RESEARCH PAPER

Ultrasound-guided interfascial rectus sheath–associated plane block in sheep: a cadaveric study

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Abstract

Objective To evaluate ovine anatomy to determine the feasibility of an ultrasound-guided interfascial rectus sheath (RS)–associated plane block in sheep cadavers.

Study design Prospective, experimental, randomized cadaveric study.

Animals A group of 22 fresh cadavers (median 52 kg, range 47–62 kg).

Methods In phase I, six cadavers underwent anatomical ($n = 2$) and ultrasonographic ($n = 4$) examination of the ventral abdominal wall and RS. Based on these findings, two lateral (one- or two-injection) approaches were defined. In phase II, 14 cadavers were randomly injected bilaterally using the one-injection approach under ultrasound guidance with 0.1% methylene blue at either high (HV, 0.5 mL kg^{-1}) or low (LV, 0.25 mL kg^{-1}) volume, targeting the plane between the *rectus abdominis* muscle (RAM) and its internal sheath. Dye distribution and nerve staining were evaluated by dissections.

Results The one-injection approach provided better visualization and consistent nerve staining. The eleventh thoracic nerve was stained in all cases, whereas the twelfth (HV = 93%; LV = 79%) and thirteenth thoracic nerves were stained only in two cadavers with HV. Dye spread was limited, suggesting compartmentalization of the interfascial plane. Further investigation in two additional cadavers included injections of methylene blue and a neutral red dye (LV each) between RAM and its internal sheath between alternate muscle bellies. The latter injections were within the same unilateral RAM, separated by tendinous intersections. Dissection showed distinct dye accumulation without mixing.

Conclusions and clinical relevance The described approach produced a limited spread and nerve staining, possibly owing to ovine-specific anatomy and the technique used, which differs from the standard rectus sheath block (RSB) technique. Targeting individual muscular compartments could be an alternative. Further studies using the traditional RSB approach are needed to confirm its applicability in this species.

Keywords locoregional anaesthesia, rectus sheath block, sheep.

Introduction

The rectus sheath block (RSB) is a locoregional technique that targets desensitization of the ventral abdominal midline (Bashandy & Elkholy 2014; St James et al. 2020; Ferreira et al. 2022). This fascial plane block involves injecting local anaesthetic between the *rectus abdominis muscle* (RAM) and the internal rectus sheath (RS), formed by connective tissue from the aponeurosis of the *transversus abdominis* (TAM), and external oblique muscles, along with the *transversalis fascia* (Bashandy & Elkholy 2014; Ferreira 2024). The goal of the RSB is to desensitize the ventral branches of the spinal nerves that traverse the TAM and penetrate the RS and RAM (Ferreira 2024). This technique has been described in dogs, pigs, calves and foals in cadaveric studies (St James et al. 2020; Calice et al. 2021; Ferreira et al. 2022; Ienello et al.

2022; Gutierrez Bautista et al. 2023). However, information regarding its clinical use is scarce and currently limited to calves, cats and dogs (Josso et al. 2022; Alterisio et al. 2023; Kamyabnia et al. 2023; Micieli et al. 2023).

Anatomical differences in RS among mammals, particularly regarding the lateral extent of RAM, may impact the technique's efficacy (Cevik et al. 2022). Variations also occur depending on the cranial or caudal position along the abdominal midline at which the RSB is performed, as the arrangement of muscular and fascial structures changes along this axis (Hermanson & De Lahunta 2018; De Lahunta et al., 2020; Ferreira 2024). Despite shared similarities in the abdominal wall structure across mammals, specific anatomical variations can influence the success and applicability of the RSB approach (Ferreira 2024). Besides, the innervation of the ventral abdomen within common veterinary species has not been fully described, and contradictory information has been reported.

The innervation of the ventral abdominal wall in sheep involves the ventral branches of the two twelfth and thirteenth thoracic nerves (T12–T13), and the first, second and third lumbar nerves (L1–L3) (Velazquez-Delgado et al. 2022; Mansour et al. 2023). These nerves constitute the clinical targets for the desensitization for ventral coeliotomies. A common locoregional technique for abdominal wall desensitization, such as epidural anaesthesia, has potential complications including hypotension, pelvic limb paralysis, infections and accidental subarachnoid space injection (Smith et al. 2021). In small animals, the RSB has been proposed within a multimodal analgesic approach for procedures involving the abdominal midline, such as umbilical hernia repair, midline laparoscopic procedures and laparotomies (Ferreira 2024). However, the RSB has not been explored in small ruminants, where surgical procedures often involve paramedian and ventral midline coeliotomies.

