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A comparative multi-site and whole-body assessment of fascia in the horse and dog: a detailed histological investigation

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Abstract

Fascia in the veterinary sciences is drawing attention, such that physiotherapists and animal practitioners are now applying techniques based on the concept of fascia studies in humans. A comprehensive study of fascia is therefore needed in animals to understand the arrangement of the fascial layers in an unguligrade horse and a digitigrade dog. This study has examined the difference between the horse and the dog fascia at specific regions, in terms of histology, and has compared it with the human model. Histological examinations show that in general the fascia tissue of the horse exhibits a tight and dense composition, while in the dog it is looser and has non-dense structure. Indeed, equine fascia appears to be different from both canine fascia and the human fascia model, whilst canine fascia is very comparable to the human model. Although regional variations were observed, the superficial fascia (*fascia superficialis*) in the horse was found to be trilaminar in the trunk, yet multilayered in the dog. Moreover, crimping of collagen fibers was more visible in the horse than the dog. Blood vessels and nerves were present in the loose areolar tissue of the superficial and the profound compartment of hypodermis. The deep fascia (*fascia profunda*) in the horse was thick and tightly attached to the underlying muscle, while in the dog the deep fascia was thin and loosely attached to underlying structures. Superficial and deep fascia fused in the extremities. In conclusion, gross dissection and histology have revealed species variations that are related to the absence or presence of the superficial adipose tissue, the *retinacula cutis superficialis*, the localization and amount of elastic fibers, as well as the ability to slide and glide between the different layers. Further research is now needed to understand in more detail whether these differences have an influence on the biomechanics, movements and proprioception of these animals.

Key words: deep fascia; dog; histology; horse; hypodermis; myofascial kinetic lines; superficial fascia.

Introduction

Fascia is defined as being "*a fibrous collagenous tissue, which is part of a body-wide tensional force transmission system*" (Schleip et al. 2012a). Superficial fascia (SF) is often defined as being an enveloping layer to be found directly beneath the skin, which might contain areolar as well as dense connective tissue (Langevin & Huijing, 2009). It divides the hypodermis, situated beneath the dermis, into a superficial and profound compartment with the superficial adipose tissue (SAT) above, and the deep adipose tissue (DAT) below (Stecco et al. 2008). The superficial compartment is regarded as being the one related to the exteroception and the profound to the interoception (Lancerotto et al. 2010). The histology of SAT shows the presence of large fat globules encased between fibrous septa. These septa, otherwise referred to as *retinacula cutis superficialis*, appear mostly oriented perpendicular to the surface of the skin, and serve to anchor the dermis to the deeper layers. The DAT has more oblique septa, referred to as *retinacula cutis profundus*, with limited elastic properties (Stecco et al. 2011a). The obliquity of these retinacula improves the slide and glide capacity of the adipose globules, and thereby facilitates the flexibility/movement between the superficial

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and profound compartments of the hypodermis (Stecco et al. 2009; Lancerotto et al. 2010). The deep fascia (DF) is a continuous sheet of dense connective tissue associated with the deeper layers (Langevin & Huijing, 2009). According to several other authors, “the deep fascia is a fibrous membrane forming an intricate network which envelops and separates muscles, forms sheaths for nerves and vessels, strengthens ligaments around joints, and binds all these structures together into a firm compact mass” (Stecco et al. 2011a; Findley et al. 2012). The DF envelops all the muscles of the body, but has different features according to which region it is sampled from (Stecco et al. 2011a). It has been documented that this complex three-dimensional (3D) network of fascia is involved in movement perception and coordination (Stecco et al. 2010), as well as the transmission of muscle force within the body (Maas et al. 2005; Meijer et al. 2006; Rijkelijkhuizen et al. 2007). The biomechanical properties of fascia tissue have been studied by a number of scientific groups (Vleeming et al. 1995; Langevin, 2006; Corey et al. 2012; Schleip et al. 2012b), and indeed fascia facilitates the functional connection between the trunk and the limbs (Vleeming et al. 1995; Fairclough et al. 2006). Observations in human samples, taken from different topographical regions within the body, show that DF presents diverse structures that seem to reflect specific functions of the lower and upper limbs, and their connection to the trunk (Stecco et al. 2008, 2009). Of recent importance is the finding of the existence of nociceptive fibers in fascia tissue (Tesarz et al. 2011; Taguchi et al. 2013). Fascia has therefore been proposed as being a source of pain in such conditions as fasciitis, as well as cases of non-specific lower back pain (Langevin & Sherman, 2007).

Fascia has also started to become an important research field in the veterinary sciences. Physiotherapy and rehabilitation have become tools of increasing importance in equine and canine medicine (Kathmann et al. 2006; Paulekas & Haussler, 2009) alongside traditional orthopedic treatment in order to treat musculoskeletal problems and to improve quality of life. Stretching, massage, myofascial release, kinesio taping or trigger point therapy are all techniques that are currently being employed by veterinary practitioners (Goff, 2009; Scott & Swenson, 2009; Wall, 2014). In a recent study, the fascia structure of the equine forelimb and the retinacula was documented, revealing differences between human and equine antebrachial fascia (Skalec & Egerbacher, 2017). In addition, the discovery of myofascial kinetic lines in the horse, as translated from the human myofascial trains, now provides an anatomical foundation for an improved understanding of fascia and by inference the biomechanics of animals (Elbrønd & Schultz, 2015).

Although fascia research continues to interest the field of veterinary medicine, there remains limited information regarding differences in both the macro- and micro-anatomical structures of fascia in diverse species. Fundamental anatomical research is therefore needed. Moreover, it seems equally relevant to examine species differences in terms of fascia directly, rather than copying concepts across from the human model.

The aim of this study was to investigate and compare the fascia of the horse and dog, to sample from a number of specific anatomically diverse regions, and to relate these findings to the Stecco human model of the upper fascia layers (Fig. 1A). The hypothesis being tested in this study was that the horse and dog fascia layers are histologically different in terms of their diffuse/widespread structure and biomechanical properties.

Materials and methods

Subjects

Eight horses and five dogs of different ages and breeds were killed for reasons unrelated to this study. Among them, one horse and two dogs were frozen for transverse sectioning. The one dog was frozen in a hanging position upside down and the other in a ventral prone/lying position. Six horses and four dogs were dissected in order to provide a topographical overview of myofascial structures. Tissues were sampled for histological study. Full-thickness specimens from the skin through to the muscle layers were collected. All the animals used in this study were kindly donated to the Department of Veterinary and Animal Sciences, University of Copenhagen. The owners were informed about the use of animals for research purposes and subsequently signed a consent form. The beagle dogs were donated after euthanization to the University of Copenhagen from a private research company. Table 1 presents and summarizes the data pertaining to the subjects used in this study.

Anatomical regions

After locally shaving the skin, tissue samples were obtained from 10 regions in the horse and six regions in the dog, considered to be functionally important and related to previously dissected equine myofascial kinetic lines (Elbrønd & Schultz, 2015; Figs 2 and 3). The samples included the layers from the epidermis and through to a depth of 2–5 cm, variation being related to the site of sampling.

