

## ORIGINAL ARTICLE



# Ostium-specific equine pulmonary vein myocardial sleeve gross anatomy and structural evidence of interatrial myocardial continuity with potential implications for ablation strategies

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## Abstract

**Background:** Pulmonary vein (PV) myocardial sleeves (MSs) harbour an arrhythmogenic substrate for atrial fibrillation. However, equine-specific data on MS distribution and wall thickness are limited yet essential for developing ablation strategies. In humans, posterior interatrial connections can compromise right-sided PV isolation.

**Objectives:** To study equine PV MS distribution, quantify ostial/branch diameters, antral lengths, characterise MS distal edge morphology, measure wall thickness and explore an interatrial myocardial continuity between the MSs of caudal branches draining through ostium III (PV III) and caudal vena cava (CaVC).

**Study Design:** Post-mortem, cross-sectional.

**Methods:** Twenty-three Warmblood hearts underwent left atrial and PV dissection. All four ostia (I–IV) were opened. From calibrated photographs, ostial and branch diameters, antral length, MS length and coverage, distal edge morphology (sharp/blurred), and myocardial thickness were assessed at standardised locations. In 6/23 hearts, a 3 × 1 cm dorsal interatrial septum (IAS) sample including PV III, and CaVC MSs was stained with Masson's trichrome and examined for myocardial continuity.

**Results:** Four principal ostia were present in 20/23 hearts and 5 in 3/23, with variable accessory vein openings. Ostia II and III were largest, I smallest. PV III showed full antral MS coverage with branch extensions, whereas MSs in other PVs were often confined to the antrum. Distal MS edges were often blurred at PV III. The MS thickness was largest at ostia II and III and smallest at ostium I. Histology showed that the dorsal interatrial myocardium was continuous with the PV III and CaVC MSs.

**Main Limitations:** Postmortem study, Warmblood cohort, few samples, no electrophysiology.

**Conclusions:** Equine PV MS anatomy is ostium-specific in extent, distal edge, and thickness. These data provide anatomical information relevant to ablation planning and to future catheter design. Morphological evidence suggests a dorsal interatrial myocardial continuity through the IAS that warrants electro-anatomical confirmation.

## KEYWORDS

atrial fibrillation, cardiac anatomy, catheter ablation, horse, interatrial septum, myocardial sleeves, pulmonary vein isolation

## 1 | INTRODUCTION

Atrial fibrillation (AF) is the most clinically relevant arrhythmia in equine medicine, compromising athletic performance and requiring targeted therapeutic interventions.<sup>1–7</sup> While medical therapy and electrical cardioversion remain first-line treatments, catheter ablation might be a promising curative option.<sup>8,9</sup> In humans, efficacy relies on the complete electrical isolation of arrhythmogenic tissue, most commonly the pulmonary vein (PV) myocardial sleeves (MSs). These MSs are extensions of the left atrial myocardium into the PVs, which can serve as a focal source of AF.<sup>10</sup> Similar mechanisms might be relevant in horses. Equine PV MSs exhibit several proarrhythmogenic features, including increased interstitial connective tissue (26.1% vs. 14.5% in the left atrium), upregulated L-type calcium channel expression, and lower bipolar voltages (1.2 mV vs. 3.4 mV in atrial tissue). Together with conduction slowing at the veno-atrial (VA) junction, these features provide a substrate for re-entry, and AF initiation and maintenance in horses.<sup>11,12</sup> Accordingly, PV isolation may be a promising therapeutic option to treat AF in horses.

Anatomically, equine PVs drain distinct lung regions and merge into collecting antra that empty into the left atrium through four principal ostia.<sup>13</sup> Kovács et al. recently reported ostial diameters and an overall range of MS length (0.4–4.8 cm),<sup>14</sup> but important details remain undefined in horses, including MS distribution across antra and first-order branches, distal edge morphology, and regional thickness of the myocardial wall at different sites.

In humans, ablation has evolved from focal, targeting discrete ectopic sites within the MS to employing circumferential strategies. In particular, wide antral circumferential ablation (WACA), in which lesions are created in the left atrium around the PVs, has demonstrated better long-term PV isolation than when ablation lines are placed strictly at the venous ostia.<sup>15–19</sup> Direct translation of this approach to horses is technically challenging because horses have markedly larger PV ostia, thicker atrial walls, and currently available catheters are designed for human cardiac dimensions.<sup>14,20</sup> Recent technical advances, particularly the development of the transeptal puncture technique and electro-anatomical mapping, now permit detailed studies of PV characteristics and targeted left atrial ablation in horses.<sup>21,22</sup>

In addition to PV anatomy, interatrial connections may also influence ablation success. In humans, posterior interatrial connections have been described as muscular continuity between the left and right myocardium, at the interatrial septum (IAS) near the fossa ovalis and the right PVs.<sup>23,24</sup> These connections are frequently epicardial, occur in a minority of patients and when present permit residual conduction or contribute to ablation failure in patients with AF.<sup>25</sup> In horses, PV III (the PV branches and antrum draining through ostium III) lies in close anatomical proximity to the dorsal IAS and the caudal vena cava

(CaVC), making this region a potential site for interatrial muscular continuity. Characterising these anatomical relationships may therefore be relevant to understanding possible structural substrates that could influence atrial conduction patterns.

This study aimed to describe the macroscopic anatomy of equine PV MSs in detail. Specifically, for each ostium, we measured ostial and branch diameters, antral length, extent of MS coverage within the antrum and extension into first-order branches, assessed the distal edge morphology (clear or blurred) and measured regional MS thickness. These anatomical data are intended to support the refinement of current ablation techniques and guide the design of catheters specifically for use in horses. Additionally, we aimed to qualitatively explore the hypothesis of a dorsal interatrial myocardial continuity in the region of the IAS, PV III, and CaVC, similar to the posterior interatrial connection described in humans.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

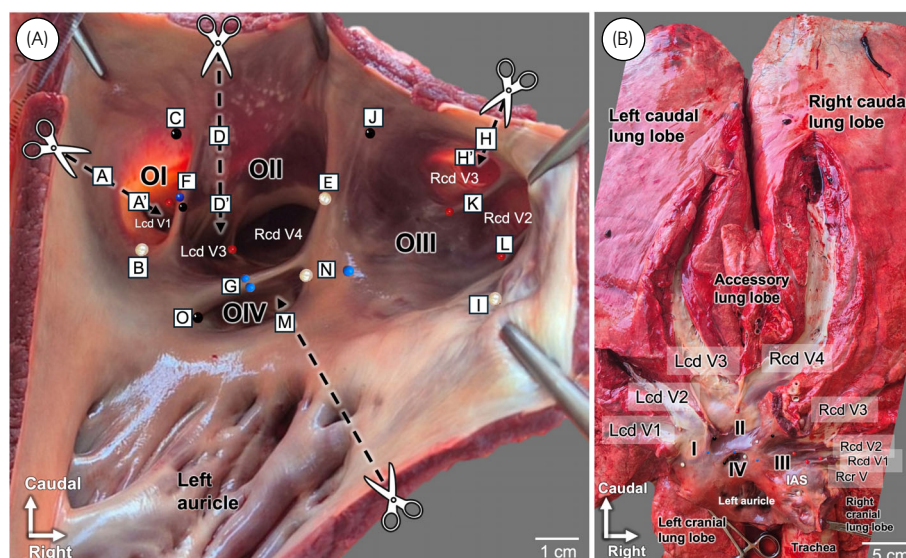
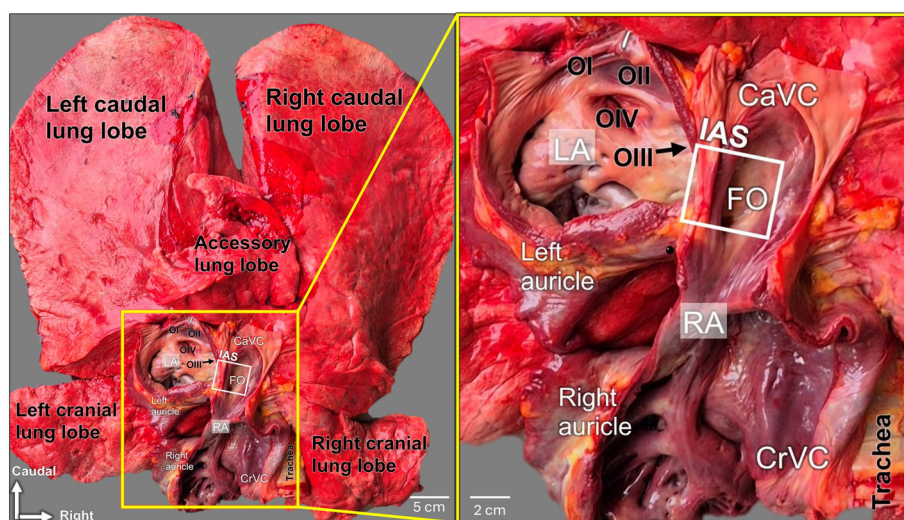
Post-mortem examination was performed on 23 adult Warmblood equine hearts within 3 h of euthanasia to minimise autolytic changes. Specimens were obtained from 18 horses euthanised for noncardiac reasons and donated by their owners for research purposes, and 5 hearts were collected from an abattoir. The study population consisted of 10 mares and 13 stallions or geldings, which varied in age from 4 to 28 years, with a mean (standard deviation, SD) of 18<sup>8</sup> years and bodyweight from 424 to 625 kg (550 (51) kg). Information regarding age, sex, and weight was unavailable for five horses from the abattoir.

