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THE PRESENCE OF MYOFIBROBLASTS, SMOOTH MUSCLE CELLS AND ELASTIC FIBERS IN THE LYMPHATIC COLLECTORS OF HORSES

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ABSTRACT

Horses are highly susceptible to lymphedema. Knowledge of the morphological components of lymphatic collectors is therefore essential to understanding the function of the lymphatic system. A better knowledge of the lymphatic system allows the development of more effective treatments against lymphedema. The composition of hypodermal and deep lymphatic collectors was investigated with immunohistochemical staining, using antibodies against proteins of the collector walls from the skin in the hind limbs of 10 healthy horses. Lymphatic collectors can be subdivided into passive (elastic fibers) and active (smooth muscle cells and myofibroblasts) components. The presence of myofibroblasts in equine lymphatic collectors has not previously been described. The high concentration of myofibroblasts, especially in the dermal collectors, suggests their possible importance in lymph flow. Myofibroblasts may act as pacemaker cells for the contraction of smooth muscle cells and probably play a role in the proliferation of smooth muscle cells during training, as there appears to be correlation between the percentage of smooth muscle cells in equine lymphatic collectors and level of physical fitness. The response of the lymphatics to stimulation may allow effective treatment of lymphedema without using pharmacological drugs. The high percentage of elastic fibers (approximately 45% in equine lymphatic collectors) indicates the importance of passive components within the lymph flow.

Horses are highly susceptible to lymphatic diseases (1). Even a lymphologically healthy horse (standing and under sedation) may show signs of lymphatic congestion from the contrast medium during lymphangiography (2). The best reported therapy for equine edema is "Equine Manual Lymph Drainage" (EMLD), a method introduced by Berens v. Rautenfeld and Rötting (3,4).

Horses have lymph capillaries and precollectors in the dermis and also dermal collectors in the deep layer of the dermis. Hypodermal collectors are present in the subcutis, and intra- or subfascial collectors can also be found (5). An understanding of the interaction between the contractile cells (e.g., smooth muscle cells and myofibroblasts) and the passive retraction system (e.g., elastic fibers) in these collectors is of clinical relevance. The elastic fibers account for nearly half of the components of the collector wall and are composed of 90% elastin (6). Elastin forms an elastic network in the extracellular matrix, which allows the tissue to stretch and change shape.

The horse represents a muscle-poor collector species (2,7-9). Smooth muscle cells, which are transformed from fibroblasts, are spindly and contain actin and myosin filaments (10,11).

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The present study also examined the myofibroblasts that have so far not been described in the lymphatic collectors of horses. Myofibroblasts are found in the extracellular matrix of the tunica interna and also have a secretory function (12). It is not known how the myofibroblasts develop. They may be modified fibroblasts or modified smooth muscle cells (13). Some researchers have suggested that they are transformed epithelial cells (14,15), which would explain their role in fibrolytic processes. Myofibroblasts contain thin filaments with focal high density, as well as large numbers of rough ER and Golgi apparatus. The basal membrane may be discontinuous or absent (16). Some fibroblast populations produce more actin fibers in hypoxic situations, leading to a transdifferentiation of fibroblasts to myofibroblasts (17).

Innervation of lymphatics is mostly sympathetic. A high sympathetic tonus can induce a convulsion of lymphatic vessels and thereby a blockade in lymph fluid. Van Helden and Zhao (18) showed that synchronous and rhythmic contraction of the lymphangions is induced by Ca^{2+}-dependent pacemaker cells. Pacemaker activity in the wall of lymphatic collectors was first shown by McCloskey et al (19). These researchers also documented that pacemaker cells are modified smooth muscle cells (20). Under electron microscopy, these cells look like myofibroblasts (5) and are positive to immunohistochemical staining against vimentin (20), as are myofibroblasts (16). The aim of this study was to provide a morphometric evaluation of the active and passive components of the lymphatic collector impulse system in the equine hind limb.