R1. *Regio colli lateralis*: caudo-ventral to the ventral part of the atlas wing, including *m. brachiocephalicus* in the horse sections, and *m. cutaneus faciei* and *m. brachiocephalicus* in the dog sections.

R2. *Regio abdominis lateralis*: cranio-ventral to the tuber coxae, including *m. obliquus externus abdominis* in the horse sections, and *m. cutaneus trunci* and *m. obliquus externus abdominis* in the dog sections.

R3. *Regio lumbalis*: paravertebral to L4, including *m. longissimus thoracis* in the horse sections, and *m. cutaneus trunci* and *m. longissimus thoracis* in the dog sections.

R4. *Regio axillaris*: caudal to the region superimposed by the tuber olecrani in a neutral posture, including *m. pectoralis ascendens* in the horse sections, and *m. cutaneus trunci* and *m. pectoralis ascendens* in the dog sections.

R5a. *Regio genus lateralis*: at the border between the cranio-distal part of *m. biceps femoris* and fascia genus at the level of the mid-distal part of the patella, including *m. biceps femoris* in both horse and dog sections.

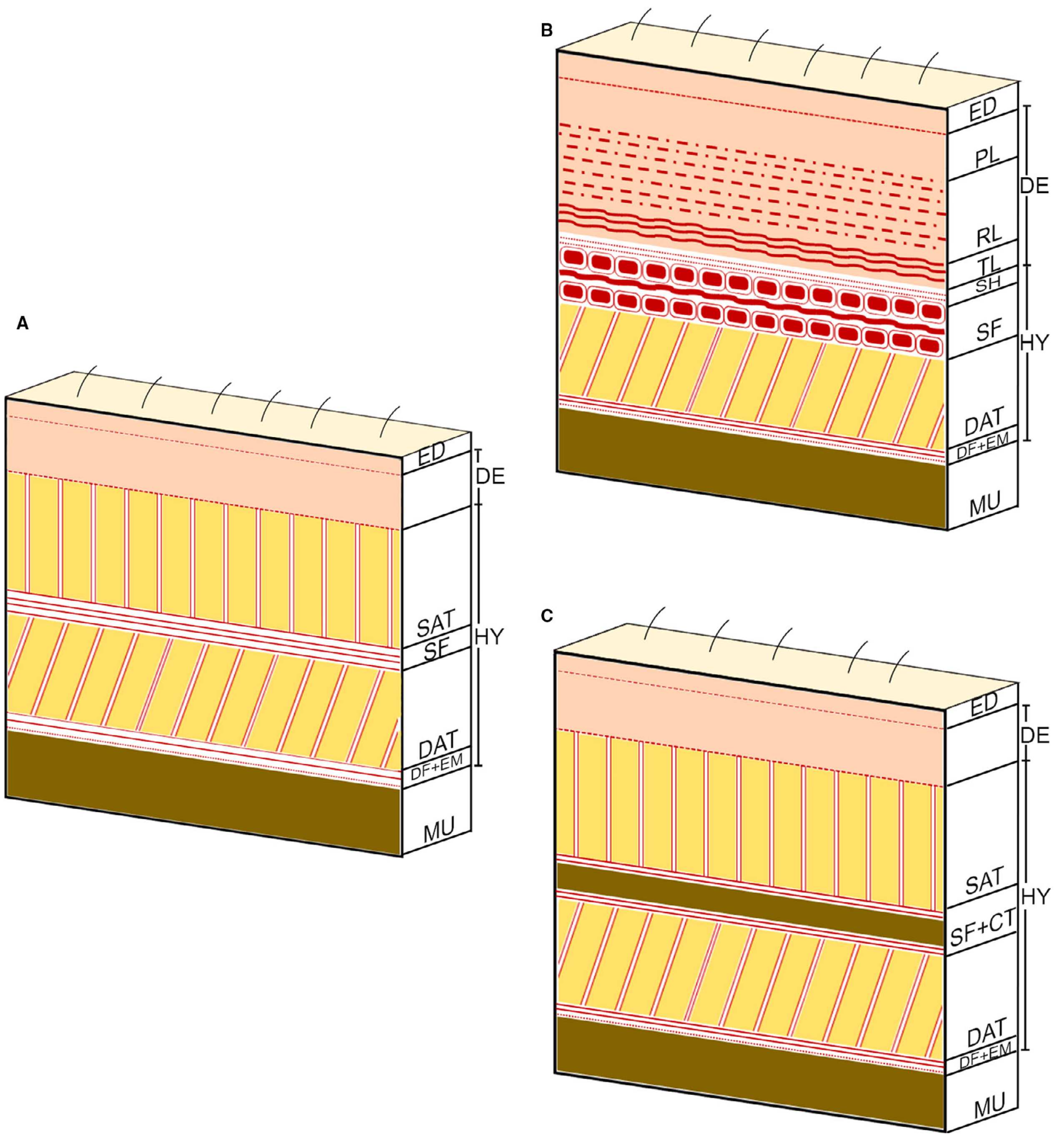

Fig. 1 Theoretical models of fascia layers. (A) The Stecco human model. (B) Horse model. (C) Dog model. DAT, deep adipose tissue; DE, dermis; DF+EM, deep fascia inclusive epimysium; ED, epidermis; HY, hypodermis; MU, muscle; PL, papillary layer of dermis; RL, reticular layer of dermis; SAT, superficial adipose tissue; SF, superficial fascia; SF+CT, superficial fascia inclusive *m. cutaneus trunci*; SH, superficial compartment of hypodermis; TL, third layer of dermis.

R5b. *Regio genus medialis* (only in the horse): at the cranio-distal edge of the *m. gracilis* at the level of the mid-distal part of the patella, including *m. gracilis* in the sections.

R6. *Regio carpi*: in the midline at the level of the intercarpal joint. In the horse sections, this region might include the *retinaculum extensorum* of carpus, the tendon from the *m. extensor carpi radialis*, and in some sections also the articular capsule of the intercarpal joint. In the dog sections, the region comprises the skin underlying the hypodermis and the joint capsule of the intercarpal joint. The *retinaculum extensorum* of carpus is situated lateral to the sampling region/area.

R7. *Regio xiphoidea* (only in the horse): at the level of the insertion of the *m. pectoralis ascendens*, including this muscle and *m. obliquus externus abdominis* in the sections.

Table 1 A description of the subjects included in the study with regard to their species, age, sex and breed

No	Animal	Age	Sex	Breed
1	Horse	17	Mare	Mixed breed
2	Horse	28	Gelding	Race horse
3	Horse	26	Gelding	Pony
4	Horse	27	Mare	Mixed breed
5	Horse	28	Gelding	Mixed breed
6	Horse	21	Mare	Welsh
7	Horse: transverse sections	2	Stallion	Icelandic horse
8	Horse: dissection	18	Mare	Fjord horse
9	Dog	2–5	Male	Beagle
10	Dog	2–5	Male	Beagle
11	Dog	2–5	Male	Beagle
12	Dog: transverse sections	2–5	Female	Beagle
13	Dog: transverse section	2–5	Female	Beagle
14	Dog: dissection	6–7	Female	Border collie

R8. *Fascia abdominis lateralis* (only in the horse): on the midpoint of a vertical line through the tuber coxae, including *m. cutaneus trunci* and *m. obliquus externus abdominis* in the sections.

