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Original article

ARTERIAL VASCULARISATION OF PIG'S AUDITORY TUBE WITH RESPECT TO *A. PALATINA ASCENDENS* – A CORROSION CAST AND MORPHOMETRIC STUDY

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Summary

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The aim of this study was to determine species-specific features of arterial vascularisation of the auditory tube of domestic pigs as a segment connecting the nasopharynx and the middle ear with regard to the increasing role of pigs as most appropriate model of human biomedical research. The arterial branches involved in the vascularisation of the auditory tube were described on corrosion casts. It was found out that in the direction of the middle ear, the tube received blood from branches of *a. palatina ascendens*, *a. meningea media*, *a. temporalis profunda caudalis* and *rete mirabile epidurale rostrale*, out of which the first of enumerated arteries was the most involved. In the majority of cases (87.5%) it gave off two branches that ramified in the lateral and medial walls of the tube. For the first time, it was demonstrated that *a. palatina ascendens* was separated from the facial artery and not from *a. lingualis*. The measurements on casts showed that the diameter of left *a. palatina ascendens* was statistically significantly larger than right one in both sexes, and that average diameters of either right or left arteries were larger in males. The results allowed concluding that *a. palatina ascendens* was predominantly involved in the arterial blood supply of porcine auditory tube.

Key words: auditory tube, corrosion cast, morphometry, vascularisation, pig

INTRODUCTION

The auditory tube (*tuba auditiva*) or Eustachian tube (*tuba Eustachii*) plays an important role in the functions of the pharynx and the organ of hearing. This is a complex structure and the only tube connecting the nasopharynx to the middle ear, responsible for ventilation and drainage of this part of the organ of hearing (Seibert & Danner, 2006; An *et al.*, 2019).

It is acknowledged that through the auditory tube, nasopharyngeal inflammations are transferred to the middle ear causing its inflammation. A substantial part of people affected with *otitis media* suffer from seriously compromised auditory perception (Gluth *et al.*, 2011; Kivekäs *et al.*, 2015; Williams *et al.*, 2016).

The vascularisation of the auditory tube in mammals is relatively poorly studied. Available literature is mainly in men, with reports of branches leaving arteries adjacent to the auditory tube – *a. pharyngea ascendens (a. carotis externa)* and two branches of *a. maxillaris – a. meningea media* and *a. canalis pterygoidei* (Hacein-Bey *et al.*, 2002; Szymanski & Agarwal, 2018).

The elucidation of specific features of porcine auditory tube vascularisation is supported by the significantly increasing body of evidence from the last 2–3 decades, providing convincing data that this animal species (including the mini pig) is the most appropriate model for biomedical research in humans (Massoud *et al.*, 1994; Pracy *et al.*, 1998; Eberlova *et al.*, 2017; An *et al.*, 2019).

The lack of information about the vascularisation of the auditory tube in domestic pigs, and the relevance of the species for human biomedical research motivated the present study that was carried out to determine the species-specific features of arterial vascularisation in this animal species.

MATERIALS AND METHODS

Animals

Head halves from 8 male and 8 female pigs, Bulgarian White × Landrace crosses, 6 months of age, weighing 95–110 kg slaughtered for meat in a licensed slaughterhouse according to the national legislation, were used (a total of 16 male and 16 female halves). After separation of heads from trunks, they were transported to the Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria, prepared for further processing by cleaning of the cut surface and dissection of common carotid arteries and placed in a refrigerator at 4 ^{o}C for 24 hours.

Permanent preparations from corrosion casts of head's arterial system

Through a cannula placed in the right and left common carotid arteries, the arterial system was perfused with physiological saline, until the fluid leaving the respective jugular vein was clear to avoid air bubbles formation at the time of resin introduction. The filling of arteries was done through the same cannulae with cold curing acrylic resin Duracryl® Plus U (Spofa Dental, Praha, Czech Republic), the liquid phase (monomer) was cooled to 4 ^{o}C, and then, additionally stained with red dye Neozapon Red 365 and fluorescent powder Rhodamine 6G, dissolved in ethanol. The coloured monomer was mixed with the powder phase of Duracryl® Plus U upon constant stirring for 40–60 s and using an original device constructed for such purposes (Tsandev *et al.*, 2019). One hundred and twenty mL of the mixture was simultaneously injected in each of carotid arteries, then the latter were ligated to prevent the backflow of the resin. On the other day (after 24 h), the skin and thicker areas of subcutaneous adipose tissue were carefully removed, and filled heads were left for 14 days at room temperature for complete polymerisation of the resin. After that, heads were placed for 48 h in a container with tap water, warmed to 37 ^{o}C for maceration of non-filled soft tissues. Then, preparations were corroded with a solution of equal parts 1% KOH and 0.5% NaOH for 5 days at 45–47 ^{o}C, rinsed on slowly running tap water and additionally cleansed by manual removal of non-corroded soft tissues. Afterwards, corrosion casts with preserved bones were processed with 50% hydrogen peroxide solution and tap water at a ratio of 1 mL peroxide per 1 liter water for 24 hours, dried and placed with the dorsal surface on the working table (on nasal and frontal bones) for dissection of heads into two parts by means of a fine hand saw for metal, first along *linea mediana ventralis* between the lower medial incisors – I_1, and then, the head was placed in the opposite position (on the ventral surface or ventral mandibular margin) by holding it carefully without moving apart the two head halves. The complete separation of the remaining part was done similarly in caudorostral direction, along the median line. The obtained equal halves were carefully cleaned and prepared for examination.