Therefore, this study aimed to: 1) identify the anatomical and ultrasonographic characteristics of the sheep's abdominal wall and 2) evaluate the spread of two volumes of methylene blue injected into the RS-associated plane in sheep cadavers, as a variant of the standard RSB technique. Our initial hypothesis was that the spread of methylene blue dye in the RS-associated interfascial space would differ significantly between the two injection volumes (high and low) in sheep cadavers. Based on the results obtained, a third objective emerged to investigate the anatomical compartmentalization of the interfascial plane. We further hypothesized that this anatomical compartmentalization could influence the distribution patterns of methylene blue dye in sheep cadavers with the latter approach.

Material and methods

A prospective, experimental, cadaveric study was conducted in two stages to investigate the anatomical conformation of the sheep's abdominal wall. Phase I assessed the feasibility of an interfascial RS-associated plane injection in this species by comparing two injectate volumes (phase II). Phase II involved evaluation of methylene blue spread by comparing two volumes of this solution. A third phase was developed based on the results observed during phases I and II, to examine the anatomical compartmentalization of the interfascial RS-associated plane.

A total of 22 fresh sheep cadavers (Aragonesa breed, median weight 52 kg, range 47–62 kg) euthanized within an approved but different study (PROEX 124.1/21) were used. All animals included in the study had no history of abdominal procedures or conditions that could alter the anatomy of the abdominal region. The referenced study was conducted after the completion of the present study, ensuring that the anatomy and fascial layers examined were not influenced by it. Euthanasia was performed with previous sedation (xylazine, 0.2 mg kg^{-1} intravenously, Xylagesic; Calier, Spain), followed by administration of embutramide, mebezonium iodide and tetracaine hydrochloride (1000, 250 and 25 mg per 50 kg body weight, intravenously, respectively; T61, Leonvet, Spain) and confirmed by thoracic auscultation after drug administration. The cadavers were positioned in dorsal recumbency, and the ventral and lateral abdominal regions were clipped from the xiphoid process to the pelvis. This anatomical study adhered to the Anatomical Quality Assurance (AQUA) checklist (Appendix SA) (Tomaszewski et al. 2017) at all stages.

Phase I: anatomical and ultrasonographic study

Anatomical dissection of two fresh cadavers was performed to study the muscular and fascial structures of the abdominal region. The procedure began with the cadavers in the dorsal recumbency. A midline incision was initially made from the xiphoid process to the umbilicus, and the abdominal skin was carefully reflected laterally and dorsally to expose the underlying structures. The muscular structures were identified based on their location, origin, insertion and the direction of their muscle fibres. Once identified, these structures, including the RAM, were described in detail. After the initial identification of the abdominal muscles, the cadavers were repositioned into lateral recumbency to facilitate the examination of deeper structures. The RAM was further dissected by making an incision along its ventral and caudal borders, and the muscle was carefully displaced cranially and dorsally. This enabled a detailed examination of the arrangement of the

fascial layers in relation to the RAM and nerve distribution. Nerve identification was performed by anatomical dissection, tracing each nerve to its exit point from the spinal column to ensure accurate identification. All dissections were conducted by the same anatomist (IGS), blinded to the treatment allocation.

Ultrasonographic evaluation was performed on four additional fresh cadavers to determine the optimal approach for nerve staining. Cadavers were positioned in dorsal recumbency. An 8−12 Hz linear ultrasound probe (M7; Mindray, GD, China) was used for phases I and II. The ultrasound probe was initially placed transversely to the midline, approximately 2 cm cranial to the umbilicus scar, to identify the *linea alba* between the xiphoid process and the umbilicus scar. It was then repositioned to the cranial third of this line and moved laterally as needed to visualize the medial border of the TAM. The RAM was identified as a hypoechoic band superficially, separated from the TAM by a double hyperechoic line. Then, two in-plane injection techniques were tested, both targeting the space between the ultrasonographically deep aspect of the RAM and its internal sheath. For the first injection (one-injection approach), the solution was deposited in the cranial third of the line between the xiphoid process and the umbilical scar, near the TAM medial border. For the second injection (two-injection approach), the probe was moved caudally after the first injection point of the one-injection approach. The second injection was performed approximately 2 cm cranial to the umbilical scar, at a more lateral location along the RAM, where the medial border of the TAM was again identified. In the one-injection approach, 0.25 mL kg^{-1} of a 1:1 mixture of 0.9% saline and 0.1% methylene blue (Proveblue 5 mg mL^{-1}; Fresenius Kabi, Spain) was injected once the target structures were visualized. Using the two-injection approach, another 0.25 mL kg^{-1} of the same solution was injected. The distribution of the dye was assessed based on previously established criteria (St James et al. 2020), where complete staining was defined as > 1 cm and the entire circumference of the nerve stained, and partial staining as < 1 cm or incomplete circumferential staining.