R9. *Regio genus* (only in the horse): proximo-dorsal to the *patella*, including *m. quadriceps femoris* in the sections.

The first six common regions to both species are very relevant with respect to the kinetic myofascial lines. The following regions, R5b, 7, 8, 9 in the horse were sampled in order to observe intraspecies regional variations.

Tissue processing for histology

The tissue samples were placed in a 4% neutral-buffered formalin immediately after sampling and fixed for at least 24 h. The samples were processed for paraffin embedding using a prolonged fascia version. This version included an extended infiltration period of 6 h to ensure adequate penetration of paraffin into the tight fibrous and dense tissues of the samples. Hereafter the samples were embedded in paraffin, cut into 4–6 μm thick sections and sampled on glass slides. The sections were stained with hematoxylin-eosin, Van Gieson, Weigert's Resorcin Fuchsin and Alcian Blue stain. Alcian blue staining was performed at pH 2.6 to specifically visualize Hyaluronan (HA).

The histological preparations were observed under a light microscope Leica DMR (Leica Microsystems, Wetzlar, Germany). Images were sampled with the Leica Application Suite (v 4.10.0). Photographs were obtained using a Ricoh GX200 and a Canon GX-1 camera. The width of the epidermis and dermis and SF were measured in the transverse recordings with ImageJ software (v 1.8.0_112). Six sections per region in the horses and three sections in the dogs were measured. Means were taken and values are presented as mean ± standard deviation.

Results

Microscopic analysis – specific to the horse

In all the equine samples analyzed, the different layers in the samples were found to be very compact, and several of them were tightly connected (Fig. 4A). The top layers in all the samples were the epidermis (0.04 ± 0.01 mm), dermis and an underlying hypodermis. The thickness of the dermis varied, being thickest in the regions on the dorsum of the trunk (R2 and 3; 5.5 ± 1.0 mm), and thinnest on the latero-ventral part of the trunk and neck (R1, 4, 7 and 8; 2.6 ± 0.6 mm) and on the extremities (R5a, 5b, 6 and 9; 3.3 ± 0.5 mm). The dermis was composed of three layers in the horse. The first layer was the superficial papillary layer, present just below the epidermis and blended into the deeper secondary layer, the reticular layer. This was composed of large irregular collagen bundles, with few interwoven elastic fibers, and hold the nerves and vessels. The third layer, the most profound, the deeper dense parallel collagen layer consisted of horizontal collagen fibers arranged in a regular pattern. Crimping effect of the regular collagen bundles was observed in this layer, as well as undulating elastic fibers detected in the Weigert's Resorcin Fuchsin stain (Fig. 4B). The dermis was separated from the hypodermis by a layer of loose collagen and elastic fibers. In this layer also, vessels and nerves of variable size were present in horizontally arranged and Alcian blue-positive islets of areolar tissue (soft irregular fascia), showing the presence of HA (Fig. 4B,D). The SF separated the hypodermis into a superficial and profound compartment, was found to be of fibroelastic origin and overlay the DAT (Figs 1A and 4B). No SAT layer was observed in the horse, except for in the lumbar region (R3) where the superficial part of the hypodermis (SH) was infiltrated with adipose tissue. Overall the morphology and number of layers in the samples varied between the regions. The SF was on the dorsolateral trunk (R1, 2, 3, 4, 7, 9) observed as a trilaminar structure (118.0 ± 0.3 μm). The three collagen layers were arranged at oblique angles, of which the two peripheral layers were sheeted around collagen bundles in the mid-layer (Fig. 4B). A crimping effect was also observed in the SF. A split/branching of the SF was observed in the R8 with the *m. cutaneus trunci*. Here the SF divided into two or more layers, which surrounded the muscle superficially and profoundly, and connected into the endo-, peri- and epimysium of the muscle. In general, HA was located within and underneath the SF (Fig. 4D). On the extremities, the SF had a simpler structure and was observed as parallel collagen fibers/bundles (R. 5a, 5b, 6). Underneath the SF, a DAT layer of variable thickness and constitution was observed. In some regions, the DAT was present as large islets of areolar tissue, which included larger vessels and nerves. Additionally, obliquely arranged retinacular structures (*retinacula cutis profundus*) including vessels and nerves were trans passing the DAT (Fig. 4C) connecting the SF to the deep fasica (DF). The DF was situated below the DAT. The DF was thick, fibrous, tightly attached to the underlying muscles, and with scattered elastic fibers (Fig. 4C). Alcian blue staining of HA was positive between the layers of DF (Fig. 4E). In the lower lateral trunk region (R7 and 8), the most superficial layer of the DF, the aponeurosis of the *m. obliquus externus*