### 2.2 | Dissection and orientation protocol

Hearts were removed along with the lungs, a long section of the aorta, and cranial and CaVC. The left and right ventricles were removed by a circumferential cut approximately 1 cm dorsal to the valvular annuli, and the right atrium was opened with a longitudinal lateral cut between the caudal and cranial vena cava, exposing the inner walls (Figure 1).

The PV ostia (I–IV) were identified from within the left atrium. First, the shape of each ostium was assessed. When the opening appeared elongated (i.e., with a visually appreciable long axis), the direction of the long axis was identified and two coloured pins (white and black) were placed at the VA junction ends of the axis. In ostia with a craniocaudal long axis (I, III), the white pin marked the cranial and the black pin the caudal margin, whereas in ostia with a

**FIGURE 1** Ventral view of the left (LA) and right (RA) atria and lung lobes after ventricular removal by a circumferential cut approximately 1 cm dorsal to the valvular annuli. The pulmonary vein ostia I–IV (OI–OIV) were exposed and the right atrium was opened by a longitudinal lateral cut between the caudal (CaVC) and cranial vena cava (CrVC). Ostium III lies adjacent to the interatrial septum (IAS) and fossa ovalis (FO), indicated by a white box. The yellow box outlines the atrial region, displayed in detail on the right side.



**FIGURE 2** Ostial orientation and dissection workflow. The right side of the images is right and the top is caudal. (A) Ventral view on the left atrium showing the four ostia (OI–OIV) and the colour-pin system. White and black pins were placed at the veno-atrial junction and marked each ostium's half-circumference along the longest axis. In ostia I and III, the white pin marked the cranial margin and the black pin the caudal margin, while in ostia II and IV, the white and black pins marked the right and left margins, respectively. A blue pin placed at the midpoint between the white and black pins served as an additional landmark. The red pins indicate the septa between the branches. Pins were digitally superimposed because this photograph was taken before pin placement. Dashed lines indicate the first incision axes of each ostium. Capital letters (A–O) indicate the measurement sites of myocardial thickness corresponding to the results in Table 3. (B) Ventral view on the left atrium and lungs after the initial incisions from the veno-atrial junction were extended into all branches and traced distally through the lung parenchyma, confirming the drainage territories of the lungs. Lcd V1–V3, left caudal pulmonary veins 1–3; Lcr V, left cranial pulmonary vein; Rcd V1–V4, right caudal pulmonary veins 1–4; Rcr V, right cranial pulmonary vein.

right-to-left axis (II, IV), the white pin marked the right and the black pin the left margin. These pins served as landmarks that bisected the ostium and were used to measure the ostial half-circumference. A blue pin was positioned at the VA junction, midway between the white and black pins to maintain the reference when measuring the distance between the white and black pins. Red pins marked the septa within each antrum (Figure 2A).

The PV ostia were dissected in the following order: ostium III, II, I, and IV. At each ostium, an initial longitudinal incision was made from

the VA junction distally into the branch indicated in Figure 2A. Additional side branches were also opened longitudinally and subsequently traced distally through the lung parenchyma to identify their drainage territories (Figure 2B). Complete assessment was possible in 20/23 hearts for ostium I, 22/23 for ostium II, 23/23 for ostium III, and 21/23 for ostium IV. In a small number of early specimens, partial lung dissection during protocol development limited evaluation of all ostia. After each cut, calibrated digital photographs (metric scale) were taken. Once all branches of an ostium were opened, the ostium was

gently tensioned to remove folds and obtain a flat measurement plane, without visible deformation of the tissue. No predefined force was applied. The ostium was then photographed for half-circumference measurements and diameter calculations. The location of the openings of the accessory veins was recorded, but the MS extent within these branches was not studied.

The PV branch labels and abbreviations followed Vandecasteele et al.<sup>13</sup>: Rcr V (right cranial PV), Rcd V1–V4 (right caudal PV branches 1–4), Lcd V1–V3 (left caudal PV branches 1–3), and Lcr V (left cranial PV). Each PV group, consisting of the antrum and its PV branches was referred to after the draining ostium: PV I, PV II, PV III and PV IV.

## 2.3 | Measurements

Digital images were calibrated and analysed using ImageJ software (version 1.53c, National Institutes of Health). A metric ruler was included in every photograph and placed in the same plane as the ostial surface to minimise parallax. Images were acquired with the camera positioned perpendicular to the measurement plane, and calibration in ImageJ was performed individually for each image prior to analysis. The ostial half-circumference was traced along the ostial margin from the white pin to the black pin, passing through the blue pin, using the segmented (polyline) tool. The ostial diameter was calculated from the half-circumference using the formula:  $\text{diameter} = (2 \times \text{half-circumference}) \div \pi$  and is reported as an equivalent circular diameter. This approach was chosen to provide a standardised comparative metric across specimens with variable ostial orientation and shape. The total antrum length was determined as the linear distance from the VA junction to the septal reference point (red pin), which defined the distal end of the common antrum. The diameter of individual PV branches, draining into the antrum, was measured by tracing the full flattened circumference.

MSs were macroscopically identified by their distinct myocardial colour. For each ostium, the shortest and longest MS lengths (from the VA junction to the distal MS margin) were measured along the antrum and were compared to the total antral length to obtain the minimal and maximal percentage MS coverage of the antrum. Coverage was classified as full coverage when the entire antrum was covered by MS, which could be with or without further extension of MS into one or more first-order branches. Coverage was classified as incomplete when the MS did not reach the distal end of the antrum, with no branch involvement, or when only part of the MS reached the distal end of the antrum. When the MS reached the first-order branches, the length into the branch was calculated as the minimum and maximum MS from the VA junction minus the antrum length. The distal edge morphology of the MS was classified as sharp (clear, abrupt demarcation between the MS and venous wall) or blurred (vague, gradual transition area). Representative examples of measurements are illustrated in Figure 3 (ostia I and II) and Figure 4 (ostia III and IV).

The MS thickness was measured in 13/23 hearts at 15 standardised sites as shown in Figure 2A: at the VA junction position of white

and black pins (representing the ostial long axis margins), at the walls between adjacent ostia, and in some PVs, at the point of the first-order branches. Not all sites were assessed in every horse, as the anatomical locations for thickness measurement were progressively expanded during the study.

To reduce observer bias, two blinded investigators independently re-measured calibrated images from two randomly selected horses. Measurements included ostial diameter, antrum length, and the shortest and longest MS length for ostia I–IV. Agreement for continuous variables was evaluated using Bland–Altman analysis, pooling values across ostia I–IV ( $n = 8$  paired observations per variable).

## 2.4 | Histology of the dorsal IAS at the level of the PV III and CaVC

In 6 of the 23 hearts, selected based on tissue availability and intactness of the collected region, a  $3 \times 1$  cm tissue block encompassing the dorsal IAS, adjacent MS of the caudal branches of PV III, and adjacent dorsal wall of the CaVC was fixed in 10% buffered formalin, paraffin embedded and sectioned at an 8  $\mu\text{m}$  slice thickness. Serial sections were stained with Masson's trichrome staining, and continuity between structures was assessed qualitatively based on myocardial fibre alignment and the presence or absence of intervening fibrous tissue. The location of collection of the sample is indicated in Figure 5A.