MATERIAL AND METHODS

Tissue samples from 10 horses (aged 2-25 years; mean age 10.8 years) were taken after slaughter/euthanasia (for other reasons). Immediately after death, the lymphatic collectors were filled directly and indirectly by injecting "Berlin Blue" (1:20 dilution with aqua dest.). Samples were taken from different locations: three specimens from the hypodermal collector system (fetlock joint, metatarsus, femur) and six specimens from the deep collector system (pastern, fetlock joint, metatarsus, tarsus, tibia and femur). Samples were subdivided into two groups: Group I was fixed in 4 % PFA and Group II in 3 % GA for 24h. The PFA fixed specimens were embedded in paraffin. The GA fixed specimens were embedded in epoxy resin (epon) after osmium treatment. Three serial sections (5 µm) were taken from the paraffin specimens, and ultrathin sections (70 nm) from the epon specimens.

Immunostaining for Light Microscopy

The paraffin sections were deparaffinized in Xylene, fixed in ethanol and rehydrated in TBS. The following monoclonal antibodies were used: anti-alpha-smooth-muscle-actin-1 (ASM), anti-vimentin and goat-anti-mouse anti-elastin (DAKO, Hamburg, Germany). After preliminary testing of several different concentrations of the antibodies and pretreatments, the following staining protocol was found to be best: In order to block the endogenous peroxidase the sections were treated with 1% H_2O_2 in PBS for 20 min in darkness at room temperature (RT). They were then treated with 10% normal rat serum (RS) in PBS for 30 min at RT, before incubation with the primary antibody anti-elastin (1:500), anti-vimentin (1:50) or anti-ASM (1:20) diluted with PBS containing 1% BSA + NaN_3 for 24h at 4°C. After washing in TBS, secondary antibody (biotinylated goat-anti-mouse IgG or biotinylated rabbit-anti-goat IgG) was applied diluted in PBS with 5% RS for 1h at RT (1:200), followed by Streptavidin peroxidase (1:200) or Streptavidin-alcalic-phosphatase (1:200) diluted in PBS for 30 min at RT. The reaction product from anti-elastin was visualized with DAB (17.5 mg in 100 ml TBS and 50 µl 30% H_2O_2) for 6 min at RT. The reaction products from anti-vimentin and anti-ASM were visualized with Fast Blue

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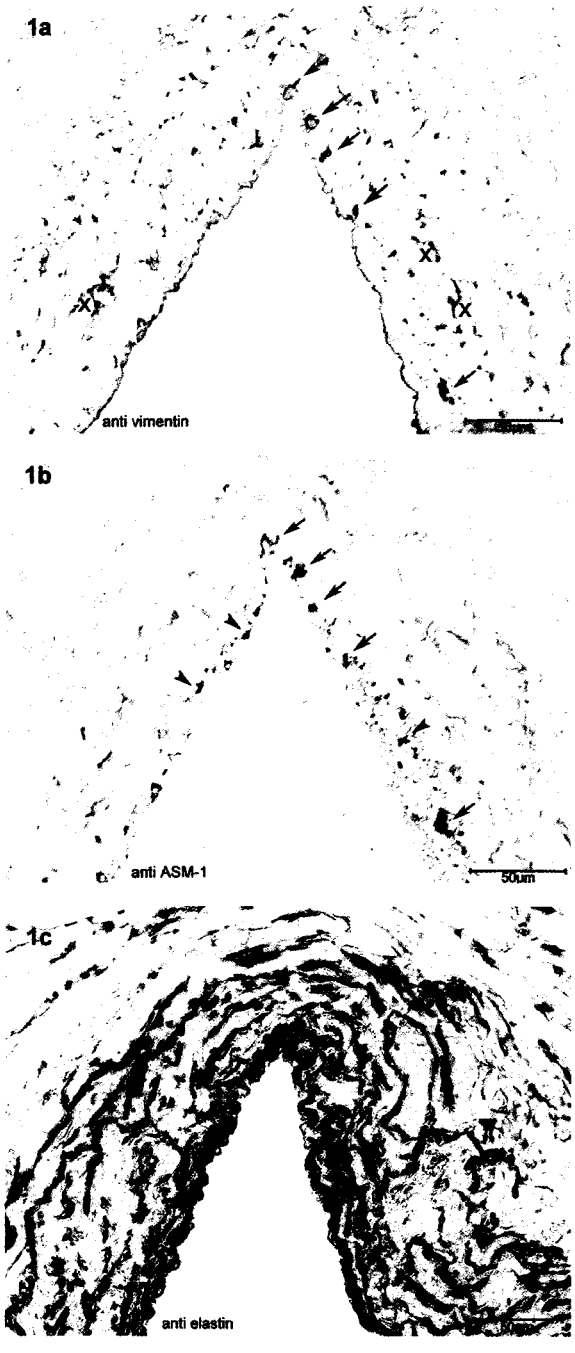