Micromorphometry of a. palatina ascendens diameter

The diameter of corrosion cast of the ascending palatine artery was measured using an eye-piece micrometer of MBS–10 microscope calibrated with standard object-micrometer (Carl Zeiss–Jena) with precision of 0.01 mm. Measurements for the right and left arteries in male and female pigs were submitted to statistical analysis.

Statistical analysis

The data were presented as mean ± SD were processed by one-way analisys of variance (one–way ANOVA) followed by Tukey-Kramer's *post hoc* test (GraphPad Prism 6 for Windows, GraphPad Software, Inc., USA). P values <0.05 were considered statistically significant.

The used anatomical terms are in compliance with Nomina Anatomica Veterinaria (2017).

RESULTS

Macroscopy of arterial corrosion casts demonstrated that the amount of introduced polymer and consequent technological processing allowed obtaining high-quality preparations. Not only the main arteries, but also their branches could be clearly and easily identified on them: the common carotid artery (*a. carotis communis)*, the external and internal carotid arteries (*a. carotis externa* and *a. carotis interna*), maxillar artery (*a. maxillaris)*, basilar artery (*a. basilaris*), the ring of arteries located at the basal brain surface (circle of Willis; *circulus arteriosus cerebri*, rostral epidural rete mirabile (*rete mirabile epidurale rostrale*) etc. (Fig. 1).

The careful examination of arteries located at the auditory tube level showed that the main artery supplying blood to the tube was the ascending palatine artery – *a. palatina ascendens*. It arose at an almost right angle from the facial artery – *a. facialis*. Along its path towards to soft palate (beneath the tympanic bulla, just to the pharyngeal part of the auditory tube), *a. palatina ascendens* followed a rostrally curved course, with well pronounced dorsal and ventral arcs that formed an angle of approximately 75–80°. After the separation of a branch for the soft palate, *a. palatina ascendens* coursed rostrodorsally to the auditory tube, where it ramified into 2, more rarely into 4 branches embracing the both walls of the tube (Fig. 1).

The observed patterns of *a. palatina ascendens* ramification were three (Fig. 2), as followed:

- Variant I

The artery was separated into a dorsal (directed along the lateral tube wall) and a ventral (directed along the medial tube surface) branches, the latter being of a visibly larger size. This variant was observed in the majority of cases: 21 (65.6%), in 7 male and 14 female pigs.

- Variant II

The separation of the artery was similar, but with a larger dorsal branch. This variant was present in 7 cases (21.9%), only in female pigs.

- Variant III

The artery gave off 4 branches, among which the two central ones were of larger diameter than peripheral ones. This variant was seen in the smallest proportion of studied preparations – 4 (12.5%), in 3 male and 1 female pigs.

