tissues, and other anatomical structures. Both techniques allow the evaluation of the vascular perfusion by means of contrast radiography (RUCKER et al., 2006). These exams help clarify the physiological and pathological aspects of an evaluated structure. In horses, these methods have been used in the diagnosis and in the therapeutic guidelines of laminitis (EASTMAN et al., 2012), and in bovine, in the study of vascular alterations due to chronic laminitis. These studies showed dilation of the lumen of the digital artery, tortuosity, and irregular course, as well as constriction of the final branches of this vessel (SINGH et al., 1994). After nonionic contrast administration on the common carotid artery, studies performed in dogs have demonstrated that cervical cranial arteriography allowed the visualization of parameters such as trajectory, caliber, uniformity, and vessel wall thickness. The method was also able to identify thrombi or hemangiomas, both in the vessel receiving the contrast and its branches (PAPUC et al., 2010). However, although the arteriographic examination presents us with a number of possibilities, it has not been used to study the vascular anatomy of the auricle in bovine.

Bovine ear cartilage features large surface vessels, but intracartilaginous capillaries are few or absent (SILVA et al., 2009). Regardless of the presence of these vessels, the bovine auricular pavilion has been used as an alternative site for the administration of drugs, mainly through subcutaneous auricular implants (PRIETSCH et al., 2014). In addition, despite being little described in the literature, field routine practice has shown that the use of this site for subcutaneous application of drugs, especially against the need to medicate animals close to the slaughter date or to reduce the residual load of these substances in products extracted from medicated animals. In spite of the low and continuous absorption via subcutaneous route (SPINOSA et al., 2011), the influence of the rich vascular bed in auricle on drug absorption should be investigated. Under these circumstances, the richer the vascularity where substances are deposited, the faster their absorption (MALAMED, 2013); therefore, when administered in the auricular pavilion, drug absorption could be anticipated.

Whether via implants or direct administration, drug application onto the auricular subcutaneous route in bovine should consider the relationship between local vascularization and absorption time. It should be noted that early-absorbed drugs can carry risks to animal health, byproduct safety, and even animal welfare. Then a sound knowledge of the ear vascular anatomy brings more than simply theoretical insights, being also useful in practical terms. Also, it might make the subcutaneous injection at the bovine auricle, whose use has already been verified in the field, an alternative route for drug administration. Another applicability of this knowledge would be highlighting the alternative use of this place for the administration of drugs according to the vascular characteristic of each of its regions (auricular pinna and base). In this context, arteriography emerges as an important tool, fully capable of contributing to the description of the vascular anatomy of the studied region.

Therefore, the use of arteriography in vascular anatomy studies (FREITAS, 2015) may assist in identifying the vascular network of the bovine auricular pavilion. Once the auricular vascularization, which has been poorly described so far, has been clarified (NICKEL et al., 1981; SCHALLER, 1999), a deeper anatomical knowledge about the area is possible with a potential contribution to clinical practice. Consequently, knowing the vascular anatomy of the bovine ear by arteriography could even minimize prospective complications from the inappropriate use of this site for therapeutic and anesthetic procedures. Moreover, despite not being within the aims of this research, the anatomical knowledge acquired from the arteriographic exams may contribute to the development of new pharmaceutical and anesthetic modalities. In doing so, subcutaneous and intravenous routes of the auricular pavilion would become popular as alternative means of medication administration in the clinical routine, being able to avoid the damage of areas used for prime cuts by the meat market, or, in a clinical scope, other more traditional routes of drug administration. For this purpose or for application in different diagnostic methods, this technique still needs to be standardized for the anatomical structure to be evaluated. Thus, this study aimed to standardize and describe the arteriography of the auricular pavilion in bovine as a basis for increasing the knowledge of the local vasculature.