Fig. 1 a: Serial section of a collector wall, stained with anti-vimentin. Arrows: myofibroblasts; crosses: fibroblasts. b: Serial section of a collector wall, stained with anti-ASM. Arrows: myofibroblasts; arrowheads: smooth muscle cells. c: Serial section of a collector wall, stained with anti-elastin. The high volume of elastic fibers is obvious.

(2 mg diluted in 4 ml APAAP) for 22 min at RT and then mounted in Glycergel^R (DAKO). As a negative control, staining was performed without primary antibody or using a monoclonal antibody of the same isotype (mouse IgG_1 negative control, DAKO) against Aspergillus niger proteins as primary antibody. Reactions were not observed with either negative control (data not shown).

Electron Microscopy

The ultrathin specimens were contrasted with uranyl acetate and lead citrate and examined by transmission electron microscopy (Zeiss EM 110; magnification 2500:1) in order to differentiate the ASM positive cells in the subendothelial layer of the collectors.

Quantification

Morphometric evaluation of the paraffin sections was performed under standard light microscopy (magnification x 40). The sections were investigated with a systematic-random-sampling system, meaning that the whole collector wall was examined, starting at a random point. ASM positive cells and elastic fibers were counted, using a special point screen.

Statistical Evaluation

ANOVA (Sigma Stat 2.0; Jandel, USA) was used for statistical evaluation. The results are presented in volume-percentage of the collector walls. Sigma Plot 2001 (Jandel, USA) was used to present the results in graphic form. Smooth muscle cells, elastic fibers and ASM-1 positive cells in the subendothelial stratum were evaluated, because almost all ASM positive cells are contractile cells. As some of the ASM positive cells in the subendothelial stratum have a different morphology from smooth muscle cells, a separate evaluation was performed under transmission electron microscopy.

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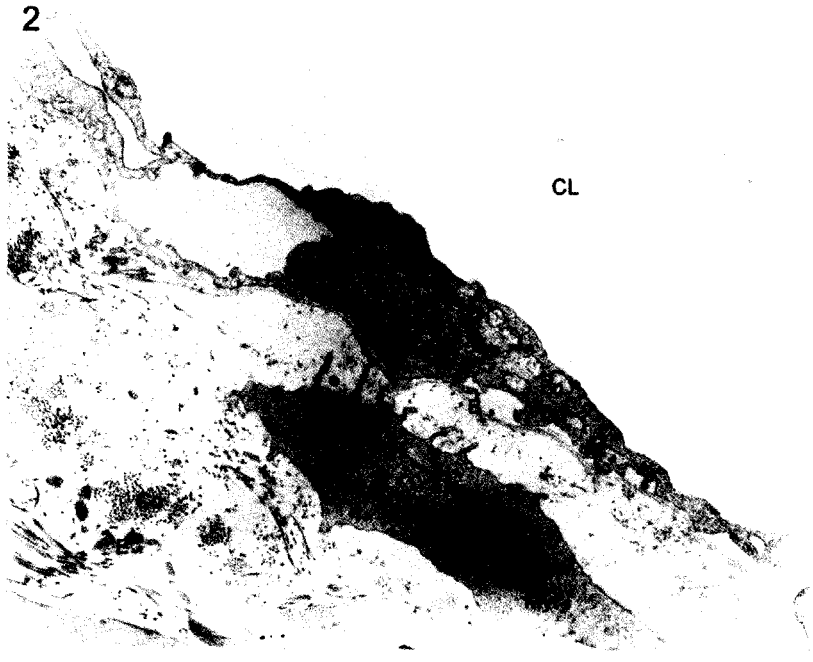

Fig. 2: TEM of a myofibroblast. My: myofibroblast; CL: collector lumen; En: endothelial cell; B: basal membrane; Co: collagen fibrils; E: elastic fibers.