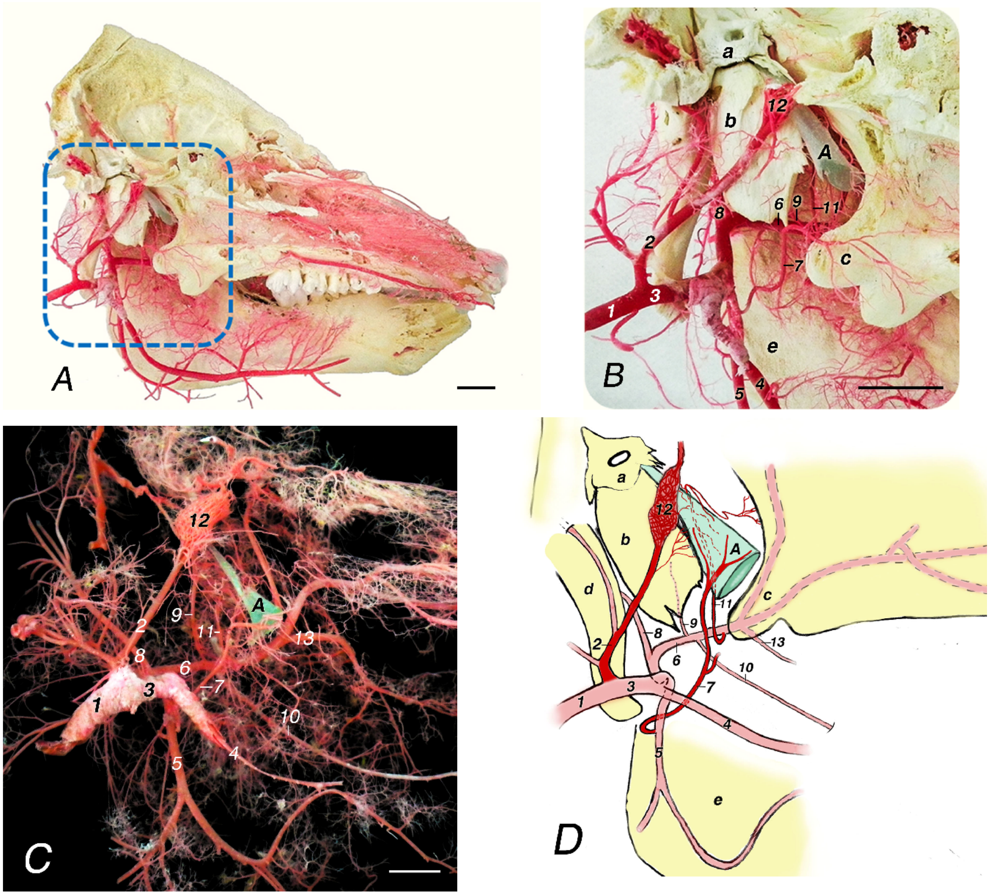

Fig. 1. A) Corrosion cast of head's arterial system – left half with preserved bones and auditory tube cast; **B)** The dotted square of A) at a higher magnification; **C)** Corrosion preparation with auditory tube cast; **D)** A general scheme of B) and C). Bar=2 cm. **A**. *Tuba auditiva*; **a**. *Pars petrosa* (*Os temporale*), **b**. *Bulla tympanica* (*Os temporale*), **c**. *Hamulus pterygoideus*, **d**. *Proc. paracodylaris*, **e**. *Mandibula*; **1**. *A. carotis communis sinistra*, **2**. *A. carotis interna*, **3**. *A. carotis externa*, **4**. *A. lingualis*, **5**. *A. facialis*, **6**. *A. maxillaris*, **7**. *A. palatina ascendens*, **8**. *A. auricularis caudalis*, **9**. *A. temporalis profunda caudalis,* **10**. *A. alveolaris inferior*, **11**. *A. meningea media*, **12**. *Rete mirabile epidurale rostrale*, **13**. *A. buccalis*.

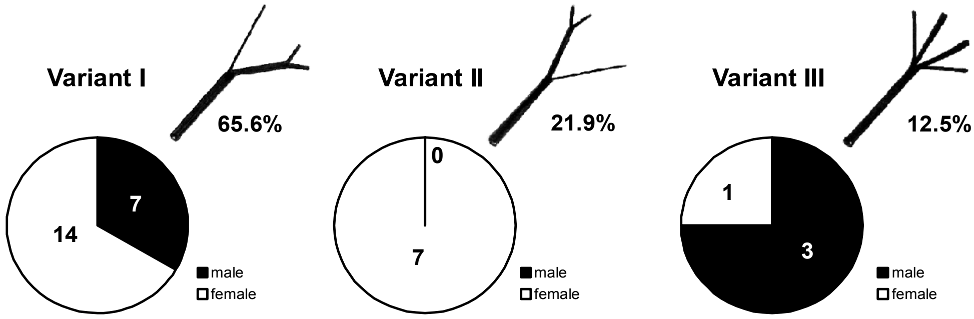

Fig. 2. Occurrence of variants of *a. palatina ascendens* ramifications in both sexes.

The area supplied with blood by *a. palatina ascendens* included about 65–70% of auditory tube wall for all three variants. The remaining relatively small part showed branches of *a. meningea media* and *a. temporalis profunda caudalis*, as well as fine arterial branches from the periphery of *rete mirabile epidurale rostrale*.