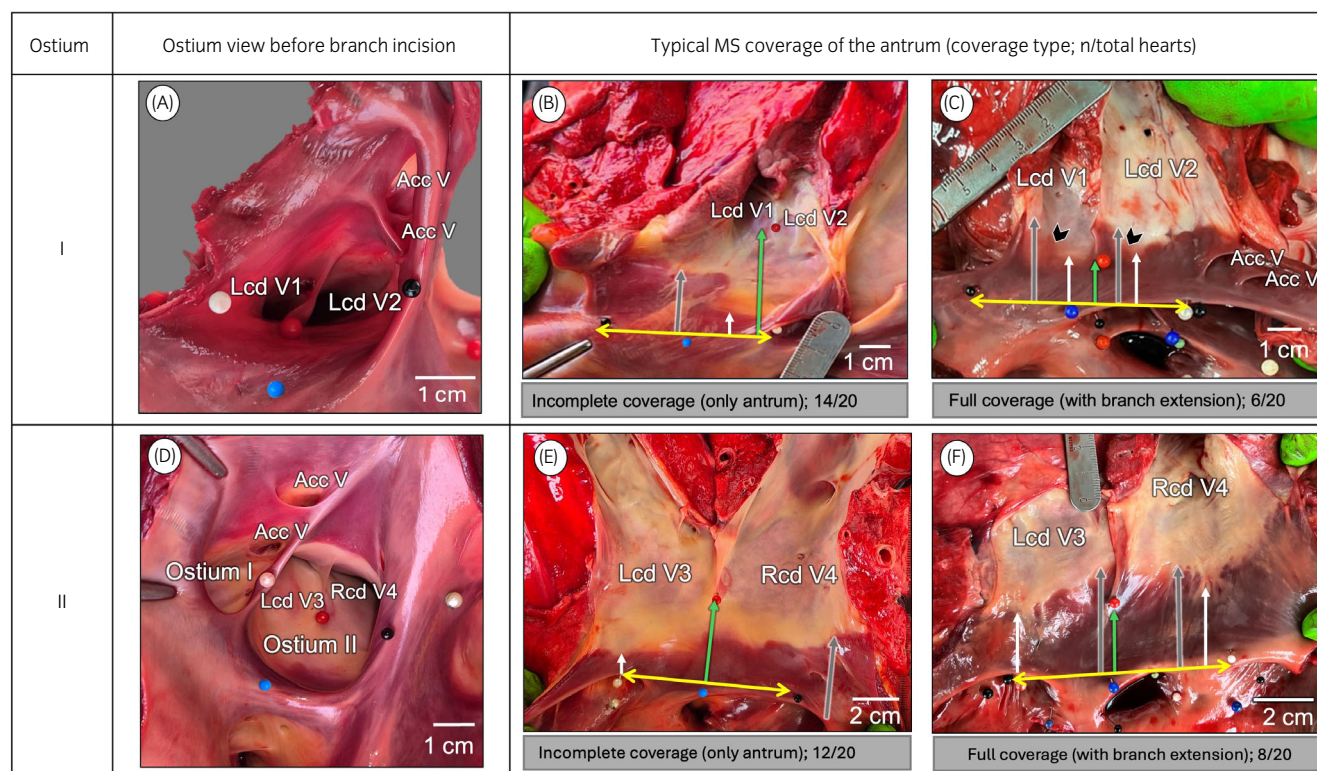
## 2.5 | Statistical analysis

Continuous data were expressed as mean (SD) for normally distributed data (Shapiro–Wilk) with  $n \geq 10$ . For data that were not normally distributed or when  $5 < n < 10$ , values were expressed as median [IQR]. For small groups ( $n \leq 5$ ), only the number of cases ( $n$ ) and range were reported. Correlations between continuous anatomical variables were assessed using Pearson's correlation and Spearman's rho for normally and non-normally distributed data, respectively. These analyses evaluated relationships between ostial diameter, antral length, shortest and longest MS length, as well as associations between sex or bodyweight and PV diameters. All tests were two-tailed, with statistical significance set at  $p < 0.05$ . Variables were paired and analysed independently by ostium. Correlation analyses were performed using SPSS (version 25, IBM). No power analysis was performed given the exploratory nature of this anatomical survey.

# 3 | RESULTS

## 3.1 | General anatomy and branching

Four main ostia (I–IV) with consistent drainage territories were observed in 20/23 horses, while 5 main ostia were observed in 3 hearts. When viewed from inside the left atrium, all ostia were more



**FIGURE 3** Pulmonary vein (PV) ostia I and II before (A, D) and after (B, C, E, F) branch incision, with the latter showing typical myocardial sleeve (MS) coverage of the antrum (including coverage type; n/total hearts) and measurement landmarks. At the veno-atrial (VA) junction, white and black pins marked each ostium's half-circumference along the longest axis (yellow double arrow), with a blue pin at the midpoint. The red pins indicate the septa between the branches. The green arrow indicates the antrum length (VA junction to the septum), and the white and grey arrows indicate the minimum and maximum MS lengths, respectively. Black arrowheads indicate the blurred distal edge of the MS. (A) Two accessory PVs (Acc Vs) opened into the antrum of ostium I, and branch orifices of the left caudal PV 1 and 2 (Lcd V1 and Lcd V2) are visible. (B) Ostium I shows incomplete MS coverage of the antrum in 14/20 hearts. (C) Ostium I shows full MS coverage of the antrum, with extension into both branches in 6/20 hearts. Here, two Acc Vs opened into the antrum. (D) Ostium II shows the antrum receiving the left caudal PV 3 (Lcd V3) and right caudal PV 4 (Rcd V4). (E) Ostium II exhibits incomplete MS coverage confined to the antrum in 12/22 hearts. (F) Ostium II displays full MS extension into Lcd V3 and Rcd V4 (8/22 hearts).

elongated than round. Ostium I had an oblique long axis running from left cranial to right caudal; ostium III had a mainly cranial-to-caudal long axis, and ostia II and IV had a right-to-left long axis.

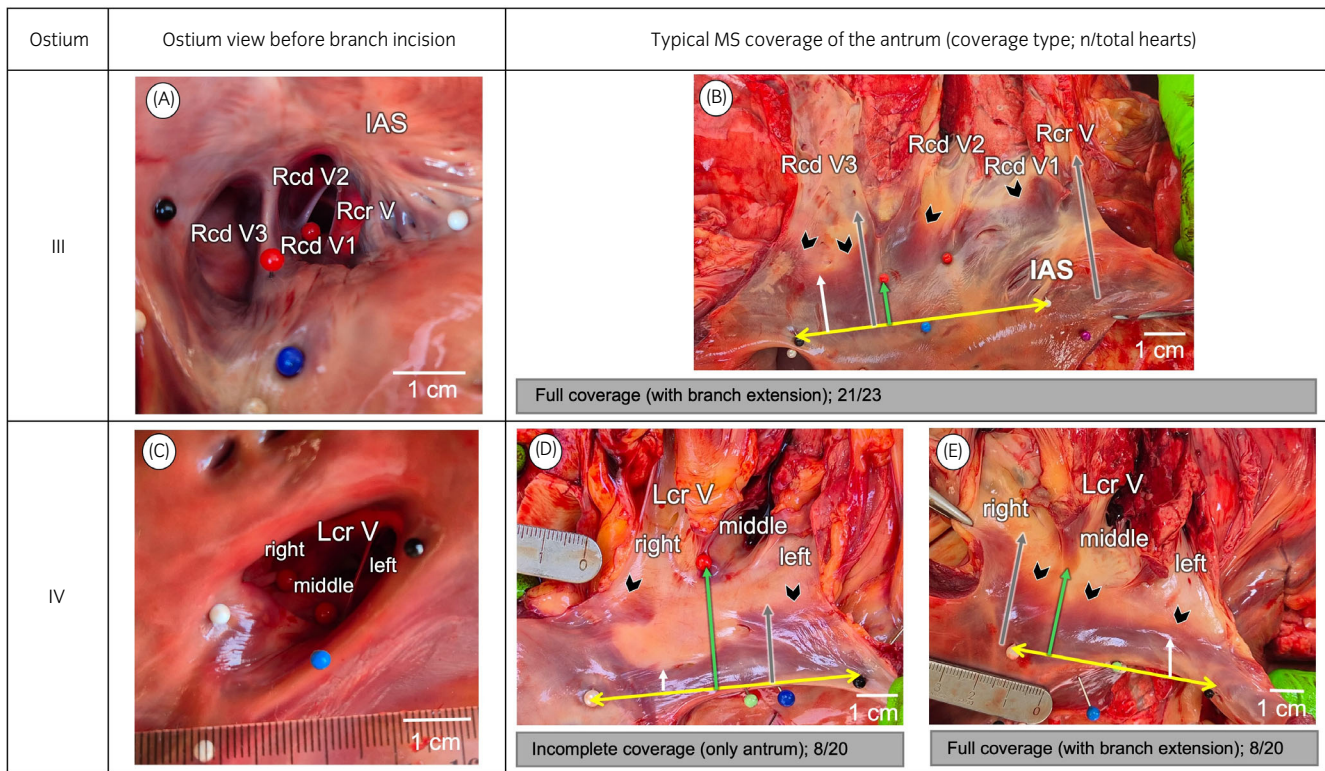
The branching patterns were consistent with previously described equine PV drainage.<sup>13</sup> Ostium I, positioned most to the left, drained two veins from the left caudal lung lobe (Lcd V1 and Lcd V2). These typically formed a common antrum (20/20 hearts). Ostium II, located most caudally, drained two veins: one from the left caudal lung lobe (Lcd V3) and one from the right caudal lung lobe (Rcd V4). These typically converged into a common antrum (20/22 hearts) or opened separately into the left atrium (2/22). Ostium III, located on the right side and adjacent to the IAS, drained the common antrum of four veins: one from the right cranial lung lobe (Rcr V) and three from the right caudal lung lobe (Rcd V1–3). The Rcr V was typically single (15/23 hearts) but was bifurcated (7/23) or trifurcated (1/23) in some specimens. The Rcd V1 and Rcd V2 mainly appeared as two separate branches (21/23 hearts), while a single trunk (2/23) or three separate branches (2/23) occurred less often. The Rcd V3 was present as single branch in all