Phase II: evaluation of methylene blue spread

In this phase, 14 fresh cadavers placed in dorsal recumbency were used to assess the interfascial RS-associated plane approach, with the one-injection approach, and compare two volumes of methylene blue. Landmark structures were RAM (near field in the ultrasound image, Fig. 1) separated by a double hyperechoic line from the TAM (far field in the ultrasound image) with the aim of placing the tip of the needle deep to the RAM, between the muscle belly and the internal RS. At this point, a test injection was performed by administering 0.2 mL of the solution and confirming the separation of the RAM from the internal RS by hydrodissection, with no intramuscular injection. Each hemiabdomen was randomly allocated to receive either 0.25 mL kg^{-1} (low volume, LV) or 0.5 mL kg^{-1} (high volume, HV) of 0.1% methylene blue (Excel; Microsoft, WA, USA). A 20 gauge, 9 cm spinal needle (spinal needle; Becton-Dickinson, Spain) was inserted in a mediolateral direction with an in-plane technique, using a new needle for

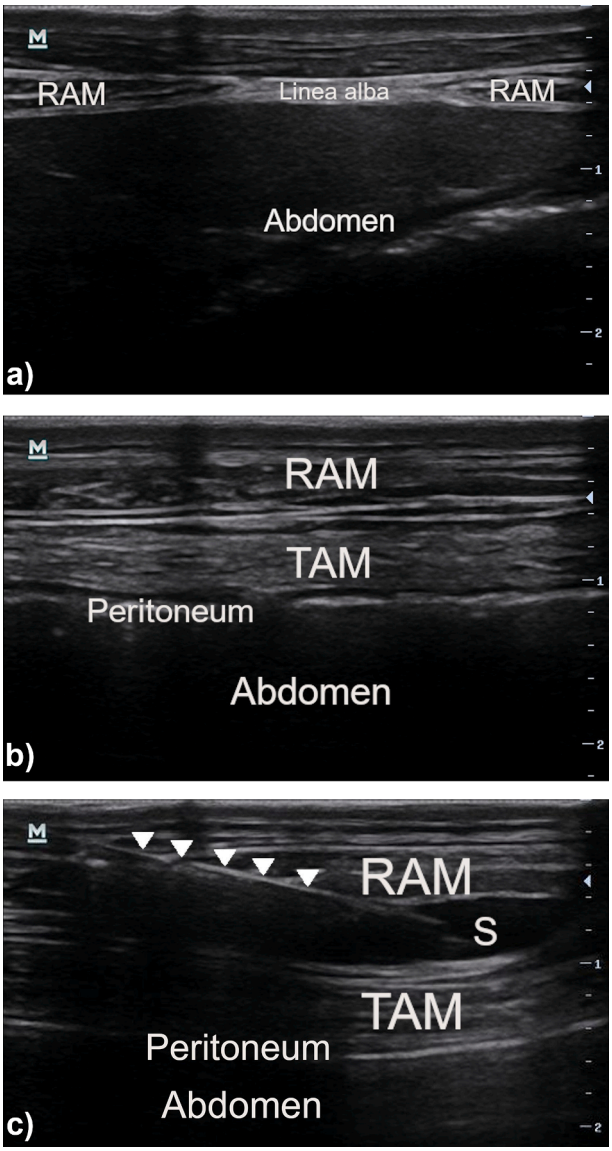

Figure 1 Transverse ultrasound images of the abdominal wall of a sheep cadaver. (a) Ultrasound image of the midline. (b) Ultrasound image after moving the ultrasound probe laterally to see the *rectus abdominis* muscle (RAM) and *transversus abdominis* muscle (TAM) prior to solution injection. (c) Ultrasound image after solution injection between *rectus abdominis* muscle and the internal rectus sheath. S, solution; Triangles, needle.

each hemiabdomen. The quality of ultrasound image and needle visualization was rated from 2 (excellent) to 0 (poor) (St James et al. 2020). Also, the number of needle redirections (defined as any adjustment in the angle or depth of the needle during the injection process) was recorded. The same investigator, who was not blinded to the treatment allocation, performed all the injections (RBD). Dissections were performed immediately after completion of both injections in each cadaver by the anatomist and a different investigator, both of whom were blinded to the injection volumes.