Material and Methods

The study was carried out at the Veterinary Hospital, College of Veterinary and Animal Science, Universidade Federal de Goiás (HV / EVZ / UFG), between June and December 2013. All the procedures were carried out according to the guidelines of the Ethics Committee on Animal Use from the UFG (process number 150/2010). Five steers were used in the analyses: three Holstein x Gir crossbreds and two Gir purebreds. The animals were about one-year-old and weighed about 200 kg (180 – 220 kg) and had no apparent changes in their auricular pavilion. Contrast injection and radiographic documentation were performed in four bovines. Of these, one crossbred and one purebred animal were used to define the procedures to be performed including an ear-base tourniquet, artery location, and quantifications of administered contrast and time between administration and radiographic imaging. After standardization, arteriographies were performed on the other two animals. The fifth animal, with the same characteristics as the crossbreds, was obtained from the Animal Pathology Department in UFG, where it had been sent for necropsy for didactic purposes. After the examination, only the head was taken to dissection and identification of blood vessels in the auricular pavilion.

For auricular arteriography, the animals were sedated with 2% xylazine hydrochloride (Dorcipec®, 2% Xylazine Hydrochloride, Vallée, Brazil) and contained in lateral decubitus, with pelvic and thoracic limbs immobilized and extended, conferring

greater safety to the procedure according to Silva et al. (2012). Afterward, all ears underwent trichotomy followed by antisepsis with degerming povidone-iodine (Riodeine Dermo Suave Degermante®, polyvinylpyrrolidone iodine in degerming solution, Rioquímica, Brazil), and topical application of 10% povidone-iodine (Riodeine Dermo Suave Tópico®, polyvinylpyrrolidone iodine in aqueous solution, Rioquímica, Brazil). At the base of the auricular pavilion, a tourniquet was performed with a 5-mm rubber tube, whilst the lateral, intermediate, and medial branches of the auricular artery were cannulated using a 23-gauge scalp vein needle. For each scalp, 1mL of sodium heparin (Hemofol®, Heparin Sodium 5000 IU, Cristália, Brazil) was used to minimize coagulation.

As a contrast, 3 mL of Iopamidol-300 (Iopamiron 300®, Iopamidol 612 mg mL^{-1}, Bayer Schering Pharma, Germany) was injected into each artery, totaling six milliliters. Radiographic images were obtained using portable x-ray unit "orange 1060HF" (EcoRay Co., Ltd., Seoul, Korea), calibrated at 42 kilovolts and 1.25 milliamperes per second, and positioned 90 centimeters away from the ear. Chassis tags of 18 x 24 centimeters, loaded with Kodak Medical X-ray Film (New York, USA), were positioned ventrally to the ear, and the rays were directed dorsally. The time between the administration of the contrast and the radiography was 30 seconds. X-ray film development was carried out using a radiographic image scanner (Fuji Computed Radiography Processor, Model CR-IR 357, Fuji Film Corporation, Tokyo, Japan). Prior to contrast administration for arteriography, the auricle was radiographed without the contrasting substance for comparative purposes.

Radiographic images were interpreted according to Ugolotti et al. (1994), and vascular anatomy description was performed according to Nickel et al. (1981) and Schaller (1999).

The common carotid artery from the animal corpse sent for necropsy was identified and isolated. Then, this vessel was injected with liquid latex until filling the auricular vessels and, later, sent for dissection. For this, the contralateral common carotid artery was ligated to avoid the liquid return. During injection, acetic acid was used for latex coagulation inside smaller caliber blood vessels. The head was kept frozen in a freezer for seven days and subsequently dissected. The auricle blood vessels and cervicoauricular muscle region were dissected from the auricle base to the horn base.

Arteriography images and photographic documentation from the auricular pavilion anatomic dissection were cataloged and compared, describing the anatomical relationships between both.