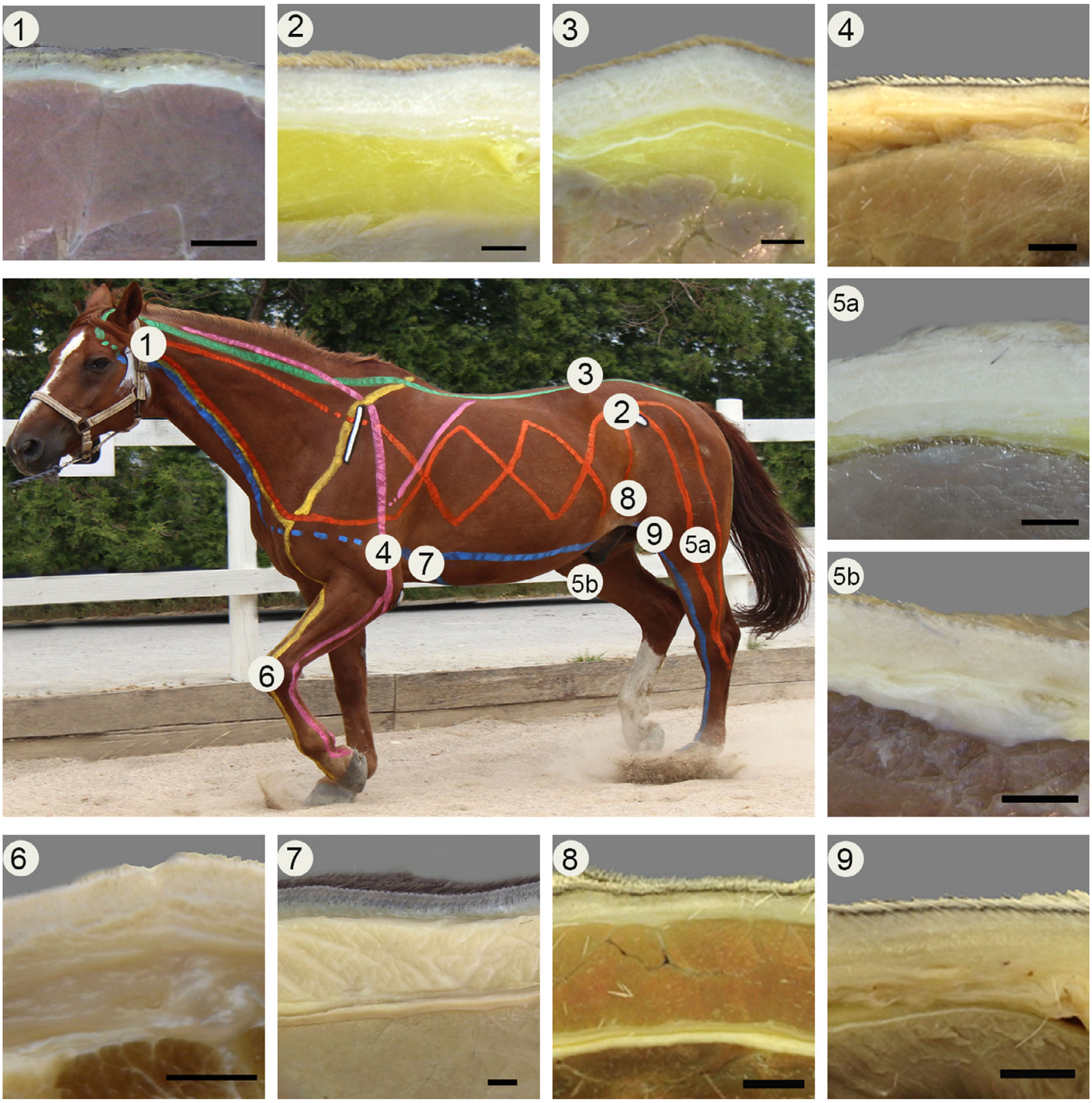

Fig. 2 Regions of sample collection. The photograph of the horse in the middle shows colored lines representing the myofascial lines* and white circles that show the sites of sample collection. The pictures at the edges show transverse cross-sections of the samples (scale bar: 0.5 cm). *For details about the colored myofascial lines, see Elbrønd & Schultz (2015).

abdominis was of elastic origin, being part of the *tunica flava abdominis*.

Additional regional variations

While dissecting the R4 region, we observed that the connection between the skin and *m. cutaneus trunci* was hard and in certain places almost impossible to separate. These locations presented as segmental lines or spots of tight collagen tissue along the trunk.

In the hindlimb fascia R5a (Fig. 5A), the SF was fused with the underlying DF. In this region, the hypodermis was infiltrated with adipose tissue and underneath a very thick layer of the DF was observed. This represented the fascia lata or the aponeurosis of *m. biceps femoris*. In R5a and 5b, the staining of HA was pronounced in the parallel collagen layer of dermis, SF and areolar tissue of the aponeurotic fascia.

In the region R6 (precarpal; Fig. 5B), the hypodermis was very thin, and the SF and DF were tightly connected and

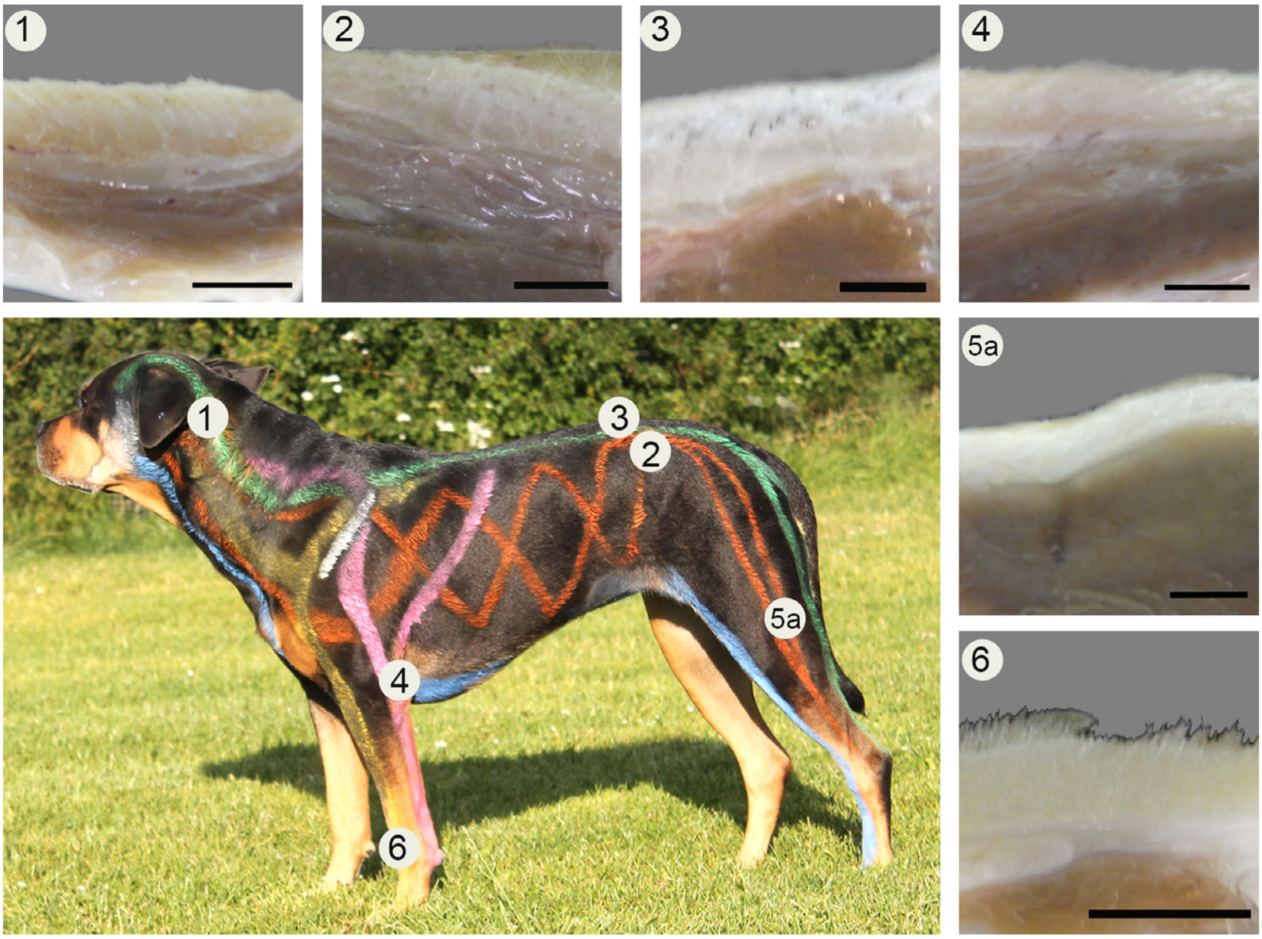

Fig. 3 Regions of sample collection. The photograph of the dog in the lower left corner shows colored lines representing the myofascial lines* and white circles that show the sites of sample collection. The pictures at the edges show transverse cross-sections of the samples (scale bar: 0.5 cm). *For details about the colored myofascial lines, see Elbrønd & Schultz (2015).

very difficult to distinguish/separate from each other. The *retinaculum extensorum* of carpus was represented in the section as being composed of a dense middle layer with compact bundles of collagen fibers. A thick layer of non-dense fibrous tissue covered the upper (closest to the skin) and lower side of this middle layer. Elastic fibers were present in relation to the non-dense layer and along the blood vessels. In this section the tendon of *m. extensor carpi radialis* including the synovial sheet was present and, below this, the joint capsule (*lamina fibrosa* and *synovialis*) was observed.