Phase III: investigation of compartmentalization of the interfascial RS plane

Following observations from phases I and II, a third phase was conducted to explore possible compartmentalization within the interfascial RS-associated plane. An additional two fresh sheep cadavers were used. For each sheep, one hemiabdomen was used for anatomical dissection to observe the fascial plane's anatomical relationships, without any distortion from fluid deposition. In the other hemiabdomen, two injections were performed in the first sheep (0.1% methylene blue at 1 mL kg^{-1} total volume, divided into two injections), to evaluate fluid-filled pocket formation. In the second sheep, alternate depositions were performed using 0.1% methylene blue (0.5 mL kg^{-1} at two injection points) and 0.5% neutral red dye (0.25 mL kg^{-1}, Neutral red; Sigma-Aldrich, Spain). Injections were made into adjacent and alternate muscle bellies within the same unilateral RAM, but separated by tendinous intersections, to assess colour dispersion and mixing. The injections were made after exposure of the external RS and RAM by a midline skin incision. A small incision was made in the external RS to allow catheter insertion. A 22 gauge venous catheter (Surflo; Terumo, Spain) connected to a 50 mL syringe was used to inject the dye between the RAM and its internal sheath, confirmed by direct anatomical visualization during dissection, and the dye was injected accordingly.

Statistical analysis

The sample size was determined primarily based on the availability of sheep cadavers, similar to studies in other species (St James et al. 2020; Ferreira et al. 2022; López-Ramis et al. 2025). In addition, similar anatomical studies were reviewed, which suggested that a minimum of five to 10 specimens would be sufficient for a descriptive study with a low expectation of anatomical variability (Ahmed et al. 2019). For the volume comparisons, an initial sample size calculation was conducted using data from the first two cadavers. Assuming a minimum detectable difference of 2 cm in nerve staining, with a standard deviation of 2 cm, a total of 16 specimens was calculated to achieve an α value of 0.05 and a power of 0.8. However, based on the observed consistency of results, a sample size of 14 cadavers was deemed sufficient to ensure statistical reliability. The data collected for statistical analysis included the number of nerves stained (both the number of animals per nerve and the percentage), the length of staining (in cm), the number of needle redirections and test doses, as well as the quality of ultrasound image and needle visualization (percentage for each category). Data normality was tested using the Shapiro–Wilk test. Parametric data are presented as mean ± standard deviation and nonparametric and ordinal variables are expressed as median (range). The Fisher's exact test was used for the presence or absence of nerve staining and evaluation of ultrasound image quality and needle visualization. The *t*-test compared nerve staining

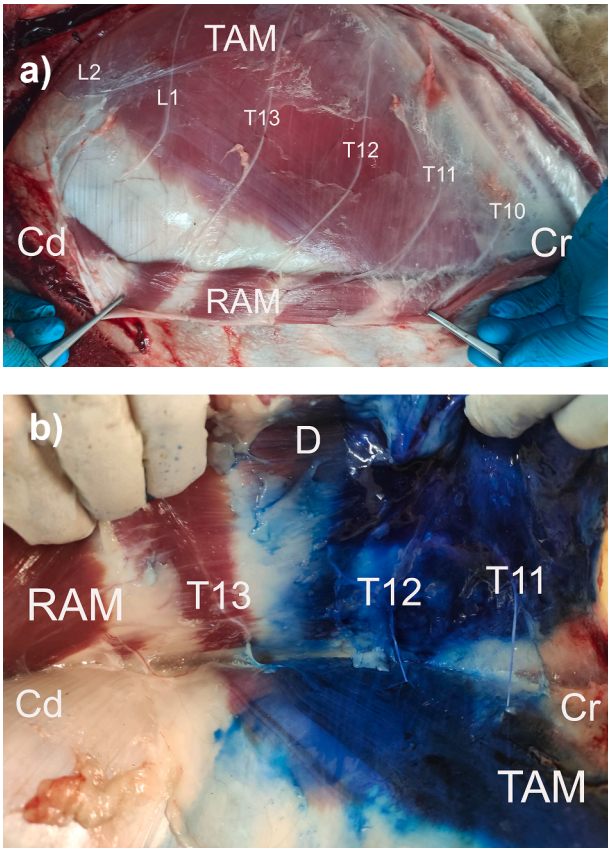

Figure 2 (a) Dissection of a sheep cadaver for identification of target structures and its disposition in the sheep. The *rectus abdominis* muscle (RAM) is reflected medially to view the thoracic and lumbar (T10 to L2) ventral nerve branches based on the vertebrae of origin, and its oblique direction. (b) Dissection of a sheep cadaver after ultrasound-guided rectus sheath injection using methylene blue dye. Dye is observed within the internal rectus sheath, between RAM and *transversus abdominis* muscle (TAM). The RAM is reflected dorsally to observe stained ventral branches of thoracic spinal nerves (T11 to T13). Cd, caudal; Cr, cranial; D, dorsal; L, lateral; M, medial.

length, number of needle redirections and test doses between LV and HV. Significance was set at $p < 0.05$.