Results

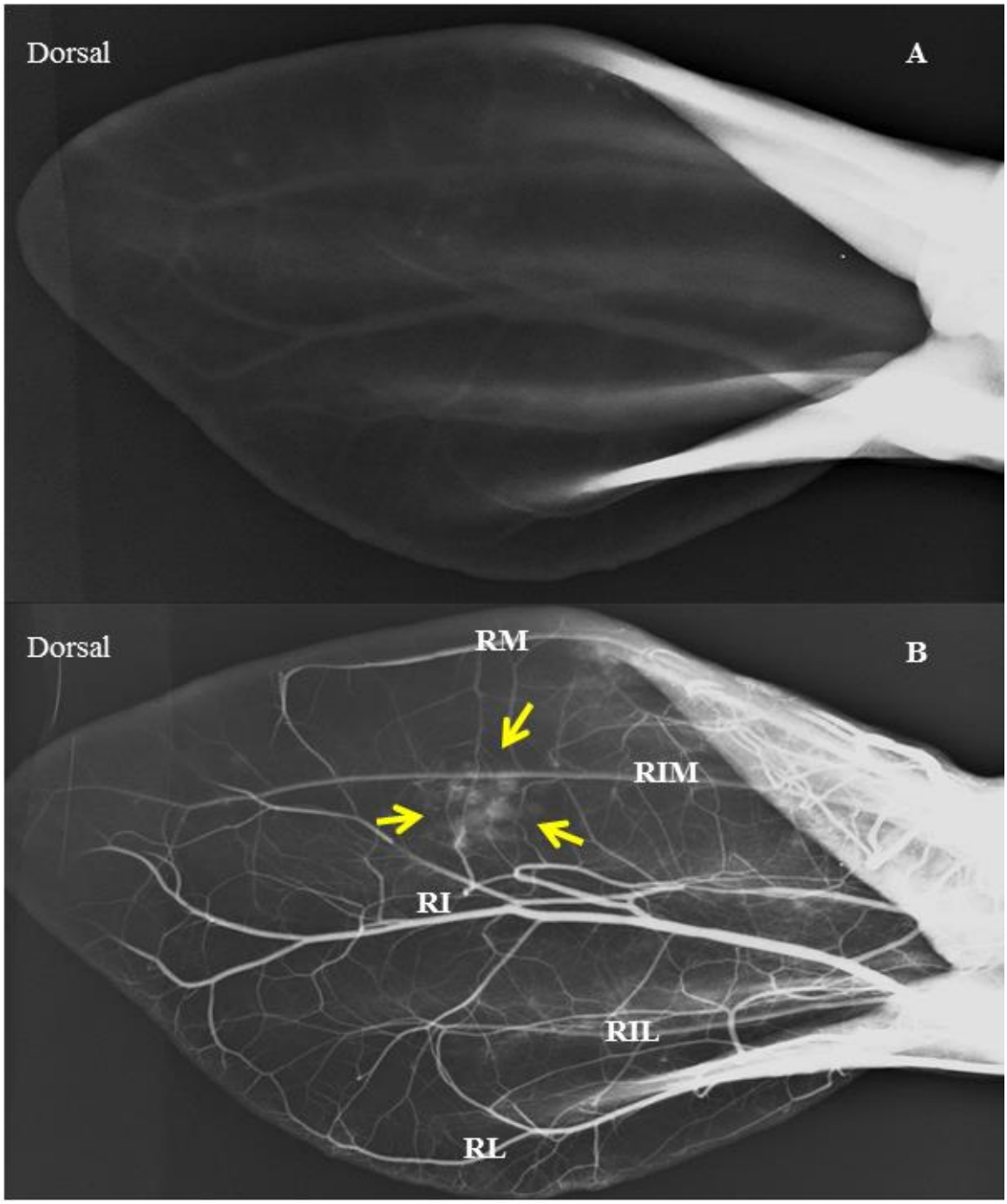
Bovine auricle radiographies without contrast injection were unable to identify local vascular network (Figure 1A). However, using contrast, they made possible the observation of the lateral, lateral-intermediate, intermediate, medial-intermediate, and medial auricular branches. (Figure 1 B).

Chemical and physical restraint was essential to immobilize the animals in lateral decubitus and avoid sudden head movements during cannulation of lateral and intermediate lateral auricular artery branches, contrast application, and radiographic examination of the ear.