RESULTS

In this study, the hypodermal (s. c.) and deep (intra- or subfascial) lymphatic collectors of the equine hind limb were investigated. The network of dermal collectors and precollectors (which are the origin of the hypodermal collectors) was not evaluated. The collector wall structure is similar to that of blood vessels, whereas the angioarchitecture of lymphatic vessels shows greater differences. Smooth muscle cells were observed mostly in the tunica media and were found sporadically with a vertical orientation in the tunica interna. Another type of ASM and vimentin positive cell was found in the subendothelial position of the internal layer (*Fig. 1a,b*). This cell type was small, tall and orientated transversely to the collector axis. These cells were evaluated separately as well as in combination with the smooth muscle cells. Deep collectors contained up to eight muscle cell layers compared to five or fewer layers in hypodermal collectors. In areas with high smooth muscle cell concentration, many ASM positive cells were also found in the subendothelial layer, arranged around the collector lumen in layers up to three cells thick. Only a few lymphatic collectors had a continuous muscle cell layer. In most serial sections of collectors, a permanent change in position and thickness of the smooth muscle cells in spiral pattern was evident.

ASM and vimentin-positive cells in the subendothelial layer were examined under transmission electron microscopy (TEM). These cells contained few actin-filaments, few "dense bodies" in the cytoplasm, a discontinuous or absent basal membrane, and large numbers of rough ER (*Fig. 2*), which are clearly characteristics of myofibroblasts.

The concentration of ASM positive (contractile) cells in the walls of hypodermal versus deep collectors of the whole hind limb were not significantly different ($p = 0.514$).

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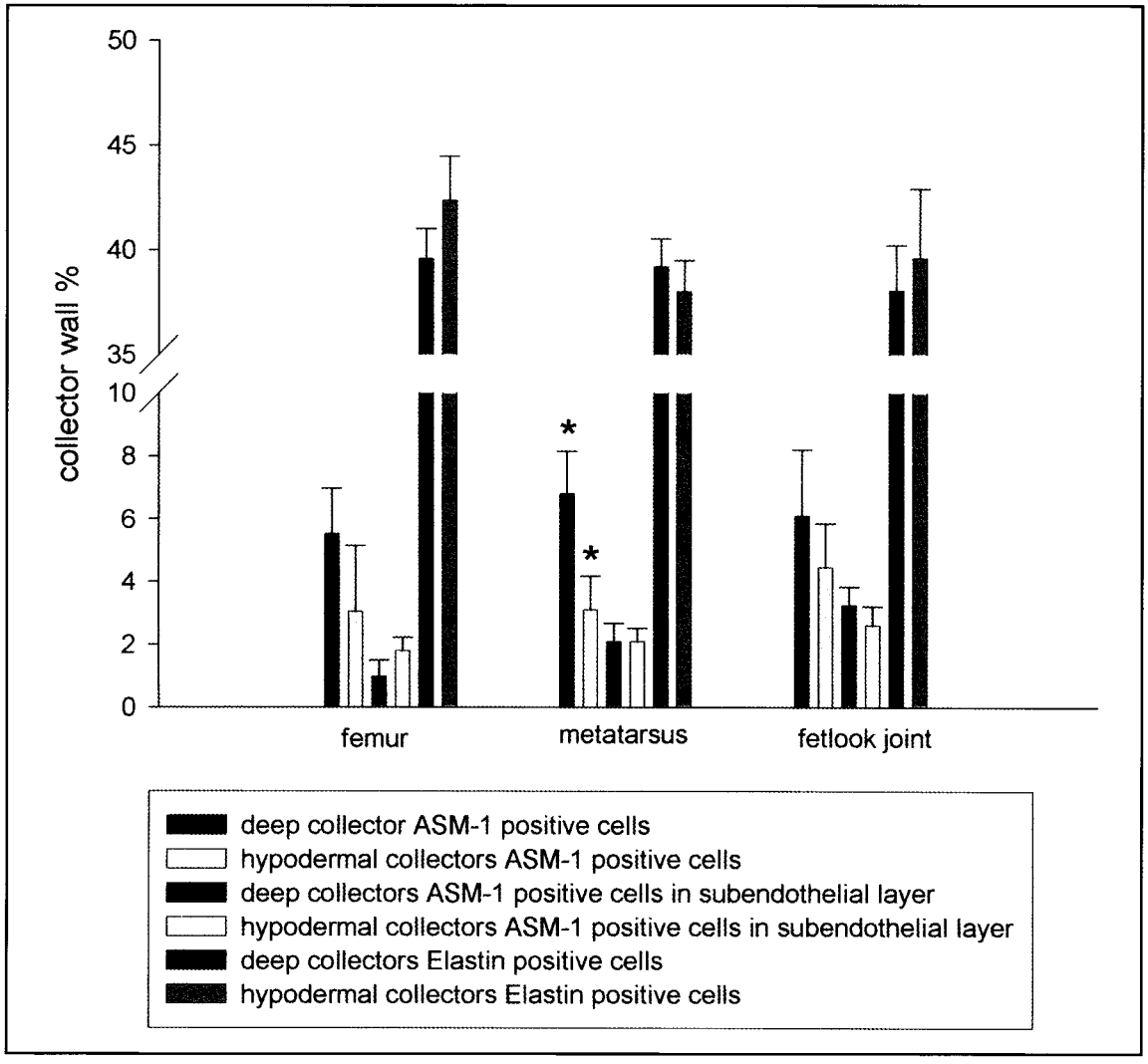