Microscopic analysis specific to the dog

The dog model of the fascia layers (Fig. 6A) was found to be very similar to that of the human presented by Stecco (Fig. 1A). The epidermis and dermis (1.0 ± 0.2 mm) were the two superficial layers and, underneath these, the hypodermis was observed. In the dog, this was irregular and loose, and the thickness varied according to the amount of adipose tissue. The SAT layer was present in all the samples, and included vertical retinacula (*retinacula cutis superficialis*) comprising larger and smaller vessels and nerves. The SF was observed as multiple layers of densely packed horizontal collagen bundles, and in relation to the *m. cutaneus trunci* was found to split as also seen in the horse (Fig. 6B). In the SF the amount of elastic fibers varied in the regions being abundant in R2, R3, R4, moderate in R5, R6 and sparse in R1. Intensely stained HA was observed in the layers of SF (Fig. 6D). The DAT layer was present under the SF. The DAT layer included oblique, horizontal and branched retinacula (*retinacula cutis profundus*). The DF was situated under the DAT, and was found to be very thin and similar to that of the SF in collagen bundles arrangement (Fig. 6C). The direction of the collagen fiber bundles was similar to that in the layers of the SF. The DF was loosely attached to the underlying muscles. Elastic fibers were scattered in the layers of the DF (Fig. 6C), and the staining of HA was intense in all the sublayers of DF (Fig. 6E).

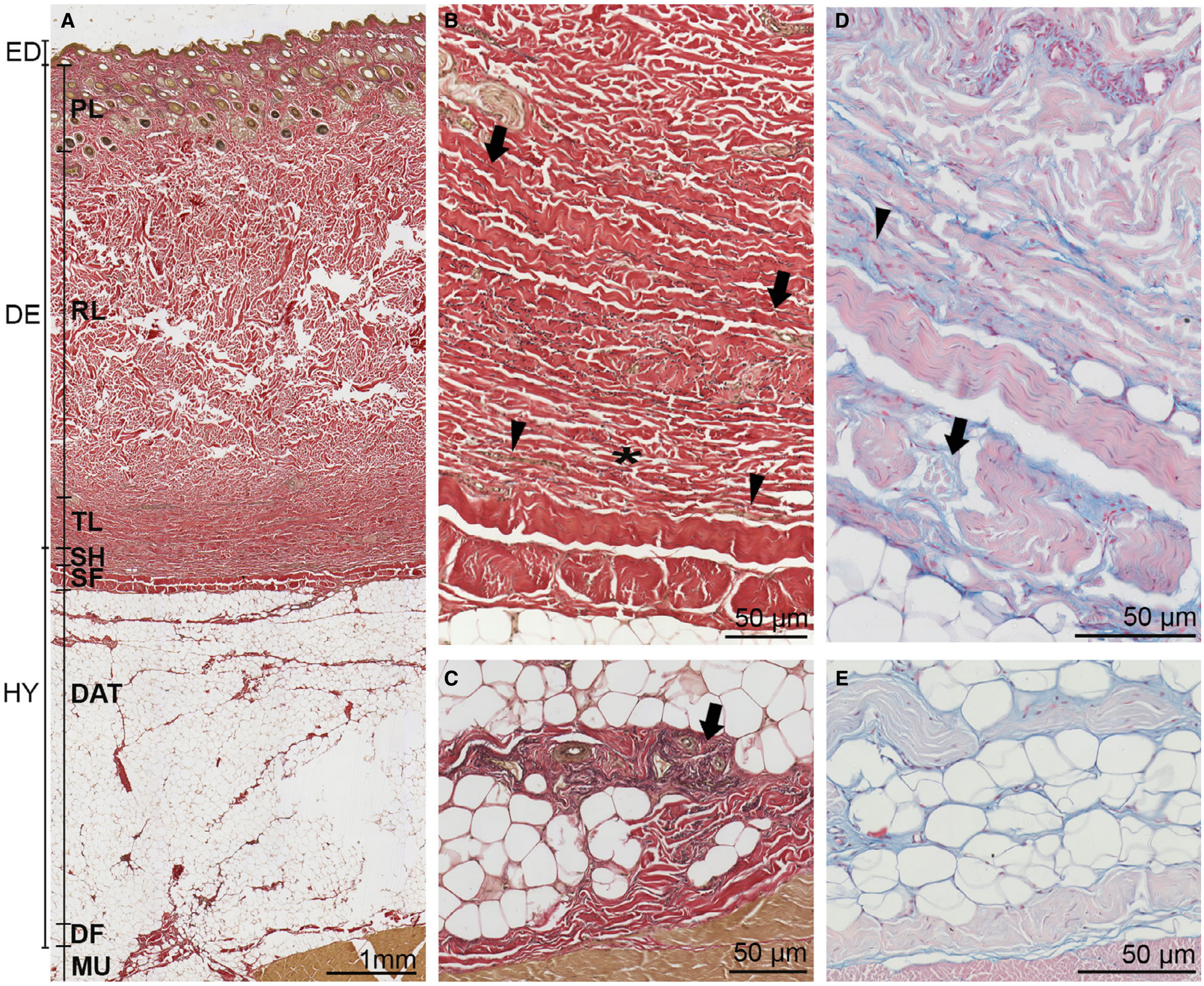

Fig. 4 Transverse-sections of *regio abdominis lateralis* (R2) of the horse. (A) Overview micrograph from skin to muscle representing the fascia layers in the horse section. DAT, deep adipose tissue; DE, dermis; DF, deep fascia; ED, epidermis; HY, hypodermis; MU, muscle; PL, papillary layer of dermis; RL, reticular layer of dermis; SF, superficial fascia; SH, superficial compartment of hypodermis; TL, third layer of dermis. (B) Loose irregular collagen bundles (asterisk) in the superficial compartment of the hypodermis separating the SF and TL. Elastic fibers, providing crimping effect, are present in between regular collagen bundles of the TL (arrows) and also in relation to the blood vessels (arrowheads). (C) The DF is tightly attached to the underlying *m. obliquus externus abdominis*. Blood vessels (arrow) are present in the *retinacula cutis profundus* of DAT. (D) Alcian blue staining, indicating the presence of Hyaluronan (HA), is intense in the layers of SH (arrowhead) and SF (arrow). (E) HA is present within the layers of DF and underlying muscle. (A–C) Weigert's Resorcin Fuchsin stain; (D, E) Alcian blue stain.

Additional regional variations

The R5a section of the dog (Fig. 7A) revealed a dense structural composition. The SAT was thin, and the DAT was absent. The DF (*fascia lata*) in this region was very thick, and was composed of dense collagen fibers and tightly attached to the epimysium of *m. biceps femoris*.

In the R6 region (Fig. 7B), the hypodermal and fascia layers were fused tightly together, so the SF and DF were difficult to distinguish. Fat agglomerations/deposits were arranged around the hair follicles in the dermis. The cephalic vein and nerve were present in the sections. Islets of areolar and adipose tissue, smaller vessels and nerves were seen in the samples. The articular capsule was present in the deepest layers.