In the absence of a pre-existing protocol for bovine auricular arteriography, two animals (four auricles) were enough to set the procedures to be performed including an ear-base tourniquet, artery location, quantification of administered contrast and time from administration to radiographic imaging.

Examination attempts without prior tourniquet application in the ear base were unsuccessful since the contrast rapidly disappeared. Therefore, for more reliable results, the tourniquet was kept until the end of the radiographic reading process. Scalp number 23 could be replaced by catheter number 24, however, using different numbers may damage the vessels with contrast reaching the extravascular spaces.

Figure 1. Ventrodorsal radiographs of the left auricle in 1-year-old crossbred bovine (*Bos taurus* x *Bos indicus*), weighing nearly 200 kg. Radiograph without contrast, impairing the identification of auricular vessels (A). Arteriography allowing the visualization of auricular vessels (B). Imaging artifact caused by the presence of *Boophilus microplus* in the region between arrows. Auricular branch of the caudal auricular artery (RL); lateral intermediate branch (RIL); intermediate branch (RI); medial intermediate branch (RIM) and medial branch of the rostral auricular artery (RM).

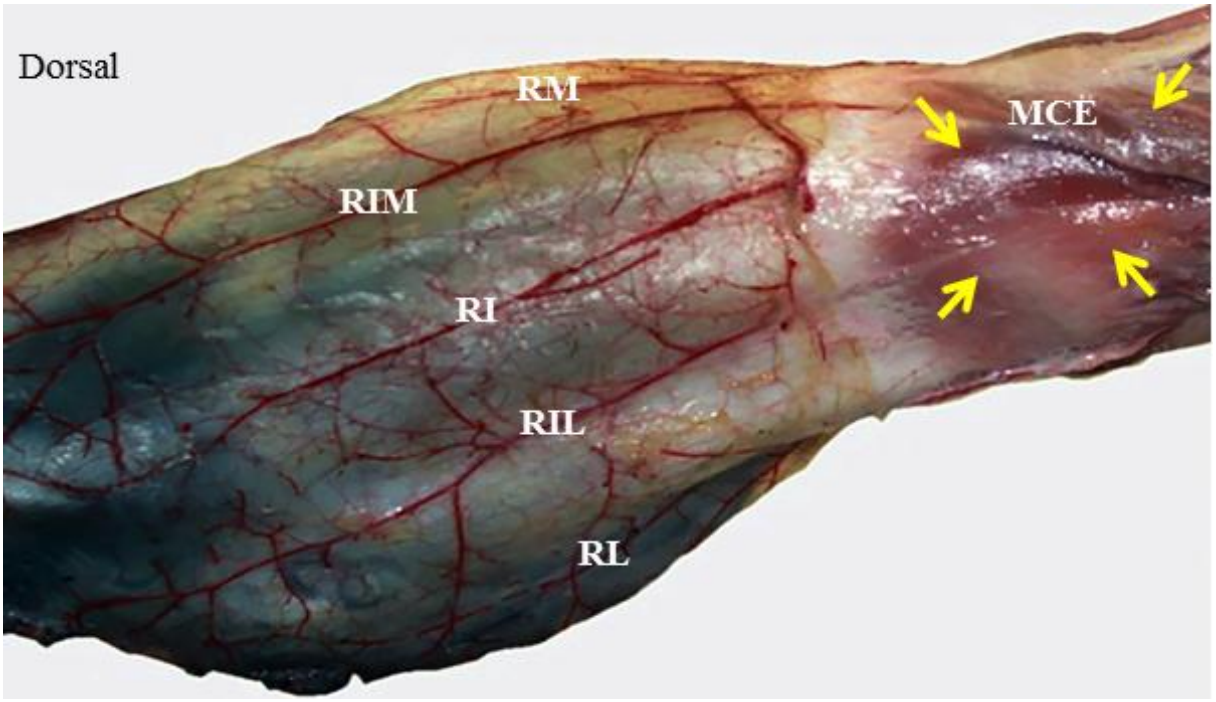


The volume of contrast used in this study (3-mL for lateral intermediate and 3-mL for intermediate auricular branches) was sufficient for examination and for vascular branch identification in a one-year-old bovine weighing nearly 200 kg. On the contrary, lower contrast volume resulted in less clear images, while excess compromised the integrity of vessels.