23 hearts. Ostium IV drained the antrum formed by the veins of the left cranial lung lobe (Lcr V), which typically appeared as two branches (18/21 hearts) or three branches (3/21 hearts). These typically formed a common antrum (20/21 hearts) and opened less frequently as two distinct veins directly into the left atrium (1/21 hearts). Accessory PVs were assessed in 22 hearts, and these veins opened into ostium I (9/22 hearts), ostium II (5/20 hearts), the left atrium (4/20 hearts), ostium I and ostium II (3/22 hearts), or ostium I and the left atrium (2/22 hearts).

### 3.2 | Ostial dimensions, MS distribution and thickness

The ostial and branch diameters are summarised in Table 1. Ostia II and III were the largest whereas ostium I was smallest.

MSs extended into the antrum of all ostia and were macroscopically identifiable as reddish myocardial extensions distinct from the pale venous wall. Table 2 reports the antral length, shortest and



**FIGURE 4** Pulmonary vein (PV) ostia III and IV before (A, C) and after (B, D, E) branch incision, with the latter showing typical myocardial sleeve (MS) coverage of the antrum (including coverage type; n/total hearts) and measurement landmarks. At the veno-atrial (VA) junction, black and white pins marked ostium's half-circumference along the longest axis (yellow double arrow), with a blue pin at the midpoint. The red pins indicate the septa between the branches. The green arrow indicates the antrum length (VA junction to the septum), and the white and grey arrows indicate the minimum and maximum MS lengths, respectively. Black arrowheads indicate the blurred distal edge of the MS. (A) Ostium III is shown pre-incision from within the left atrium, demonstrating its anatomical position immediately adjacent to the interatrial septum (IAS). The intact ostium shows the orifices of three right caudal pulmonary vein branches (Rcd V1, Rcd V2, and Rcd V3) and the right cranial pulmonary vein (Rcr V). (B) Ostium III shows full MS coverage of the antrum with extension into first-order branches in 23/23 hearts. (C) Ostium IV is shown pre-incision from within the left atrium, showing the left cranial PV (Lcr V) ostium before opening the three branches (right, middle and left) in this case. (D) Ostium IV shows incomplete MS coverage, confined to the antrum in 8/20 hearts. (E) Ostium IV shows incomplete MS coverage with partial extension into the Lcr V in 8/20 hearts.

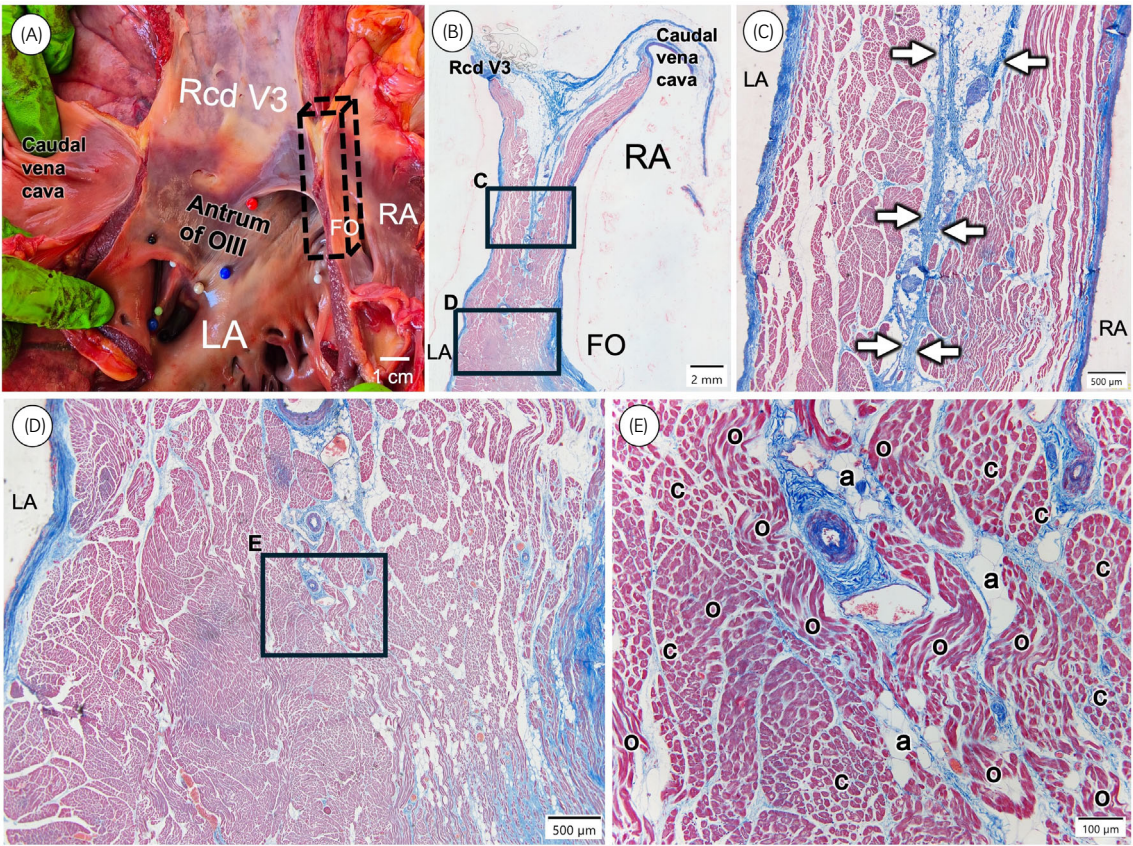
longest MS lengths, and corresponding minimum and maximum percentage coverage of the antral length for each ostium and its principal branches. PV III most often showed full antral coverage by the MS, with frequent extension into the branches. PVs II and IV generally showed incomplete MS coverage of the antrum with variable branch involvement. PV I typically showed a MS confined to the antrum without branch extension. The distal MS edge was more often blurred than sharp, particularly at PV II and III. Representative examples of typical antral MS coverage are illustrated in Figure 3 for PV I and PV II and in Figure 4 for PV III and IV. MS thickness at the VA junction differed by location (Table 3). The thickest myocardial wall was at ostia II and III, and the thinnest was consistently at ostium I.

Bland-Altman analysis demonstrated the following bias (observer 1 – observer 2) and 95% limits of agreement: ostial diameter ( $n = 8$ )  $-0.5$  mm ( $-2.8$  to  $1.8$  mm); antrum length ( $n = 8$ )  $-0.5$  mm ( $-2.7$  to  $1.8$  mm); shortest MS length ( $n = 8$ )  $0.9$  mm ( $-2.7$  to  $4.5$  mm); and longest MS length ( $n = 8$ )  $-0.7$  mm ( $-2.3$  to  $0.9$  mm). Relative to the mean values, interobserver bias  $<5\%$ , and

limits of agreement corresponded to  $\pm 10$ – $20\%$ , depending on the variable.

### 3.3 | Histology of the dorsal IAS

In all six examined specimens, sections across the dorsal part of the IAS at the caudal limb of the fossa ovalis showed a single myocardial bundle with myofibres oriented in multiple directions. Both the MSs of the caudal branches of PV III and CaVC consisted mainly of fibres in a longitudinal cut (oriented parallel to the long axis direction of the PV III). A fibroadipose plane, highlighted by Masson's trichrome, separated the PV III and CaVC MSs dorsally. However, both MSs fused more ventrally into a common myocardial mass within the dorsal IAS. This fusion occurred through gradual changes in myofibre orientation until both MSs fused together as one bundle, highly suggestive of anatomical muscular continuity between PV III and the CaVC MSs (Figure 5B–E).