Results

Phase I: anatomical and ultrasonographic study

Dissection revealed the cutaneous muscle of the trunk externally, with fibres oriented ventrally. Once removed, RAM was exposed, showing fibres parallel to the *linea alba* and divided by tendinous intersections. The RAM was pyramidal, polygastric, widest caudally and narrowing towards the costal arch. It was covered cranially by the pectoralis major and laterally by the external oblique muscle, whose muscle fibres originated on the lateral aspect of ribs and were arranged in a craniocaudal and dorsoventral direction. Dissection of the pectoralis muscle showed that the external oblique muscle extended to the midline, forming part of the external lamina of RS. The RAM extended laterally, differing from other species with a more ventrally localized muscle band, generally. Deep to the RAM, TAM had fibres oriented caudoventrally, inserting into the *linea alba*, forming the internal lamina of the RS (Fig. 2a and b). When elevating the RAM dorsally, the ventral branches of the spinal nerves were observed coursing within the muscular part of the TAM, emerging to enter the RAM with an oblique trajectory. The distance between these nerve roots increased progressively as they emerged further caudally along the animals' intervertebral foramina. The TAM's muscular portion approached the midline more cranially; while progressing caudally, the muscular portion ran increasingly laterally from the midline. Fat deposition and apparent trabecular connective tissue were noted between muscle structures.

Caudally, upon lifting the external oblique muscle, the internal oblique muscle with fibres oriented caudocranially and dorsoventrally was identified as a small-sized muscle and an aponeurosis of insertion that did not reach the midline.

The anatomical dissection showed spinal nerves traversing the TAM under its fascia and penetrating the individual muscle bellies of RAM in an oblique trajectory, at increasing distances from the costal arch as they moved caudally. Notably, these nerve fibres entered the RAM in a relatively lateral position (Fig. 2a).

The anatomical findings suggested that the ultrasound probe should be placed lateral to the *linea alba* to better

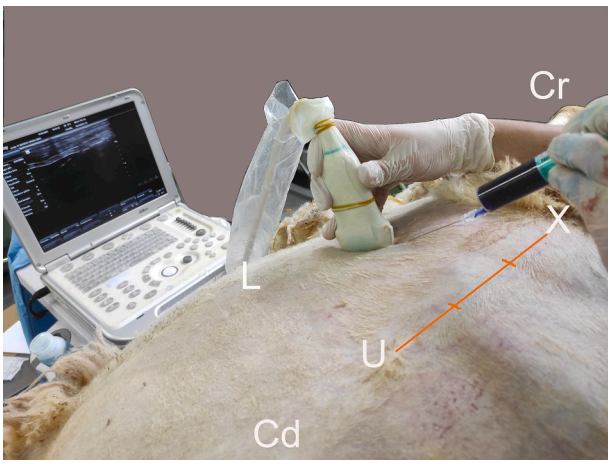

Figure 3 View of the ultrasonographic approach for rectus sheath block in sheep, in the proximal third of the distance between the *umbilicus* scar and the *xiphoid appendix* and moved laterally to see the target structures (*rectus abdominis* muscle, internal sheath of the *rectus abdominis* muscle, *transversus abdominis* muscle). Cd, caudal; Cr, cranial; L, lateral; U, umbilicus scar; X, xiphoid appendix.

Table 1 Staining scores of ventral nerve branches of spinal nerves after ultrasound-guided rectus sheath injections performed with two volumes of 0.1% methylene blue methylene blue (0.25 or 0.50 mL kg^{-1}) in 14 sheep cadavers. Score 0, partially stained when the nerve was stained < 1 cm or not completely circumferentially. Score 1, completely stained when ≥ 1 cm of nerve stained and entire circumference of the nerve was stained. T, thoracic vertebra.