Discussion

This is the first study that presents a detailed histological analysis of the fascia layers in specific regions of the horse and dog, as well as highlighting interspecies discrepancies and comparing them with the human fascia model, as presented by Stecco (Fig. 1; Stecco et al. 2011a). In order not to confuse the reader when referring to human fascia

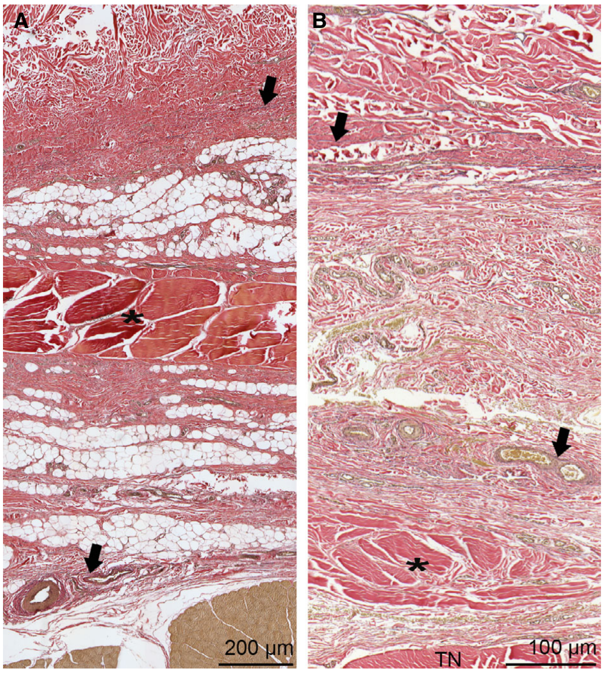

Fig. 5 Sections of horse limbs. (A) *Regio genus lateralis* (5a). The hypodermis is infiltrated with adipose tissue underneath which the *fascia lata* (asterisk) is present in the form of the deep fascia. Elastic fibers (arrows) are present in the dermis and hypodermis layer in relation to blood vessels and loose areolar tissue. (B) *Regio carpi* (R6). The *retinaculum extensorum* of carpus is present in this section being composed of a dense middle layer (asterisk) with compact bundles of collagen fibers. A thick layer of non-dense fibrous tissue covered the upper and lower sides of the middle layer. Elastic fibers (arrows) are present in the dermis and non-dense layers in relation to blood vessels. TN, tendon of *m. extensor carpi radialis*. (A, B) Weigert's Resorcin Fuchsin stain.

literature, the authors have used the Anglified anatomical descriptions: the SF and the DF instead of the anatomically correct terminology *fascia superficialis* and *fascia profunda*.

With this study, we confirmed our hypothesis, that the horse and dog fascia are histologically different with respect to the presence, tightness and morphology of the different layers. The thicknesses of the different layers of the horse and dog skin were found to be in accordance with previous reported values (Wong et al. 2005; Young et al. 2018). A difference in the dermal layers of the dog and horse was confirmed with the presence of the deeper dense parallel collagen layer (the third layer of dermis), referred to as the accessory Cordovan layer, being only present in horses (Dellmann, 1993; Wakuri et al. 1995). The present study confirmed that this deep layer of dermis was compact and tightly attached to the superficial compartment of the hypodermis, and it was therefore difficult to distinguish it from the SF. The major reason for this was the absence of the SAT and *retinacula cutis superficialis*. Only a thin layer of loose collagen and elastic fibers marked this boundary. This finding was true for all the equine regions, except for the lumbar region R3, in which a thicker SAT was present and separated the third layer of the dermis and the SF. In contrast to the horse, the dog fascia was loose and flexible with a well-developed SAT and *retinacula cutis superficialis*. These results are supported by previous studies of the equine and canine integument (Banks, 1993; Dellmann, 1993; Wakuri et al. 1995; Wong et al. 2005; Miller et al. 2012).

In the horse, the flexibility of the skin is provided by a sparse amount of elastic fibers mostly confined to the third layer of the dermis, in which crimping of the collagen bundles was also observed. This arrangement could possibly compensate for the loss of flexibility associated with the absence of the SAT and *retinacula cutis superficialis*. Elastic fibers provide the connective tissue with resilience, permitting long-range deformability and passive recoil without energy input. This elastic function complements collagen fibrils, which impart tensile strength (Kielty et al. 2002). The presence of HA in the superficial compartment of the hypodermis allows free movement of the skin over the underlying SF. In the deeper layers of the horse, the flexibility is provided by the SF, the DAT and the obliquely arranged *retinacula cutis profundus*, visualized by the presence of HA. The elastic-strain energy (from stretching muscle–tendon complexes and myofascial tissues) is an important factor in the biomechanics of the horse. It contributes to the force required to elevate the center of mass during movement (Alexander & Bennet-Clark, 1977; Alexander, 2002). The present results support the hypothesis that the biomechanics may be reflected in the fascia morphology, as previously also referred to by Schleip et al. (2010). All of the previous references (Alexander & Bennet-Clark, 1977; Alexander, 2002; Kielty et al. 2002; Schleip et al. 2010) lend weight to our suggestion that criss-crossing of the collagen fibers in the third layer of the dermis and SF enables a mechanism of elastic recoil with superior capabilities. This mechanism transforms, stores and reduces the energy consumption during locomotion as also described by Clayton (2016), and is beneficial for endurance in terms of long and repeated periods of physical activity in horses. The SAT was only present in the lumbar region (R3) of the horse, and we suspect this to be related to insulation of underlying vital organs, for example kidneys, and to their behavior to lean backwards into prevailing winds, snow, rain, etc. as the SAT works as an insulator and reservoir of energy, water and electrolytes (Lancerotto et al. 2010; Miller et al. 2012). Additionally, we suspect that in the horse this lumbar SAT tissue, which in dissection was found to be extended into the paravertebral thoracic and lumbar region, is involved in tension bearing associated with the saddle and rider. The SAT has been found to exhibit a high structural stability, and to possess elastic and plastic properties, as shown in a compression test by Lancerotto et al. (2010). This group found that fat lobes of SAT quickly returned to their original position and shape after pressure displacement. In the dog, all the