Furthermore, image quality was compromised when radiographies were taken in less than or greater than 30 seconds after contrast application, or even when the distance between the apparatus and the region being X-rayed was other than 90 cm. Likewise, establishing the sequence of actions without regard to the animal age and breed caused a rupture of blood vessels, diffusion of contrast into the subcutaneous space, and loss of image sharpness for small blood vessels. In addition, venous vessels could also be stained during the examination.

The dissection of an Anatomical specimen made it possible to clarify the vascular network of the auricle (Figure 2), enabling comparisons with the arteriographic findings. The repletion of the arterial bed with latex substantially increased the diameter of blood vessels, facilitating dissection and identification of smaller caliber arteries and veins.

Figure 2. Anatomical specimen showing the arterial vessels of the left ear of a 1-year-old crossbred bovine (*Bos taurus* x *Bos indicus*), weighing nearly 200 kg. One should note a poor subcutaneous vascularization between the auricular pavilion and the cornual process bases (arrows). Cervicoauricular muscle (MCE); Auricular branch of the caudal auricular artery (RL); lateral intermediate branch (RIL); intermediate branch (RI); medial intermediate branch (RIM) and medial branch of the rostral auricular artery (RM).



The dissection of the superficial cervicoauricular muscle, between the auricular pavilion base and the cornual process base, showed the latter has a poor subcutaneous vascularization while a rich vascularization is observed near the auricular pavilion.

Discussion

A simple radiographic examination impairs the vascular assessment of bovine auricle since soft tissues and blood vessels show similar radiographic density (BURK; ACKERMAN, 1996). Thereby, the arterial network should be highlighted for arteriography with radiopaque contrast since it

emphasizes the difference in generated radiographic density (POTTER et al., 2013).

Auricular arteriography standardization requires important procedures including animal immobilization in lateral decubitus, radiographic imaging without contrast injection, ear base tourniquet, auricular artery cannulation, and contrast ideal volume. Changes in such procedures, with no regard to animal age and breed, may compromise the final result. Among the complications observed in the present study, which may occur in other studies, are rupture of blood vessels, diffusion of contrast into subcutaneous spaces, and poor visualization of small blood vessels by low quality images. Younger or older animals, from different breeds, and with distinct auricle sizes may require lower or higher contrast volume. Using a low contrast volume may result in less clear images, while excess compromise vessel integrity.

The use of tourniquet at the base of the auricular pavilion was fundamental for the procedure success and should also be maintained during the radiographic assessment. As mentioned in digital venography of equine (BRUNNER et al., 2008; D'ARPE; BERNARDINI, 2010; RUCKER et al., 2006) and bovine (BOOSMAN et al., 1989), this methodological detail cannot be overlooked. Neglecting this detail may impair the examination even with the use of higher contrast volume and serial radiographs. Once dismissed, contrast may dissipate rapidly, and even if some blood vessels are identified, radiographic images might be affected. Conversely, with tourniquet use, radiographic images must be obtained in a pre-established time. In the venography of equine digits, a maximum of 45 seconds was considered ideal for good quality images (RUCKER et al., 2006). In this study, however, 30 seconds were enough to proceed with the arteriography of the bovine auricular pavilion with a satisfactory result, which might have been due to the lower anatomical complexity of this Anatomical specimen.

In addition to highlighting the bovine ear main arteries, drawing on arteriovenous anastomoses, the arteriography also evidenced many other veins, what hinders the identification of main arteries. Besides that, the arteriographic examination could also identify blood drainage since the contrast injected into the caudal auricular artery reached the caudal auricular vein. In short, the bovine auricular pavilion presented a complex vascularization, being irrigated by the medial auricular branch of the rostral auricular artery and by the lateral, lateral intermediate, and medial intermediate auricular branches of the caudal auricular artery (NICKEL et al., 1981). Such vascular network also includes the deep auricular artery and the intermediate branch, also deriving from the caudal auricular, being the latter more evident in small ruminants (SCHALLER, 1999).