Fig. 3: Volume percentage of anti-ASM and anti-elastin positive cells in the collector walls.

There were also no significant differences between different locations on the same leg in individual horses ($p = 0.433$ in all horses). However, a clear distinction was seen when comparing samples of different heights from hypodermal and deep collectors of different horses. The outcome was only significant for the metatarsus (*Fig. 3*, $p < 0.05$), but the results showed an increase of the total number of ASM positive (contractile) cells in the deep collectors in contrast to the hypodermal collectors. Also, the number of contractile cells in the hypodermal collectors tended to decrease from the distal to the proximal part of the limb, although the decrease was not statistically significant. To summarize, the myofibroblasts (ASM and vimentin positive cells) were found at a higher level in the hypodermal collectors. In the deep collectors, the smooth muscle cells (only ASM positive) were predominant.

The percentage of elastic fibers in the collector walls of the equine hind limb ranged from 37.1% to 42.4% (*Fig. 3*). Sections

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containing little elastin alternated with parts containing larger amounts of elastin. The latter was true for areas where the lymphatics crossed a joint. The deep collectors showed only scarce embedding in the tissue, in contrast to the hypodermal collectors. Neither a continuous elastic internal membrane nor an elastic external membrane was present.

DISCUSSION

The lymphatic collectors of the equine hind limb can be subdivided into superficial (epifascial, subcutaneous, hypodermal) and deep (intra-, subfascial) collectors, similar to the human classification system (21,22). Additionally, the horse reveals the presence of another dermal collector net. Therefore, the superficial collectors must be divided into dermal and hypodermal collectors (1).

In the present study, only the hypodermal net of the superficial collectors was investigated. Horses have far fewer smooth muscle cells in their collectors than humans do (2,9); they represent a muscle poor collector type. Also in contrast to humans (22), hypodermal collectors of horses contain fewer smooth muscle cells than deep collectors.