**FIGURE 5** Dorsal interatrial septum (IAS) at the caudal limb of the fossa ovalis (FO) demonstrating dorsal separation and ventral fusion of the myocardial sleeves (MSs) from the caudal branches of pulmonary vein III and the caudal vena cava (CaVC). (A) Gross view of the sampling site after opening the pulmonary vein III from the veno-atrial junction distally. The dashed box outlines the 3 × 1 cm tissue block including the dorsal interatrial septum and the MSs of the pulmonary vein III and CaVC. (B) Histological slide stained with Masson's trichrome showing the dorsal IAS myocardium in continuity with both pulmonary vein III and CaVC MSs. Dorsally the two MSs show a separation by fibroadipose tissue. Black boxes indicate the areas magnified in (C) and (D). (C) 4× magnification of the interface demonstrates a fibroadipose plane between the pulmonary vein III and CaVC MSs (white arrows). (D) 4× magnification of the dorsal interatrial wall showing fusion of the CaVC and pulmonary vein III MSs into a single myocardial mass at the dorsal interatrial septum. The black box marks the region magnified in (E). (E) 20× magnification of the IAS highlights fibres in cross-section (c), oblique fibres (o), and adipose tissue (a). LA, left atrium; RA, right atrium; Rcd V3, right caudal pulmonary vein 3.

**TABLE 1** Pulmonary vein ostial and first-order branch diameters (mm). Values are expressed as mean (SD) for normally distributed data when  $n \geq 10$ .

| Ostium | Diameter (mm) | Branching at first septum     | Diameter (mm) |
|--------|---------------|-------------------------------|---------------|
| I      | 26 (6)        | Left caudal pulmonary vein 1  | 12 (4)        |
|        |               | Left caudal pulmonary vein 2  | 19 (7)        |
| II     | 42 (6)        | Left caudal pulmonary vein 3  | 20 (7)        |
|        |               | Right caudal pulmonary vein 4 | 21 [7]        |
| III    | 41 (11)       | Right caudal pulmonary vein 3 | 19 (3)        |
|        |               | Right caudal pulmonary vein 2 | 15 (4)        |
|        |               | Right caudal pulmonary vein 1 | 9 [4]         |
|        |               | Right cranial pulmonary vein  | 13 (3)        |
| IV     | 29 (7)        | Left cranial pulmonary vein   |               |

*Note:* For non-normally distributed data or when  $5 < n < 10$ , values are given as median [IQR]. Diameters were derived from half-circumference measurements ( $\text{diameter} = \text{circumference}/\pi$ ) as a standardised comparative metric; values assume circular geometry and may underestimate maximal dimensions in ovoid ostia.

**TABLE 2** Myocardial sleeve (MS) extent into the antrum and main pulmonary vein branches with distal edge morphology.

| Antrum coverage by MS |         |                                                   |       |                                                          |                                                            |  |               |                                      |                            |                                                                                                  |
|-----------------------|---------|---------------------------------------------------|-------|----------------------------------------------------------|------------------------------------------------------------|--|---------------|--------------------------------------|----------------------------|--------------------------------------------------------------------------------------------------|
|                       |         | Incomplete                                        |       |                                                          |                                                            |  |               |                                      |                            |                                                                                                  |
|                       |         | Total MS length from VA junction (min to max; mm) |       | Confined to the antrum (n/total; min to max % of length) | Partially extends into branches (n/total; min % of length) |  | Main Branches | MS extension into branches (n/total) | MS length distal to antrum | Distal edge pattern as sharp (s) and blurred (b) (n/total)                                       |
| I                     | 20 (9)  | 12 (8) to 19 (10)                                 | 6/20  | 14/20; 40 (27) to 65 (24)                                | 0/20                                                       |  | Lcd V1        | 5/20                                 | (1–11) to (9–24)           | (s) 5/20<br>(b) 15/20<br>(s) 5/20<br>(b) 15/20                                                   |
|                       |         |                                                   |       |                                                          |                                                            |  | Lcd V2        | 6/20                                 | 4 [7] to 13 [9]            |                                                                                                  |
| II                    | 27 (14) | 22 (11) to 30 (13)                                | 8/22  | 12/22; 48 (20) to 82 [26]                                | 2/22; min (55–90)                                          |  | Lcd V3        | 8/22                                 | 7 [12] to 11 [4]           | (s) 13/22<br>(b) 9/22<br>(s) 17/22<br>(b) 5/22                                                   |
|                       |         |                                                   |       |                                                          |                                                            |  | Rcd V4        | 8/22                                 | 19 [9] to 30 [16]          |                                                                                                  |
| III                   | 23 (6)  | 30 (8) to 41 (10)                                 | 23/23 | 0/23                                                     | 0/23                                                       |  | Rcd V3        | 23/23                                | 6 (5) to 18 (8)            | (s) 9/23<br>(b) 14/23<br>(s) 8/23<br>(b) 15/23<br>(s) 6/20<br>(b) 14/20<br>(s) 12/20<br>(b) 8/20 |
|                       |         |                                                   |       |                                                          |                                                            |  | Rcd V2        | 18/23                                | 20 (7) to 28 (7)           |                                                                                                  |
|                       |         |                                                   |       |                                                          |                                                            |  | Rcd V1        | 16/20                                | 15 (9) to 23 (11)          |                                                                                                  |
|                       |         |                                                   |       |                                                          |                                                            |  | Rcr V         | 15/20                                | 17 (9) to 24 (9)           |                                                                                                  |
| IV                    | 20 (8)  | 13 (7) to 24 (11)                                 | 5/21  | 8/21; 34 [30] to 62 [34]                                 | 8/21; min 59 [15]                                          |  | Lcr V         | 13/21                                | (0–15) to 10 (10)          | (s) 10/21<br>(b) 11/21                                                                           |

Note: For each pulmonary vein ostium (I–IV), the table reports antral length (mm), total MS length from the veno-atrial (VA) junction (min to max, mm), and antral length covered by the MS, classified as: Full length coverage (antrum fully covered with or without the pulmonary vein branches reached) and incomplete antral coverage with MS confinement to the antrum (no branch involvement) or partial MS extension into branches without full antral coverage. For each of the main branches, the table reports the number of horses with MS extension (n/total), distal MS length beyond the antrum (min to max, mm), and distal edge morphology classified as sharp (s; abrupt transition) or blurred (b; gradual transition). Values are expressed as mean (SD) for normally distributed data when  $n \geq 10$ . For non-normally distributed data or when  $5 < n < 10$ , values are given as median [IQR]. For small groups ( $n \leq 5$ ), only the number of cases (n) and range are reported. Lcd V1–V3, left caudal pulmonary veins 1–3; Lcr V, left cranial pulmonary vein; Rcd V1–V4, right caudal pulmonary veins 1–4; Rcr V, right cranial pulmonary vein.

**TABLE 3** Equine myocardial thickness, measured at the pulmonary vein veno-atrial (VA) junction and at the myocardial sleeve of the ostium in some branches, as indicated by locations A–O in Figure 2A.

| Ostium | Measurement site                             | MS thickness (mm) | Number of horses; range (min–max, mm) |
|--------|----------------------------------------------|-------------------|---------------------------------------|
| I      | A: VA junction (at ostium I incision)        | 4.0 [0.3]         | 9; 4.0–4.6                            |
|        | A': Lcd V1 ostium (at the ostium I incision) | —                 | 3; 1.0–2.0                            |
|        | B: White pin                                 | 4.0 [0.8]         | 6; 3.0–4.0                            |
|        | C: Black pin                                 | 3.0 [0.8]         | 6; 3.0–4.0                            |
| II     | D: VA junction (at ostium II incision)       | 6.0 [0.7]         | 9; 3.0–9.0                            |
|        | D': Lcd V3 ostium, (at ostium II incision)   | —                 | 2; 1.0–2.0                            |
|        | E: White pin: wall between ostium II and III | 8.0 [1.5]         | 7; 7.0–12.0                           |
|        | F: Black pin: wall between ostium I and II   | 4.0 [0.0]         | 7; 3.0–4.0                            |
|        | G: Blue pin: all between ostium II and IV    | 4.0 [1.0]         | 7; 4.0–5.0                            |
| III    | H: VA junction (at ostium III incision)      | 6.9 (0.9)         | 11; 5.6–8.0                           |
|        | H': Rcd V3 ostium (at ostium III incision)   | 2.6 (0.8)         | 11; 1.0–3.3                           |
|        | I: White pin                                 | 8.0 [1.5]         | 7; 7.0–9.0                            |
|        | J: Black pin                                 | 8.0 [1.0]         | 8; 7.0–9.0                            |
|        | K: Septum between Rcd V3 and Rcd V2          | 2.6 (0.4)         | 13; 2.0–3.1                           |
|        | L: Septum between Rcd V2 and Rcr V           | 2.2 (1.0)         | 13; 1.0–4.0                           |
| IV     | M: VA junction (at ostium IV incision)       | 5.2 [0.5]         | 10; 5.0–6.0                           |
|        | N: White pin: wall between ostium III and IV | 6.0 [1.0]         | 5; 6.0–7.0                            |
|        | O: Black pin                                 | 7.0 [1.0]         | 7; 6.0–7.0                            |

Note: Data are presented for up to 13 horses, with the actual number of cases included at each site indicated in the table. Values are expressed as mean (SD) for normally distributed data when  $n \geq 10$ . For non-normally distributed data or when  $5 < n < 10$ , values are given as median [IQR]. For small groups ( $n \leq 5$ ), only the number of cases ( $n$ ) and range are reported. Lcd V1, left caudal pulmonary vein 1; Lcd V3, left caudal pulmonary vein 3; Rcd V2, right caudal pulmonary vein 2; Rcd V3, right caudal pulmonary vein 3; Rcd V4, right caudal pulmonary vein 4; Rcr V, right cranial pulmonary vein.