The orientation of the auditory tube and its two walls: medial and lateral (Tsandev *et al.*, 2020) allowed observing the branches of abovementioned arteries and of rete mirabile, ramifying in the tube and surrounding soft tissues.

The dorsal branch of *a. palatina ascendens* was dichotomously branched at *ostium pharyngeum tubae auditivae* in a substantial part of cases (87.5%), supplying blood to the major part of the ventromedial auditory tube surface, surrounding soft tissues and the pharyngeal orifice, in whose rostral and caudal part was observed a fine capillary network (Fig. 1).

The dorsomedial segment of the tube (up to *ostium tympanicum tubae auditivae*) was vascularised by small-sized horizontally located 4–5 branches coming from the periphery of *rete mirabile epidurale rostrale*, and at a lesser extent, by very short small-sized branches of *a. meningea media*, located in the vicinity.

A. meningea media was involved in the vascularisation of the caudolateral tube wall. Along its course to the cranial cavity, it curved anteriorly to *bulla tympanica* (at the base of the skull) and most commonly, gave off three well visible branches that mainly penetrated in soft tissues laterally to the tube and in the soft palate. The caudolateral auditory tube wall received also blood from their terminal branches.

The caudal part of the tube (the dorsal one-third of its length), was supplied by branches of *a. temporalis profunda caudalis*, whose diameter was small. Around the pharyngeal orifice of the tube located caudolaterally, the vascularisation was from branches of *a. palatina ascendens*.

Measurements of diameters of *a. palatina ascendens* on corrosion casts are given in Table 1. The right artery in male pigs was with slightly larger diameter – by 0.06 mm that in females. On the left, the arterial lumen was also larger in male pigs – by 0.09 mm. In both sexes, the diameter of the left artery was statistically significantly larger than that of the right one. In males, the size of right and left arteries was bigger than respective values in females, yet insignificantly.

Table 1. Diameter (mm) of *a. palatina ascendens dextra* and *sinistra* in both sexes. Data are presented as mean±SD (n=16)

Animals	Right	Left
Male	0.83±0.097	0.97±0.12**
Female	0.77±0.07	0.88±0.09*

*P<0.05; **P<0.01 – statistically significant differences between right and left arteries for a given sex.

DISCUSSION

The results of corrosion cast observations showed that *a. palatina ascendens* participated in the vascularisation of the larger part of the auditory tube wall in domestic pigs. The present study provides original data for its separation and branching pattern in this animal species.

Opposite to most literature reports that in pigs, *a. palatina ascendens* is either a branch of *a. lingualis* (Becker, 1960; Ghoshal, 1975; Barone, 1996; Waibl & Wilkens, 1996), or exits independently from *a. carotis externa* (Kowalczyk & Frackowiak, 2019), in all studied cases, it separated from *a. facialis*.

The results demonstrated that in most of cases – 28 (87.5%), the ascending palatine artery was branched dichotomously as it attained the auditory tube into two various-sized branches with different location as seen in variant I and variant II. The incidence of the third variant was considerably lower – 4 cases (12.5%): in this pattern, *a. palatina ascendens* gave off four branches, with larger size of medial compared to lateral ones. These facts allowed concluding that in both mentioned variants the number of branches of larger size was equal to those of smaller size: 1:1 in the dichotomous type and 2:2 – in the dispersed type. The fact could be hardly explained, yet it may be assumed that most probably, this is associated to provision of optimum vascularisation of the auditory tube. Although a small part of the auditory tube wall was supplied with blood by branches of other arteries – *a. meningea media* and *a. temporalis profunda caudalis*, as well as by *rete mirabile epidurale rostrale*, for practical purposes, e.g. for comparative and functional investigations, *a. palatina ascendens* should be used as the main primary blood vessel for porcine auditory tube, especially around its pharyngeal orifice. This could be interpreted also in support of the statement of Eberlova *et al.* (2017) that corrosion casts from pigs could be used in human anatomy learning. Therefore, it is important to note that the resin used in our study was far less expensive than plastics discussed in the mentioned research, and that quality of obtained casts was not inferior.

Morphometry of casts demonstrated that the diameter of *a. palatina ascendens* was larger in male pigs. Also, left arteries in both sexes were larger than right ones. The results provide proofs for sex-specific differences and support facts for different size and weight of paired organs e.g. brain hemispheres, testes etc. (Vankov & Ovcharov, 2008; Ocklenburg & Güntürkün, 2012). That is why, the established difference in the size of right and left arteries is objective and understandable.

In conclusion, the presented original data on the vascularisation of the porcine auditory tube could be relevant for future human biomedical research.

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