This study investigated the active (smooth muscle cells and myofibroblasts) and passive (elastic fibers) vasomotion factors in the walls of lymphatic collectors in healthy horses. A quantitative morphometric evaluation of the contractile cells and elastic fibers was necessary. The antibody against alpha-smooth-muscle-actin (ASM) that was used marked all cells containing alpha-actin, including smooth muscle cells as well as subtypes of fibroblasts and myofibroblasts. Myofibroblasts are also positive to immunohistochemical staining against anti-vimentin (15, 19). Therefore this antibody was used to differentiate the two types of cells (*Fig 1a,b*). The demonstration of contractile myofibroblasts (12,23) in the subendothelial layer of the lymphatic collector wall of the equine hind limb is new. Eyden (16) had characterized myofibroblasts in humans, which are identical to these equine cells. The concentration of ASM positive cells (smooth muscle cells and myofibroblasts) between deep and hypodermal collectors found in this study differs. However, the small number of ASM positive cells in both types of collectors does not allow a functional interpretation. More important with regard to the function of myofibroblasts is the study by Risse (24), which demonstrated a decrease of ASM positive cells (smooth muscle cells and myofibroblasts) in the subendothelial layer of horses with elephantiasis. Further research is necessary to clarify the function of myofibroblasts. In sheep mesenteric lymphatic vessels, McClosekey et al (20) postulated that these cells possess a pacemaker activity. Dinenno et al (25) and Moreau et al (26) have shown that training influences vessel development. The energy working on the endothelia releases mitogens and concurrent proliferation of smooth muscle cells in the blood vessels (27). It is very probable that this is also true for lymphatic vessels. Because myofibroblasts are proliferative cells (13), a transformation into smooth muscle cells under these conditions seems probable.

The interaction between smooth muscle cells and myofibroblasts requires further investigation. In dermal collectors of horses there are some myofibroblasts but an absence of smooth muscle cells (data not shown). Thus, myofibroblasts must have their own functional significance. This was also demonstrated by Goldberg and Rabinovitch (28) and Alberts et al (29), who reported that myofibroblasts can change the volume of amorphic fluid in the subendothelial layer because they can produce glycoproteins, which bind tissue fluids. Therefore, myofibroblasts in lymphatic collectors could resorb lymph fluid from the lumen to protect collectors against insufficiency. Harland (5) postulated that the increasing interstitial pressure in the vessel walls forces the contraction of myofibroblasts and smooth muscle cells.

The high volume of elastic fibers (approx.

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45%; *Fig. 1c*) in deep and hypodermal collector walls of the equine hind limb had not previously been demonstrated. Gerli et al (30) showed elastic subendothelial fibers in human initial lymphatic vessels, which should empty the initial lymphatic vessels through retractive force. Another indication of the importance of elastic parts in lymph dynamics was shown by Risse (24). In insufficient lymphatic collectors of horses with elephantiasis, elastic fibers and ASM positive cells are decreased. The elastic fibers of the superficial collectors are in contact with the elastin layer of the deep equine dermis. This layer works like a compression stocking, especially in the dermal net of the superficial collectors. At present it is unclear where the forces that induce primary elastic stretching and emptying of collectors originate. The number of contractile cells in horses is much lower than in humans (2,9,22). Therefore, the joint-pump must also play an important role in the transport of lymph fluid. The pastern joint is more important than the tarsus because the vessels show a plantar direction on the distal joint but a lateral/medial direction on the proximal joint.

The lymphatic system warrants further investigation as it plays a role in many diseases, e.g., chronic cellulitis, chronic lymphangitis, "swollen legs" as well as tendonitis, rhabdomyolysis syndrome and laminitis (31,32).

Horses show a greater affinity for lymphedema than other domestic animals, but similar to humans. Meyer (2) traced this affinity to a weaker smooth muscle cell pump in the collectors of horses compared to humans (22). On the other hand, in horses MLD produces a much better effect than in humans (4), since the subcutis is thicker in humans (32). Thus, in human extremities only hypodermal collectors can be stimulated to contract with MLD. In contrast, deep (intra- and subfascial) collectors can also be stimulated in horses.

In human medicine, MLD has been established in sports medicine, orthopedics and traumatology for many years (33, 34). Establishment of MLD in equine medicine would be beneficial because it is a doping-free method that gives good results. For example, in the early stage of trauma (first week), EMLD leads to better resorption of inflamed edema and therefore better wound healing. This shortens the convalescence period and is thus of interest in sport.