Sheep	Volume (mL kg^{-1})	Spinal nerves			
		T10	T11	T12	T13
1	0.25	0	1	1	0
	0.5	0	1	1	1
2	0.25	0	1	1	0
	0.5	0	1	1	0
3	0.25	0	1	1	0
	0.5	0	1	1	1
4	0.25	0	1	1	0
	0.5	0	1	1	0
5	0.25	0	1	1	0
	0.5	0	1	1	0
6	0.25	0	1	0	0
	0.5	0	1	1	0
7	0.25	0	1	1	0
	0.5	1	1	1	0
8	0.25	1	1	0	0
	0.5	0	1	0	0
9	0.25	1	1	1	0
	0.5	0	1	1	0
10	0.25	0	1	0	0
	0.5	0	1	1	0
11	0.25	0	1	1	0
	0.5	0	1	1	0
12	0.25	0	1	1	0
	0.5	0	1	1	0
13	0.25	0	1	1	0
	0.5	0	1	1	0
14	0.25	0	1	1	0
	0.5	0	1	1	0

visualize the target structures, with the optimal position being in the proximal third between the xiphoid process and the umbilicus scar. This positioning improved the visibility of RAM, its internal sheath and TAM on ultrasound, as the muscular belly of TAM was closer to the *linea alba* in a more cranial direction (Fig. 3). Subsequent ultrasonographic evaluation revealed then the presence of *linea alba* in the midline, with the RAM (superficial in the ultrasound image) laterally. Moving the probe laterally, approximately 7−10 cm from the midline, and close to the costal arch, facilitated visualization also of the TAM (deep in the ultrasound image) superficial to the hyperechoic peritoneal line (Fig. 1b).

For the two-point injection, the probe was moved caudally towards the umbilical scar and then laterally another 10 cm to visualize the same structures as for the first injection point. However, target visualization was less clear, and no nerve staining was achieved with the second injection, owing to anatomical separation of nerve branches.

Phase II: evaluation of methylene blue spread

Both volumes of methylene blue consistently stained the roots of the eleventh (T11) and twelfth (T12) thoracic nerves in all cases (Fig. 2b). T11 was stained in all animals with both volumes, with a mean length of 4.5 ± 1.7 cm for LV and 4.0 ± 1.5 cm for HV, whereas T12 was stained in 11/14 (79%) animals with LV (5.1 ± 1.8 cm) and in 13/14 (93%) animals with HV (5.5 ± 1.8 cm). Additionally, T10 was stained in two animals with LV (3.2 ± 0.3 cm) and one with HV, whereas T13 was stained in two animals with HV (4.0 ± 1.4 cm). However, no staining of L1 was observed (Table 1). There were no significant differences in nerve staining length, or the number of nerves stained between LV and HV. The image quality was judged as excellent in 72%, good in 21% and poor in 7% for LV, and excellent in 64% and good in 22% for HV (Table 2). Needle visualization was judged as excellent (85%) or good (15%) for LV, and excellent in 79% of observations for HV. Most of the

Table 2 Evaluation of ultrasound (US) quality and US needle visualization during US-guided rectus sheath injections of 0.1% methylene blue (0.25 or 0.50 mL kg^{-1}) in 14 sheep cadavers. For US, image quality was categorized as follows: excellent, clear double line, distinct muscle layer and internal rectus sheath separation post-injection; good, muscle and peritoneum identifiable, no clear double line; and poor, poor visibility of the landmarks. Needle visualization was classified as excellent, with full visualization of the shaft and tip; good, with clear visibility of only the tip; and poor, where the tip's position was discernible only after administering a test dose.

Sheep	Volume (mL kg^{-1})	US image quality	US needle visualization	Number of test doses	Number of needle redirections
1	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
2	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
3	0.25	Good	Excellent	1	1
	0.5	Good	Excellent	1	1
4	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
5	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
6	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
7	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
8	0.25	Poor	Good	3	2
	0.5	Poor	Good	2	1
9	0.25	Good	Good	1	1
	0.5	Good	Excellent	1	0
10	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
11	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
12	0.25	Excellent	Excellent	1	0
	0.5	Excellent	Excellent	1	0
13	0.25	Excellent	Excellent	1	0
	0.5	Poor	Good	2	1
14	0.25	Good	Excellent	1	0
	0.5	Good	Good	1	0

sheep required only one administration of test dose without needle redirection (13 for LV, 12 for HV).

An unexpected finding was the formation of fluid-filled pockets or compartments containing the injected dye, consistent across volumes. These sacs appeared to match the boundaries of RAM muscle bellies and were accompanied by trabecular connective tissue. Additionally, trabeculae of apparent connective tissue were observed during anatomical dissection and evaluation of the staining. The observed fascial compartmentalization did not exactly correspond to the tendinous intersections of the RAM; instead, they were present in the insertion aponeurosis of the internal oblique muscle. Numerous trabeculae of connective tissue were also observed.