### 3.4 | Associations between variables

No significant association was found between sex or bodyweight and PV ostial diameter in the Warmblood study sample. Correlation analysis showed that ostial diameter was not associated with antral length or MS length at any ostium (all  $r/p < 0.25$ ,  $p > 0.05$ ). At ostium III, antral length positively correlated with both shortest ( $r = 0.85$ ,  $p < 0.001$ ) and longest MS length ( $r = 0.63$ ,  $p = 0.001$ ), while other ostia showed no association. Within each ostium, shortest and longest MS lengths were positively correlated ( $r/p = 0.75$ – $0.83$ , all  $p < 0.001$ ), indicating organised sleeve distribution.

## 4 | DISCUSSION

This study provides an ostium-specific macroscopic characterisation of equine PV MSs and establishes a structural framework relevant for future electrophysiological studies and development of catheter ablation strategies for horses. While previous studies established the presence of MSs and their overall length,<sup>11,14</sup> our study additionally quantified the MS length, antral coverage, and regional thickness variations for each ostium.

Four principal ostia were identified in most hearts, each with lobe-specific drainage. In some horses, accessory veins opened into

the left atrium, consistent with prior equine anatomical descriptions.<sup>13</sup>

The mean ostial diameters in our study (I–IV: 2.6, 4.2, 4.1 and 2.9 cm) were generally similar to post-mortem values reported by Kovács et al. (I–IV: 3.02, 3.27, 3.34 and 1.81 cm),<sup>11</sup> and in vivo electro-anatomical reconstruction values reported by Kjeldsen et al. using a mainly impedance-based mapping system (I–IV: 3.1, 4.2, 5.1 and 3.5 cm).<sup>14</sup> Our ostium II measurement matched the in vivo mean and exceeded the post-mortem values. Ostia III and IV were smaller than the in vivo mean but larger than in the post-mortem study, and ostium I was smaller compared to both other studies. These differences likely reflect the methodology used. In vivo mapping delineates the PV ostia under physiological loading and may overestimate dimensions due to catheter-induced wall displacement and impedance effects, whereas post-mortem measurements depend on how the tissue is handled. Despite this, all studies agree on rank order, with ostia II and III being the largest and I and IV being smaller, which supports the validity of our measurements while providing anatomical reference data that may be useful for future procedural planning.

Human patients with AF show increased PV ostial diameters and greater total PV ostial volumes.<sup>26,27</sup> Our study included horses without documented cardiovascular disease; however, subclinical cardiac pathology could not be excluded and arrhythmogenic remodelling may enlarge the PV ostia. Whereas in humans, males typically have larger PV ostia than females,<sup>28</sup> we did not identify an association

between sex or bodyweight and ostial diameter, and no association was found between bodyweight and PV diameter, consistent with previous equine studies.<sup>14</sup> This might be related to the small sample size of our study.

PV MSs were macroscopically identified as distinct, reddish myocardial cuffs, visually separable from the paler venous wall. This appearance is consistent with sleeve-bearing regions described in the cranial and caudal equine vena cava,<sup>29</sup> and with grossly identifiable PV MSs in humans.<sup>30</sup> MSs were present at all PV ostia but varied systematically with location. PV III most often exhibited full antral coverage with long MSs (30 (5) mm to 42 (10) mm) extending into first-order branches, indicating a larger amount of excitable substrate analogous to the superior PVs in human patients.<sup>30–32</sup> The antral size positively correlated with sleeve extent at PV III, a relationship not observed in PV I, II and IV. In contrast, PV I typically had a small diameter and short MS (12 (9) mm to 19 (9) mm), confined to the antrum, suggesting a smaller amount of arrhythmogenic tissue. These findings suggest that veins with extensive MS coverage may represent anatomically relevant targets for ablation in horses, a hypothesis that requires functional validation. The distal edge morphology of the MS may further inform ablation targets. Sharp endings likely represent abrupt demarcation between myocardial and venous tissue, potentially creating discrete electrical boundaries, whereas blurred endings may reflect the gradual tapering of the MS, with fibre thinning, reorientation, and transition into connective and adipose tissues. In dogs, such regions show slow, heterogeneous conduction, fractionated electrograms, and multiple breakthrough sites within the MS, consistent with local micro-re-entry.<sup>33</sup> Our data highlight the predominance of blurred endings of MSs at PV III, reinforcing the potential arrhythmogenic importance. However, without histological analysis and functional confirmation of these regions, this remains a hypothesis that requires validation in future tissue and mapping studies.

The large equine PV ostial diameters and thicker myocardial walls at the VA junction compared to those in humans create constraints for ablation procedures. In humans, WACA provides more durable isolation than strictly ostial approaches.<sup>19</sup> However, in horses, creating a complete circumferential lesion is challenging because point-by-point ablation is time-consuming<sup>20</sup> and the myocardium at the VA junction is thick. In this study, the myocardial wall thickness at the VA junction was the thickest around ostia II and III. Ostium III was consistently over 5.6 mm and reached up to 12 mm at the wall between ostia II and III. Durable ablation requires transmural (full thickness) lesions, and thicker atrial tissue is known to be associated with a higher risk of acute reconnection after ablation in humans.<sup>27</sup> In vitro equine studies have shown that optimal radiofrequency settings of 40 W for 60 s achieve mean (SD) lesion depths of 4.8 (1.6) mm,<sup>34,35</sup> which might be below the local equine myocardial wall thickness measured at ostium II and III in the present study. These anatomical findings suggest that achieving transmural lesions at these sites may be technically challenging and optimisation of energy settings or alternative energy modalities such as laser ablation<sup>36</sup> will require functional validation.

Energy delivery tools also pose limitations. Point-by-point radiofrequency around a large equine ostium is time consuming and

technically demanding. Single-shot ablation systems (e.g., balloon-based or multielectrode systems) offer an alternative approach by delivering simultaneous circumferential lesions in a single application, potentially reducing procedural time and improving lesion uniformity compared with point-by-point ablation.<sup>37–39</sup> However, current human devices are size-limited relative to equine anatomy. In this study, ostia II and III exceeded 40 mm in diameter, which is larger than the maximum array size of the current single-shot systems. Currently available pulsed-field ablation catheters are offered in two diameters (31 and 35 mm),<sup>40</sup> and contemporary cryoballoon systems are limited to fixed balloon diameters of 23 or 28 mm.<sup>41</sup> Myocardial wall thickness is another limiting factor. Pulsed-field ablation on pig hearts produces a lesion depth of a maximum of 5.8–8.2 mm depending on the energy settings,<sup>42</sup> which may be insufficient to achieve transmural lesions at equine ostia II and III. Achieving durable PV isolation requires validation but will likely require large-diameter ablation tools and higher energy delivery combined with careful power reduction in thin-walled regions to avoid perforation.

In addition to ostial diameter and wall thickness constraints, anatomical variations such as the opening of PV branches directly into the left atrium without formation of a common antrum should also be considered for planning ablation. This scenario was observed in a minority of cases, specifically in two hearts at ostium II and 1 heart at ostium IV. Ablation of such an anatomical configuration would require using two circles of point-by-point ablation or smaller-sized balloon catheters compared to other ostia. These anatomical variations further underscore the importance of developing individualised ablation strategies tailored to each horse's unique vascular anatomy.