To conclude, the lymphatic system plays a role in many conditions encountered in equine veterinary practice. Knowledge of its structures is therefore necessary to develop effective therapeutic methods such as EMLD. The new proof of the myofibroblasts in equine collector walls presented in this study shows a possible way to train the lymphatic system and may allow for therapy as well as prophylactic treatment.

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REFERENCES

1. Berens v. Rautenfeld, D, V Schacht: Fundamentals of Comparative Lymphology. In: *Textbook of Lymphology*. 1st ed. Földi, M, E Földi, S Kubik (Eds.), Urban & Fischer, München, Jena (2003), 180-194.
2. Meyer, KC: Die Angioarchitektur peripherer Lymphgefäße an den Gliedmaßen des Pferdes - eine indirekt lymphographische, raster - und transmissionselektronen-mikroskopische Untersuchung. Hannover, Tierärztl. Hochsch., Diss. (1988).
3. Berens v. Rautenfeld, D, AK Rötting, K Rothe, et al: Manuelle Lymphdrainage beim Pferd zur Behandlung der Beckengliedmaße – Teil 1: Anatomische Grundlagen und Behandlungsstrategien. Pferdeheilkunde 16 (2000), 30-36.
4. Rötting, AK, D Berens v. Rautenfeld, T Schubert, et al: Manuelle Lymphdrainage beim Pferd zur Behandlung der