Phase III: investigation of compartmentalization of the interfascial RS plane

Injection of 1 mL kg^{-1} methylene blue, divided into two injections, revealed that the dye formed a well-defined pouch as a result of connective tissue retention, preventing cranial and caudal diffusion but successfully staining nerves within the pouch (Fig. 4a). Following the administration of both methylene blue and neutral red dye, it was observed that the dyes neither mixed nor progressed beyond insertions separating the muscle bellies of the RAM (Fig. 4b).

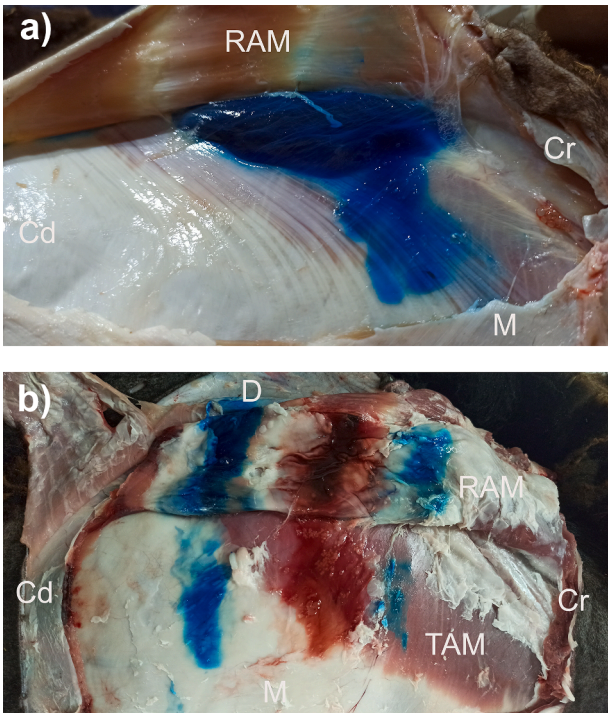

Figure 4 Dissection of a sheep cadaver after injection of methylene blue dye between the *rectus abdominis* muscle (RAM) and its internal rectus sheath, for evaluation of dye distribution in the interfascial plane. (a) Observation of a fluid-filled sac between the RAM and its internal sheath after injection of 0. 5 mL kg^{-1} of methylene blue. (b) Distribution of dye solution after injection of alternate methylene blue dye and neutral red in adjacent RAM muscle bellies, without colour mixing (0.25 mL kg^{-1} each). Cd, caudal; Cr, cranial; D, dorsal; M, medial; TAM, *transversus abdominis* muscle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Discussion

The anatomical investigation revealed practical relevant differences in the muscular disposition of the TAM in sheep compared with other species such as dogs or calves (St James et al. 2020; Ferreira et al. 2022), which impacted the ultrasound-guided approach. Specifically, the dorsolateral termination of the muscular portion of the TAM suggests a divergence in ultrasound visualization in this area compared with other species. The lateral dorsal extension of the RAM results in the intersection between this muscle and TAM occurring at a more dorsolateral point within the abdominal wall compared with other species (St James et al. 2020; Ferreira et al. 2022; Ienello et al. 2022; Gutierrez Bautista et al. 2023), despite significant anatomical variability reported in dogs. Although the classical RSB technique positions the needle tip medial to the TAM's medial border, we modified our injection approach to target the more dorsally located spinal nerves, farther from the midline. This deviation from the standard RSB technique was intended to enhance nerve staining. Consequently, the feasibility of the conventional RSB approach, as described in other species, remains uncertain in sheep. However, our results may indicate a required adaptation for its effective use in this species.

The evaluation of the ultrasound-guided approach when comparing the spread of the injectate revealed the absence of staining of T13—L1. This lack of staining might be attributed to the spinal nerves' oblique trajectory and their entry into the RAM from a greater distance caudal to the costal arch (Mansour et al. 2023). This anatomical configuration, as previously described in sheep (Velazquez-Delgado et al. 2022), suggests that the separation of nerve roots may hinder successful staining. This may theoretically limit the efficacy of this block, although previous studies have described a dermatome overlap between spinal nerves of the abdominal wall (Linzell 1959; Velazquez-Delgado et al. 2022). However, the overlapping of nerves on dermatomes could partially overcome the failure to block each nerve (Velazquez-Delgado et al. 2022). Moreover, deviation from the standard RSB technique may have influenced the spread pattern.