In this study, we also identified an anatomical substrate that may affect ablation durability beyond the PVs themselves. Histological analysis showed that while the MSs of the caudal branches of PV III and the CaVC were separated dorsally by fibroadipose tissue, they fused more ventrally into a common myocardial mass within the dorsal IAS. This suggests a sleeve-to-sleeve fusion, without a single continuous MS connecting the vessels. In humans, posterior interatrial muscular connections near the fossa ovalis, close to the right PVs can permit residual conduction and can compromise PV isolation.<sup>25</sup> This mechanism may also be relevant in horses. The CaVC is a known re-entry hotspot in horses,<sup>43–45</sup> and the adult equine IAS at the fossa ovalis and septal pouches contains a muscular core with variable fibrous and adipose content that may itself be arrhythmogenic.<sup>46</sup> Together, these features suggest that the MS fusion within the dorsal IAS observed in this study may provide a structural substrate capable of supporting macro-re-entry or provide a bypass pathway after attempts to isolate PV III. Although this remains speculative, future isolation strategies for ostium III in horses should include testing for conduction between the PV III and the CaVC after ablation, verifying the block across the IAS, and tailoring the lesion depth and width to the local thickness (6.9 (0.9) mm). Prospective electro-anatomical mapping with pacing manoeuvres, ideally combined with connexin immunohistochemistry, is warranted to validate the functional significance of this indirect septal pathway and optimise ablation efficacy.

This study has several limitations. The work was conducted on post-mortem tissue, and although dissections were performed within the first 3 h of euthanasia, subtle changes in tissue dimensions may have occurred, meaning that the absolute values may not fully reflect the *in vivo* anatomy. Additionally, our sample size was limited to 23 horses, with some missing data. A larger sample size would better capture the range and frequency of anatomical variations in the population. This study only included Warmblood horses, and breed-related differences cannot be excluded. Importantly, all hearts were obtained from horses without known cardiovascular disease, and no horses with AF were included. Structural remodelling associated with AF may alter PV size and MS characteristics; therefore, extrapolation of the present findings to horses with clinical AF requires dedicated investigation. Additionally, the lack of histological confirmation limits the correlation between gross appearance and microscopic myocardial content, especially when evaluating distal edge morphology. The ostial diameter was calculated from half-circumference assuming circular geometry. Because many ostia were ovoid, the reported values represent equivalent circular diameters rather than true long- or short-axis anatomical dimensions and may have underestimated true maximal dimensions. Photographs were obtained after gentle manual tensioning to flatten the ostia. As the applied force was not standardised, minor measurement variability related to tissue handling cannot be excluded. The MS length and thickness were measured macroscopically from photographs. Very thin distal strands may have been missed. MS thickness was measured at specific points to provide an idea of the thickness distribution around the VA junction of each ostium; however, this approach cannot fully capture spatial heterogeneity. Furthermore, accessory ostia were recorded but not quantified for MS extent. Interobserver agreement was assessed only for continuous measurements and in two horses, limiting the precision of reliability estimates. Categorical variables were not formally subjected to interobserver statistical testing. Finally, this was an anatomical and histological study, and functional electrophysiology correlates were beyond the scope of this study but are critical for future translational work. Future studies should combine MS histology with high-resolution electro-anatomical mapping in horses with and without arrhythmias to directly relate myocardial structure to conduction behaviour and arrhythmia susceptibility.

## 5 | CONCLUSION

PV MSs were present at all four ostia but demonstrated systematic ostium-specific variations in extent, morphology, and thickness. PV III exhibits the most extensive MS architecture: a long MS with full antral coverage, frequent branch extension, predominantly blurred distal edges, and a thick wall at the VA junction. These anatomical features suggest increased structural complexity and may justify prioritisation of this region during ablation procedures, pending functional validation. However, the substantial myocardial thickness at PV III may complicate the achievement of durable transmural block. The observed myocardial continuity between PV III and the CaVC

provides anatomical evidence of a potential structural substrate that warrants electrophysiological investigation. In contrast, PV I had a shorter MS confined to the antrum and a thinner wall than other ostia, likely suggesting comparatively lower arrhythmogenic substrate and a region where isolation may be more achievable with standard catheter techniques. Compared with humans, horses have much larger PV ostial diameters and thicker myocardial walls, underscoring anatomical considerations relevant for the development and optimisation of equine ablation strategies.

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## CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicting interests.

## AUTHOR CONTRIBUTIONS

**Lara Ibrahim:** Conceptualization; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Inne Van Hooydonk:** Conceptualization; investigation; methodology; visualization; writing – review and editing. **Pieter Cornillie:** Conceptualization; writing – review and editing; supervision. **Gunther van Loon:** Conceptualization; validation; writing – review and editing; supervision.

## DATA INTEGRITY STATEMENT

Lara Ibrahim had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis.

## ETHICAL ANIMAL RESEARCH

This study adhered to the ethical guidelines outlined by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium.

## INFORMED CONSENT

Informed consent was obtained from the owners of all horses euthanised for noncardiac reasons and donated for research purposes. Hearts collected from an abattoir were obtained with the permission of the abattoir operator.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at <https://doi.org/10.6084/m9.figshare.33228723>.<sup>47</sup>