The visualization of a wide range of blood vessels by arteriography has a practical potential, such as refusing the use of subcutaneous auricular implants in the bovine pavilion, when aiming at a slow absorption, given its rich vascularization. Moreover, in order to fully enlighten images, this technique should be associated with the dissection of blood vessels within the region in question. This would make the results of the arteriographic examination more tangible and consequently measurable. Even though the description of the ear vasculature has been established (NICKEL et al., 1981; SCHALLER, 1999), some aspects need to be considered. An argument justifying this concern is the lack of studies approaching the local vasculature. A few studies (GETTY, 1986; NICKEL et al., 1981; SCHALLER, 1999) have described blood supply and drainage but have not raised the description of such intricate vascular mesh formed by thin vessel anastomoses, seen here in the arteriography. If disregarded in clinical practice, it may underestimate the time taken for drug absorption.

Still, the complex vascular network found in the arteriographic examination of the auricular pavilion has not yet been scrutinized in the

anatomical treatises (DYCE et al., 2010; GETTY, 1986; KÖNIG; LIEBICH, 2011). Therefore, this consistent vascular network may partially justify a hypothetical early absorption of drugs when indiscriminately administered or implanted in this area. It could lead to shorter duration of action or even poisoning, mainly by endectocides when formulated in high concentrations. Differently, the arteriography standardization of bovine auricular pavilion associated with cervicoauricular muscle dissection (between the pavilion and cornual process bases) pointed to a poor subcutaneous vascularization. These results are believed to contribute to the selection of the most suitable sites for liquid drug administrations in the case of the auricle, especially when a prolonged release of certain active principles is aimed. Additionally, the dissection of the cervicoauricular muscle subcutaneous tissue validates the site selection for implantation of subcutaneous auricular devices aiming slow absorption, i.e. the base of the ear (PRIETSCH et al., 2014).

Based on the arteriography and dissection results, along with their contribution to clinical practice, the cervicoauricular muscle region of bovines can be suggested as a place for the administration of prolonged release drugs. Contrary to what was observed in the pavilion area, which has a great vascular bed and, consequently, a more intense absorptive capacity. Notwithstanding, it is worthy to remark that neither therapeutic nor pharmacological indications were the goals of this study, being only a practical link of the generated knowledge. Nevertheless, further studies should be carried out on serum concentrations for drugs to be administered in these two auricular regions, so that effective and adequate comparisons can be made about the absorptive characteristics of each studied site (pinna and cervicoauricular muscle).

Finally, after cross checking with literature background, these results show us that the choice of the bovine auricular pavilion should be analyzed carefully in the case of administration of different therapeutic protocols, from hormones to synchronize the estrous cycle (TREGASKES et al., 1994) to endectocides, antibiotics, and insecticides (BAGGOT, 1988). Therefore, in theory, the auricle has been tested in drug delivery systems, so the elucidation of its vascular characteristics, via arteriographic examination associated with the dissection of the region, can streamline the availability and arrival of certain drugs into its action site. Thus, knowing the local vasculature provides more resources for the correct choice of place to administer drugs in the auricle. And, as a consequence, it contributes to the efficiency of treatments by allowing, according to the purpose of the therapy, accelerating or prolonging the drug release, as well as reducing potential toxicity against a gradual absorption of active principles (PRIETSCH et al., 2014) when deposited correctly in areas of slow absorption. Furthermore, the results obtained in this study allow us to suggest the auricular pavilion as another alternative site for drug administration routes, including through slow intravenous injection and especially during transurgical anesthetic readministration, without compromising the main vessels.

Conclusions

The arteriography of auricular pavilion highlighted a complex vascular network, which in terms of practical application makes this body part unfavorable for the administration of prolonged-release drugs.

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