In terms of injection techniques, the one-injection approach yielded better staining results and improved muscle plane visualization compared with the two-injection approach. Although a two-injection approach might theoretically enhance coverage, our findings indicated that moving the

injection site caudally led to poorer visualization of the ultrasound landmarks and reduced staining success. This observation underscores the importance of precise injection site placement to optimize block efficacy. For interfascial plane blocks, it is assumed that a larger volume will result in a more widespread distribution of the injectate (Fischer 2024). However, this assumption may vary depending on the fascial plane and the species evaluated (Bruggink et al. 2012). In the transversus abdominis plane block in cats, the use of two injections involved more nerve staining despite the use of lower volumes (Garbin et al. 2022). Therefore, we assessed two volumes, 0.25 and 0.5 mL kg^{-1}, as in other species (St James et al. 2020; Ferreira et al. 2022).

Several factors such as collagen arrangement of fascial layers, presence or absence of adipose tissue and contractile elements also play a role in fascial plane blocks (Chin et al. 2021; Fischer 2024), and differences between species has been described (Ahmed et al. 2019). A relevant finding of the present study was the lack of diffusion of the injectate solution used despite using larger volumes and different approaches. Fascial plane blocks target the layers of deep *fascia*, which should be loosely connected to allow them to be easily separated through hydrodissection by the injectate (Chin et al. 2021; Fischer 2024). In the present study, a dense macroscopic arrangement of the connective tissue forming the aponeurotic portion of the RS was observed, similar to that in horses (Ahmed et al. 2019), which probably prevented the diffusion of dye solution in these sheep. Overall, this macroscopic anatomical disposition with more compact connective tissue arrangement likely accounted for the absence of staining in distant nerve branches (Chin et al. 2021). Although the injections were administered between RAM and TAM rather than following the standard RSB approach, the compartmentalization we observed is unlikely to result solely from this technical variation. In fact, the phase III findings, despite medial injection, support the possibility of true anatomical compartmentalization within the interfascial RS-associated plane in sheep. However, the feasibility of the standard RSB approach and potential differences in dye spread, as documented in other species, remain to be investigated in sheep.

In the present study, the rate of success of T13 nerve staining was very low and no staining of the lumbar nerves was obtained. This may preclude the use of this block for procedures involving the caudal part of the abdomen. However, the percentage of nerve staining in the present study does suggest potential sensory blockade of the ventral midline abdominal wall cranial to the *umbilicus*, and the umbilical area (St James et al. 2020; Ferreira et al. 2022; Ienello et al. 2022).

This study has several limitations. First, it used cadavers, although the animals were euthanized immediately before the study with all procedures completed within 1 hour in all cases. However, the conditions of the present study may not entirely reflect the conditions *in vivo*. The sample size calculation, based on nerve staining counts, may limit its accuracy, especially given the restricted dye spread suggesting anatomical compartmentalization. A limitation of the study is that the investigator performing the injections was not blinded because of practical challenges of administering visually distinguishable volumes. However, the anatomical dissections were performed by a different investigator and the anatomist, who used objective measurements (e.g. number and length of stained nerves) to minimize bias. The concentration of 0.1% methylene blue was chosen to achieve optimal dye visualization, aiming to simulate the viscosity and spread characteristics of the local anaesthetic solutions typically used in clinical practice. However, the viscosity of the dye solution was not determined in this study, which could potentially influence the injection spread. Although this approach does not replicate the standard RSB as originally described in other species, it may provide insight into the challenges and potential adaptations required for this interfascial plane block in sheep *in vivo*.

In conclusion, the anatomical and ultrasonographic evaluation revealed unique characteristics of the RS and abdominal wall in sheep. Owing to the different anatomical conformation in this species, the interfascial RS-associated plane block may not be feasible because spread of the anaesthetic solution is prevented. Alternatively, the injection of local anaesthetic solution at each muscular pocket would be required. Further research is warranted to investigate the feasibility of the standard RSB technique and the possible limited diffusion of the anaesthetic solution in this species.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

Preliminary results were presented at the Association of Veterinary Anaesthetists (AVA) Autumn Meeting, London, 18–20 September 2024.

No artificial intelligence tools were used in the preparation of this manuscript.

Authors' contributions

RB: conception and design of the study, ultrasound evaluation, anatomical dissection and evaluation, RSB injections, data management, statistical analysis and preparation of the drafted and final versions of the manuscript. MR: conception and design of the study, anatomical dissection and evaluation and critical revision of the manuscript. IGS: anatomical dissection and evaluation, study design and critical revision of the manuscript. IAGS: revision of study design and critical revision of the manuscript.

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Received 27 September 2024; accepted 26 August 2025.

Available online 1 September 2025

Supporting Information

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaa.2025.08.041.