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## REFERENCES

- Slack J, Boston RC, Soma LR, Reef VB. Occurrence of cardiac arrhythmias in Standardbred racehorses. *Equine Vet J*. 2015;47:398–404.
- Deem DA, Fregin GF. Atrial fibrillation in horses: a review of 106 clinical cases, with consideration of prevalence, clinical signs, and prognosis. *J Am Vet Med Assoc*. 1982;180:261–5.
- Rose RJ, Davis PE. Paroxysmal atrial fibrillation in a racehorse. *Aust Vet J*. 1977;53:545–9.
- Decloedt A, Van Steenkiste G, Vera L, Buhl R, van Loon G. Atrial fibrillation in horses part 1: pathophysiology. *Vet J*. 2020;263:105521.
- Kjeldsen ST, Nissen SD, Buhl R, Hopster-Iversen C. Paroxysmal atrial fibrillation in horses: pathophysiology, diagnostics and clinical aspects. *Animals*. 2022;12:698.
- Decloedt A, Van Steenkiste G, Vera L, Buhl R, van Loon G. Atrial fibrillation in horses part 2: diagnosis, treatment and prognosis. *Vet J*. 2021;268:105594.
- Nath LC, Elliott AD, Weir J, Curl P, Rosanowski SM, Franklin S. Incidence, recurrence, and outcome of posttrace atrial fibrillation in Thoroughbred horses. *J Vet Intern Med*. 2021;35:1111–20.
- van Loon G, Van Steenkiste G, Vera L, Decloedt A. Catheter-based electrical interventions to study, diagnose and treat arrhythmias in horses: from refractory period to electro-anatomical mapping. *Vet J*. 2020;263:105519.
- Buschmann E, Van Steenkiste G, Duytschaever M, Boussy T, Vernemmen I, Ibrahim L, et al. Successful caudal vena cava and pulmonary vein isolation in healthy horses using 3D electro-anatomical mapping and a contact force-guided ablation system. *Equine Vet J*. 2024;56(5):1068–76.
- Haissaguerre M, Jais P, Shah DC, Takahashi A, Hocini M, Quiniou G, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med*. 1998;339:659–66.
- Kjeldsen ST, Nissen SD, Saljic A, Hesselkilde EM, Carstensen H, Sattler SM, et al. Structural and electro-anatomical characterization of the equine pulmonary veins: implications for atrial fibrillation. *J Vet Cardiol*. 2024;52:1–13.
- Linz D, Hesselkilde E, Kutieleh R, Jespersen T, Buhl R, Sanders P. Pulmonary vein firing initiating atrial fibrillation in the horse: oversized dimensions but similar mechanisms. *J Cardiovasc Electrophysiol*. 2020;31:1211–2.
- Vandecasteele T, van Loon G, Vandeveld K, De Pauw B, Simoens P, Cornillie P. Topography and ultrasonographic identification of the equine pulmonary vein draining pattern. *Vet J*. 2016;210:17–23.
- Kovacs S, Racz B, Sotonyi P, Bakos Z. Morphological and histological investigation of the conduction system in the equine atrial muscle sleeve of pulmonary veins. *Equine Vet J*. 2024;56:1059–67.
- Kjeldjojo P, O'Neill MD, Lim PB, Malcolm-Lawes L, Whinnett ZI, Salukhe TV, et al. Pulmonary venous isolation by antral ablation with a large cryoballoon for treatment of paroxysmal and persistent atrial fibrillation: medium-term outcomes and non-randomised comparison with pulmonary venous isolation by radiofrequency ablation. *Heart*. 2010;96:1379–84.
- Skeete J, Sharma PS, Kenigsberg D, Pietrasik G, Osman AF, Ravi V, et al. Wide area circumferential ablation for pulmonary vein isolation using radiofrequency versus laser balloon ablation. *J Arrhythm*. 2022;38:336–45.
- Nair GM, Birnie DH, Nery PB, Redpath CJ, Sarrazin J-F, Roux J-F, et al. Standard vs augmented ablation of paroxysmal atrial fibrillation for reduction of atrial fibrillation recurrence: the AWARE randomized clinical trial. *JAMA Cardiol*. 2023;8:475–83.
- Sousa PA, Barra S, Saleiro C, Khoeiry Z, Adão L, Primo J, et al. Ostial vs. wide area circumferential ablation guided by the Ablation Index in paroxysmal atrial fibrillation. *Europace*. 2023;25(6):eud160. <https://doi.org/10.1093/europace/euad160>.
- Tilz RR, Heeger CH, Vogler J, Eitel C, Feher M, Phan HL, et al. Wide antral circumferential vs. ostial pulmonary vein isolation using pulsed field ablation-the butterfly effect. *Front Cardiovasc Med*. 2023;10:1217745.
- Buschmann E, Van Steenkiste G, Duytschaever M, Segers P, Ibrahim L, van Loon G, et al. In vitro characterization of radiofrequency ablation lesions in equine and swine myocardial tissue. *Sci Rep*. 2024;14:22877.
- Vernemmen I, Buschmann E, Van Steenkiste G, Demeyere M, Verhaeghe LM, De Somer F, et al. Intracardiac ultrasound-guided transseptal puncture in horses: outcome, follow-up, and perioperative anticoagulant treatment. *J Vet Intern Med*. 2024;38:2707–17.
- Vernemmen I, Van Steenkiste G, Buschmann E, Cornelis K, Schauvliege S, Ibrahim L, et al. Development of an atrial transseptal puncture procedure in horses to access the left heart: an ultrasound-guided jugular vein and transhepatic approach. *Equine Vet J*. 2025;57(1):243–55.
- Ho SY, Anderson RH, Sanchez-Quintana D. Atrial structure and fibres: morphologic bases of atrial conduction. *Cardiovasc Res*. 2002;54:325–36.
- Platonov PG, Mitrofanova L, Ivanov V, Ho SY. Substrates for intra-atrial and interatrial conduction in the atrial septum: anatomical study on 84 human hearts. *Heart Rhythm*. 2008;5:1189–95.
- Yoshida K, Baba M, Shinoda Y, Harunari T, Tsumagari Y, Koda N, et al. Epicardial connection between the right-sided pulmonary venous carina and the right atrium in patients with atrial fibrillation: a possible mechanism for preclusion of pulmonary vein isolation without carina ablation. *Heart Rhythm*. 2019;16:671–8.
- Kato R, Lickfett L, Meiningner G, Dickfeld T, Wu R, Juang G, et al. Pulmonary vein anatomy in patients undergoing catheter ablation of atrial fibrillation: lessons learned by use of magnetic resonance imaging. *Circulation*. 2003;107:2004–10.
- Gebhard C, Krasniqi N, Stahli BE, Klaeser B, Fuchs TA, Ghadri JR, et al. Characterization of pulmonary vein dimensions using high-definition 64-slice computed tomography prior to radiofrequency catheter ablation for atrial fibrillation. *Cardiol Res Pract*. 2014;2014:179632.
- Niculescu MC, Niculescu V, Sisu AM, Ciobanu IC, Daescu E, Petrescu CI, et al. Study of the diameter and number of the pulmonary veins orifices. *Rom J Morphol Embryol*. 2006;47:133–5.
- Ibrahim L, Buschmann E, van Loon G, Cornillie P. Morphological evidence of a potential arrhythmogenic substrate in the caudal and cranial vena cava in horses. *Equine Vet J*. 2025;57:256–64.
- Szczepanek E, Bolechala F, Koziej M, Jasinska KA, Holda MK. Morphometric characteristics of myocardial sleeves of the pulmonary veins. *J Cardiovasc Electrophysiol*. 2020;31:2455–61.
- Kholová I, Kautzner J. Anatomic characteristics of extensions of atrial myocardium into the pulmonary veins in subjects with and without atrial fibrillation. *Pacing Clin Electrophysiol*. 2003;26:1348–55.
- Tagawa M, Higuchi K, Chinushi M, Washizuka T, Ushiki T, Ishihara N, et al. Myocardium extending from the left atrium onto the pulmonary veins: a comparison between subjects with and without atrial fibrillation. *Pacing Clin Electrophysiol*. 2001;24:1459–63.
- Hocini M, Ho SY, Kawara T, Linnenbank AC, Potse M, Shah D, et al. Electrical conduction in canine pulmonary veins: electrophysiological and anatomic correlation. *Circulation*. 2002;105:2442–8.
- Waks JW, Josephson ME. Mechanisms of atrial fibrillation – reentry, rotors and reality. *Arrhythm Electrophysiol Rev*. 2014;3:90–100.
- Kuhne M, Suter Y, Altmann D, Ammann P, Schaer B, Osswald S, et al. Cryoballoon versus radiofrequency catheter ablation of paroxysmal atrial fibrillation: biomarkers of myocardial injury, recurrence rates, and pulmonary vein reconnection patterns. *Heart Rhythm*. 2010;7:1770–6.
- Krist D, Linz D, Schotten U, Zeemering S, Leenen D. A novel laser energy ablation catheter for endocardial Cavo-tricuspid isthmus ablation and Epicardial ventricular lesion formation: an in vivo proof-of-concept study. *Front Med Technol*. 2022;4:834856.

37. Kuck KH, Furnkranz A, Chun KR, Metzner A, Ouyang F, Schluter M, et al. Cryoballoon or radiofrequency ablation for symptomatic paroxysmal atrial fibrillation: reintervention, rehospitalization, and quality-of-life outcomes in the FIRE AND ICE trial. *Eur Heart J*. 2016;37:2858–65.
38. Ernst S. Multielectrode pulmonary vein isolation versus single tip wide area catheter ablation-paroxysmal atrial fibrillation: is there any mystery in pulmonary vein isolation? *Circ Arrhythm Electrophysiol*. 2016;9:e004077.
39. Monnig G, Eckardt L. Multielectrode pulmonary vein ablation catheter (PVAC((R))) current data on results and risks. *Herzschrittmacherther Elektrophysiol*. 2014;25:236–40.
40. Ollitrault P, Chaumont C, Font J, Manning M, Conti S, Matusik PT, et al. Superior vena cava isolation using a pentaspline pulsed-field ablation catheter: feasibility and safety in patients undergoing atrial fibrillation catheter ablation. *Europace*. 2024;26(7):euae160. <https://doi.org/10.1093/europace/euae160>
41. Andrade JG, Dubuc M, Guerra PG, Macle L, Rivard L, Roy D, et al. Cryoballoon ablation for atrial fibrillation. *Indian Pacing Electrophysiol J*. 2012;12:39–53.
42. Verma A, Neal R, Evans J, Castellvi Q, Vachani A, Deneke T, et al. Characteristics of pulsed electric field cardiac ablation porcine treatment zones with a focal catheter. *J Cardiovasc Electrophysiol*. 2023;34: 99–107.
43. Buschmann E, Van Steenkiste G, Vernemmen I, Demeyere M, Schauvliege S, Decloedt A, et al. Caudal vena cava isolation using ablation index-guided radiofrequency catheter ablation (CARTO 3) to treat sustained atrial tachycardia in horses. *J Vet Intern Med*. 2025; 39:e17251.
44. Van Steenkiste G, Boussy T, Duytschaever M, Vernemmen I, Schauvliege S, Decloedt A, et al. Detection of the origin of atrial tachycardia by 3D electro-anatomical mapping and treatment by radiofrequency catheter ablation in horses. *J Vet Intern Med*. 2022; 36:1481–90.
45. Buschmann E, Van Steenkiste G, Vernemmen I, Demeyere M, Schauvliege S, Decloedt A, et al. Lesion size index-guided radiofrequency catheter ablation using an impedance-based three-dimensional mapping system to treat sustained atrial tachycardia in a horse. *Equine Vet J*. 2025;57:1009–16.
46. Ibrahim L, Vernemmen I, Buschmann E, van Loon G, Cornillie P. Morphological variations of the interatrial septum and potential implications in equine cardiology. *Sci Rep*. 2025;15:16500.
47. Ibrahim L. Ostium-specific equine pulmonary vein myocardial sleeve gross anatomy and structural evidence of interatrial myocardial continuity with potential implications for ablation strategies. *Figshare*. 2026. <https://doi.org/10.6084/m9.figshare.33228723>.

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