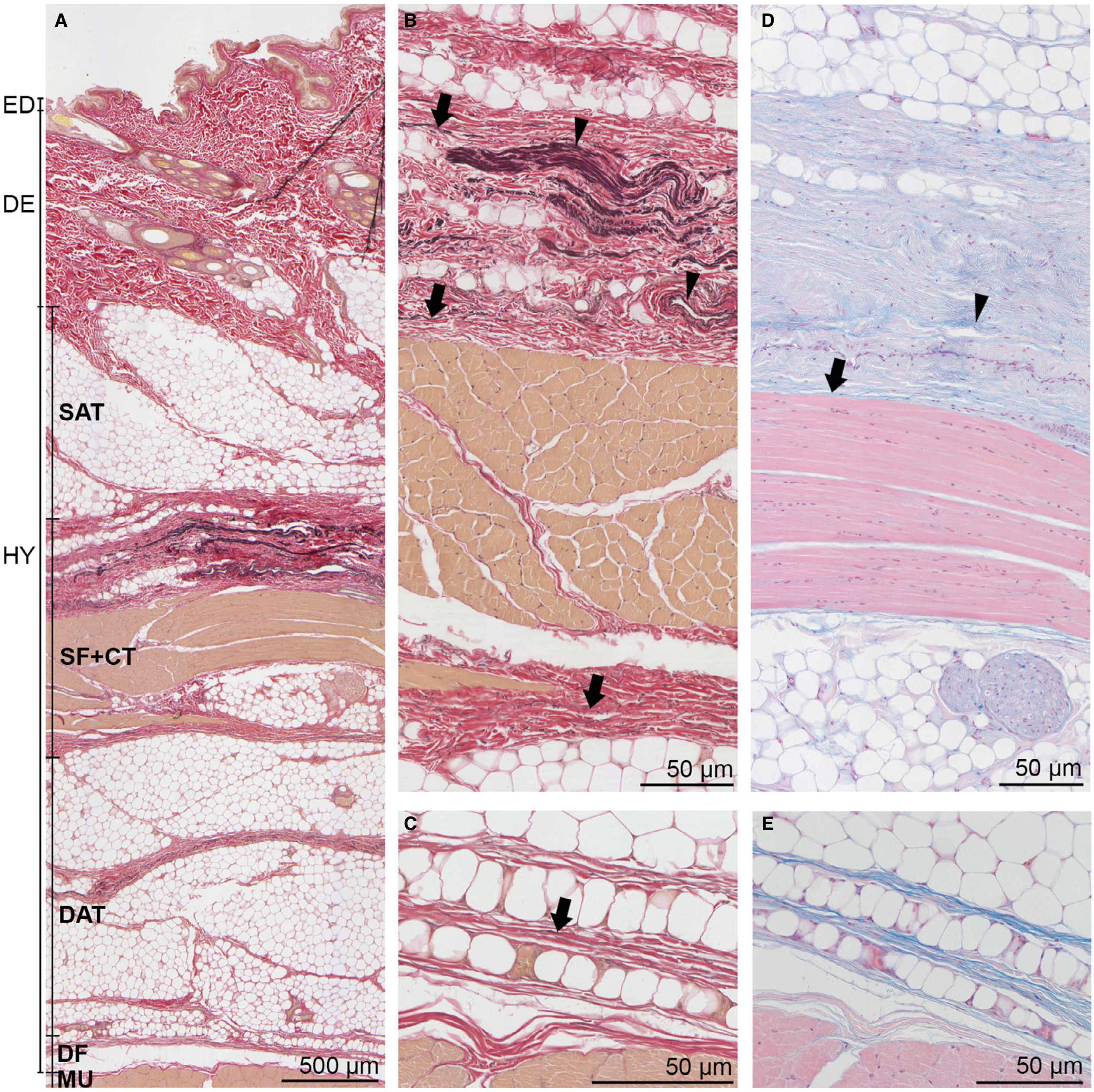

Fig. 6 Transverse sections of *regio abdominis lateralis* (R2) of the dog. (A) Overview micrograph from skin to muscle representing the fascia layers in the dog section. DAT, deep adipose tissue; DE, dermis; DF, deep fascia; ED, epidermis; HY, hypodermis; MU, muscle; SAT, superficial adipose tissue; SF, superficial fascia; SF+CT, superficial fascia inclusive *m. cutaneus trunci*. (B) Elastic fibers are present along the regular collagen fibers in SF (arrows) and along the blood vessels (arrowheads). (C) The DF is loosely attached to the underlying *m. obliquus externus abdominis*. Elastic fibers (arrow) are present in the layers of DF. (D) Alcian blue staining, indicating the presence of Hyaluronan , is intense in the layers of SF in relation to muscle (arrow) and blood vessels (arrowhead). (E) Hyaluronan is present within the layers of DF. (A–C) Weigert's Resorcin Fuchsin stain; (D, E) Alcian blue stain.

regions studied enclosed SAT, and we suggest that in the dog it not only provides insulation to a harsh environment but also helps to improve shock absorption during the landing phase as the exchange of ground reaction force transmits into the skin and underlying structures (Dellmann, 1993, Professor G. Fisher, personal communication). In the dog, this SAT morphology would serve to improve the flexibility in the superficial part of the hypodermis as compared with the horse.

The SF in both the horse and dog was found to be a flexible transmission zone between the superficial and profound compartment of the hypodermis. It was additionally found to comprise a well-developed slide and glide capacity as revealed by the prominent deposition of HA, just like in

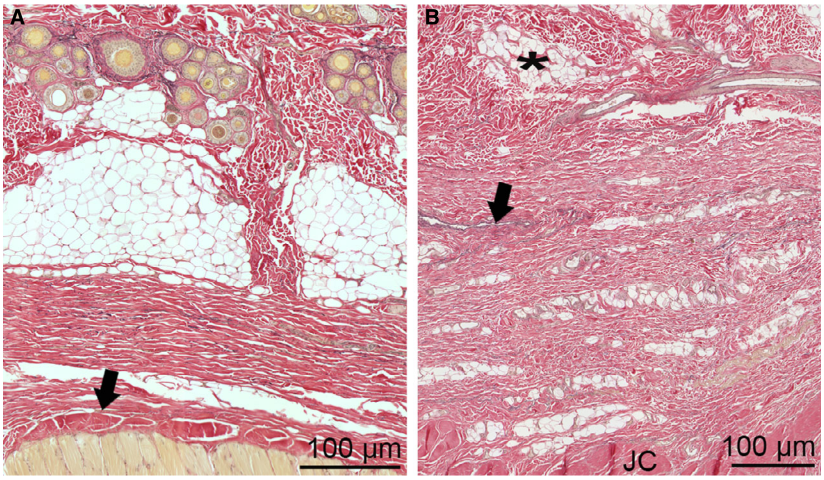

Fig. 7 Sections of dog limbs. (A) *Regio genus lateralis* (5a). Superficial adipose tissue is present below the dermis and the deep fascia (*fascia lata*; arrow) in this region, which is thick and composed of dense collagen fibers and tightly attached to the epimysium of *m. biceps femoris*. The superficial fascia and deep fascia is fused. (B) *Regio carpi* (R6). Fat deposits (asterisk) are present in the hypodermis of the carpus. Elastic fibers (arrow) are present in the loose areolar tissue in relation to blood vessels . The articular capsule (JC) of the intercarpal joint is present in the section. (A, B) Weigert's Resorcin Fuchsin stain.

humans (Stecco et al. 2011a,b). In the horse, the SF was trilaminar in the neck and trunk with a strict composition, while in the dog it was multilayered by interwoven collagen fibers, loosely packed and mixed with abundant elastic fibers as seen in the human (Stecco et al. 2011a). In the horse and dog, the SF in the extremities merged together with the DF and it was difficult to distinguish, something that has also been described in humans (Stecco et al. 2011a). In the horse, this trilaminar SF, showing a crimping pattern of the collagen bundles, supports skin tension from different directions (longitudinal and transverse) and might thereby provide a supportive role for the substitution of SAT function, present in the dog and human. The equine SF also forms a solid structure with the skin to protect against external forces (Nakajima et al. 2004). The tightness of the connection between the equine SF and skin (during walking – trotting – galloping – rolling – movement in general) additionally supports its role as a part of the equine elastic recoil system as mentioned above. The presence of a thin layer of areolar tissue rich in HA, between the third layer dermis and the SF, in the superficial compartment of the hypodermis indicates the possibility to slide and glide in the layer of structures above the SF in the horse. In contrast to this, the dog presented an intense and diffuse distribution of HA in and between the dermal layers as well as in the deeper layers, which indicates a high degree of flexibility and a capacity of all the layers to slide and glide. *Musculus cutaneus trunci* was present within the layers of the SF in both the horse and the dog. The general perception of the *m. cutaneus trunci* is to cause the skin to shiver, the panniculus reflex, but recent equine studies suggest that the equine *m. cutaneus trunci* might also play a role in response to pain and hypersensitivity of the horse's back to a rider and saddle (van Iwaarden et al. 2012; Essig et al. 2013). Based on the findings of the non-fatigable panniculus reflex in the horse (Essig et al. 2013), and the muscle being a part of the lateral myofascial kinetic line (Elbrønd & Schultz, 2015), and the recent morphological and topographical findings, we suggest that the muscle takes part in stabilization of the trunk at fast speed such as trot and canter.

In both the dog and the horse, the DAT layer appeared to be very different from the SAT. In the horse, the DAT was less well defined and the *retinacula cutis profundus* were less consistent and mostly obliquely orientated. In the dog, the *retinacula cutis profundus* were well defined, branched, oblique and with multiple layers. The elastic properties of the *retinacula cutis profundus* were tested in humans by Lancerotto et al. (2010), who concluded that the obliquity of the septa allows for sliding and gliding of the subcutaneous tissue on the DF in conjunction to the DAT layer, being easily disgregated. Additionally, Nakajima et al. (2004) referred to the DAT as a mobile layer that isolates the musculoskeletal movements. In the horse, the DAT layer showed significant differences in terms of thickness and presence between regions, i.e. being thick in regions R2, R3, R7, R8, yet thin in R4, whilst present in the form of areolar tissue in R1, R5a, R5b, R6 and R9. Additionally, a reduction in the extremities was present in the dog, which reaffirms the findings of Stecco et al. (2011a). Along with the regional differences, the DAT in this study showed great individual variations in both the horse and the dog in terms of fat content and thickness, as observed in humans (Stecco et al. 2011a). Stecco (2015) marked the DAT as a watershed between the exteroceptive (including skin, SAT, SF) and the interoceptive, proprioceptive systems (including DF and muscles). The sites, in the limbs, where the DAT is absent, the proprioceptive and exteroceptive systems combine to facilitate the proprioception of form, volume and surface of various objects. The oblique arrangement, limited elastic properties and easy lateral displacement of the *retinacula cutis profundus* allows the thick horse skin and SF to slide and glide mainly on the top of the DF. This confirms our hypothesis of the biomechanical reflection to the histology, as the horse demands a high stability and tight fascial structures in order to optimize endurance activities at low metabolic costs. Well-defined, multilayered and branched septa with a high deposition of HA may be the reason for the high dog skin flexibility as compared with that of the horse.

Overall, our findings show that the equine and canine DF are very similar to the human fascia as previously described (Stecco et al. 2011a). We found evidence in the dissections as well as at the microscopical level that the muscles have a high degree of flexibility in relation to the fascia, and can slide and glide in several directions due to the presence of areolar tissue and high amounts of HA between the fascia layers, as described previously (McCombe et al. 2001). A number of expert authors describe the DF of the limbs as being a highly organized tissue, with different regional specializations (Geneser, 1986; Stecco et al. 2008; Skalec & Egerbacher, 2017). Our results show that the DF of the horse and the dog limb are also well defined, and that they have highly organized layers with regional modifications. The fasciae of the limbs are formed of two or three layers of parallel collagen fiber bundles in humans (Benetazzo et al. 2011) and, in our study, we found that it was composed of several layers. Because the SF and DF fuse at bony prominences, separating the layers can be difficult, as in humans (Stecco et al. 2011a). The present results support the hypothesis that the multilayered structure of the DF of the limbs probably ensures resistance to pressure without consequent damage and adaptation to volume changes during muscle contraction and movement (Stecco et al. 2008; Skalec & Egerbacher, 2017). The capacity of the various collagen layers to slide over each other may change in cases of overuse syndrome, trauma or surgery, all possible causes of myofascial pathologies. It has previously been accepted that joint retinacula were isolated elements, which was at least until recently when these structures were shown to be a reinforcement of the DF (Abu-Hijleh & Harris, 2007; Stecco et al. 2010). Our findings in the equine R6 confirm that the *retinaculum extensorum* of the carpus is not an independent or microscopically separable structure. Furthermore, a recent study on the equine thoracic limb (Skalec & Egerbacher, 2017) also supports our findings through anatomical dissections and microscopical examinations. In humans, thoracic compared with pelvic limb fascia exhibits a major difference in the presence and distribution of elastic fibers. In the DF of the thoracic limb, many elastic fibers are reported (Stecco et al. 2007), mixed with the collagen fiber bundle, whereas in the pelvic limb elastic fibers are only present in the areolar tissue between the different fibrous layers (Stecco et al. 2008). The difference in quantity of elastic fibers between the upper and lower limbs in humans is due to their different function as thoracic limbs as they are not involved in locomotion. The elastic fibers are thought to ease the precise and fine movements of the human thoracic limbs (Stecco et al. 2008). Our histological examinations of the equine and canine fore- and hindlimb sections show that elastic fibers are predominantly mixed with the areolar connective tissue, also described by Skalec & Egerbacher (2017) in equine forelimbs. This difference might be explained by the fact that humans are bipedal, whilst horses and dogs are quadrupeds and engage all four limbs in locomotion, as also suggested by Skalec & Egerbacher (2017). The prominent distribution of HA in the areolar tissue in the extremities supports the slide and glide concept of the DF layers. However, we did not observe the carpal extensor retinaculum in the dog as the retinaculum is situated laterally to the sampling area over the carpal joint. In the horse, the R7 and R8 *tunica flava* is part of the DF.

Conclusion

This study has shown that both the horse and dog fascia have similarities, but they also clearly have differences in terms of their histology. These differences are thought to reflect the biomechanics of the two species. We conclude that in the horse, an absence of SAT, tight fascia and the presence of elastic fibers, located around collagen fiber bundles serving to provide a crimping effect in the third layer of the dermis, not only supports the compact stature but also provides an energy-efficient system for long-distance running. Whereas, in the dog, the presence of SAT with a well-defined *retinacula cutis superficialis* and a loose structure of the fasciae provide flexibility and looseness to the whole body, and helps facilitate survival in harsh conditions, something of importance for a carnivore/predator. Whether these differences have a consequence for the functional anatomy or for myofascial release and treatment of these two species now remains to be established.

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Conflicts of interest

The authors know of no conflicts of interest in connection with this study.

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