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Beckengliedmaße – Teil 2: Behandlungen und Untersuchungen bei Pferden mit "chronischer Phlegmone". Pferdeheilkunde 16 (2000), 37-44.
5. Harland, MM: *Immunhistochemisch-morphometrische und ultrastrukturelle Charakterisierung tiefer und oberflächlicher Lymphkollektoren der Beckengliedmaße des Pferdes*. Hannover, Tierärztl. Hochsch., Diss. (2003).
6. Montes, GS: Structural biology of the fibres of collagenous and elastic systems. Cell Biol. Int. 20 (1996), 15-27.
7. Kaindl, F, E Mannheimer, L Pleger-Schwarz, et al: Histologie der praefaszialen Lymphgefäße der Extremitäten. In: *Lymphographie und Lymphadenographie der Extremitäten*. Band 87. Glauner, R (Ed.), Thieme, Stuttgart, (1960), 4-17.
8. Poberai, M, A Gellert, I Nagy, et al: Vergleichende histologische Untersuchungen über die Struktur der Wand der Lymphgefäße. III. Histologischer Bau der Wand der peripheren Lymphgefäße. Acta morphol. Acad. Sci. hung. 11 (1962), 229-238.
9. Laue, E: Makroskopische, licht- und elektronenmikroskopische Untersuchung über das Lymphgefäßsystem des Pferdes vom Huf bis zum Carpalgelenk. Berlin, FU, Fachbereich Veterinärmedizin, Diss. (1987).
10. Gabbiani, G, E Rungger-Brändle: The fibroblast. In: *Handbook of Inflammation*. 3rd edition. Glynn, LE (Ed.), Elsevier, Amsterdam (1981), 1-50.
11. Fawcett, DW: Muscular tissue. In: *A Textbook of Histology*. 11th edition. Bloom, W, DW Fawcett (Eds.), Saunders, Philadelphia (1986), 265-310.
12. Liang, W, P McDonald, B McManus, et al: Histamine-induced Ca(2+) signaling in human valvular myofibroblasts. J. Mol. Cell Cardiol. 35 (2003), 379-388.
13. Ghadially, FN: Myofibroblasts and myofibroblastoma. In: *Ultrastructural Pathology of the Cell and Matrix*. 3rd edition, 2. Ghadially, FN (Ed.), Butterworths, London, Boston, Singapore, Sydney, Toronto, Wellington (1988), 872-881.
14. Beltinger, J, BC McKaig, S Makh, et al: Human colonic subepithelial myofibroblasts modulate transepithelial resistance and secretory response. Am. J. Physiol. 277 (1999), 271-279.
15. Yang, AL, SJ Tsai, MJ Jiang, et al: Chronic exercise increases both inducible and endothelial nitric oxid synthase gene expression in endothelial cells of rat aorta. J. Biomed. Sci. 9 (2002), 149-155.
16. Eyden, B: The myofibroblast: An assessment of controversial issues and a definition useful in diagnosis and research. Ultrastruct. Pathol. 25 (2001), 39-50.
17. Stenmark, KR, E Gerasimovskaya, RA Nemenhoff, et al: Hypoxic activation of adventitial fibroblasts: role in vascular remodeling. Chest 122 (2002), 326-334.
18. Van Helden, DF, J Zhao: Lymphatic vasomotion. Clin. Exp. Pharmacol. Physiol. 27 (2000), 1014-1018.
19. McCloskey, KD, HM Toland, MA Hollywood, et al: Hyperpolarisation-activated inward current in isolated sheep mesenteric lymphatic smooth muscle. J. Physiol. 521 Pt 1 (1999), 201-211.
20. McCloskey, KD, MA Hollywood, KD Thornbury, et al: Kit-like immunopositive cells in sheep mesenteric lymphatic vessels. Cell Tiss. Res. 310 (2002), 77-84
21. Petrenko, VM, IA Pastukhova: Structure of lymphatic vessels in the human thighs. Morfologiia 118 (2000), 50-53.
22. Schacht, V: *Topometrische und klinisch orientierte Anatomie des oberflächlichen Lymphgefäßsystems der unteren Extremitäten des Menschen*. Hannover, Med. Hochsch., Diss. (2000).
23. Bogatkevich, GS, E Tourkina, CS Abrams, et al: Contractile activity and smooth muscle-(alpha) actin organisation in thrombin-induced human lung myofibroblasts. Am. J. Physiol. Lung Cell Mol. Physiol. 28 (2003), 28.
24. Risse, M: *Zur Pathogenese der akuten Lymphangitis und Elephantiasis beim Pferd – Eine histologische, immunhistochemische und transmissionselektronen-mikroskopische Studie*. Hannover, Tierärztl. Hochsch., Diss. (2004).
25. Dinenno, FA, H Tanaka, KD Monahan, et al: Regular endurance exercise induces expansive arterial remodeling in the trained limbs of healthy men. J. Physiol. 534 (2001), 287-295.
26. Moreau, KL, AJ Donato, DR Seals, et al: Arterial intima-media thickness: site-specific associations with HRT and habitual exercise. Am. J. Physiol. Heart Circ. Physiol. 283 (2002), 1409-1417.
27. Shigematsu, K, H Yasuhara, H Shigematsu, et al: Direct and indirect effects of pulsatile shear stress on the smooth muscle cell. Int. Angiol. 19 (2000), 39-46.
28. Goldberg, BD, M Rabinovitch: Connective Tissue. In: *Cell and Tissue Biology*. Weiss, L (Ed.), Urban & Schwarzenberg, Baltimore, München (1998), 170.
29. Alberts, B, D Bray, J Lewis, et al: Die extrazelluläre Matrix bei Tieren. In: *Molekularbiologie der Zelle*. 3 Aufl. Alberts

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B, D Bray, J Lewis, et al (Eds.), VCH-Verlagsgesellschaft, Weinheim, New York, Basel, Cambridge (1995), 1148-1175.
30. Gerli, R, L Ibba, C Fruschelli: Morphometric analysis of elastic fibres in human skin lymphatic capillaries. Lymphology 22 (1989), 167-172.
31. Rötting, AK: *Manuelle Lymphdrainage, Erprobung an den Extremitäten des Pferdes.* Berlin, Hannover, Diss. med. vet., (1999).
32. Berens v. Rautenfeld, D, K Rothe: Lymphsystem und Manuelle Lymphdrainage. In: *Anatomie und Propädeutik des Pferdes.* 2. Aufl. Wissdorf, H, H Gerhards, B Huskamp, et al (Eds.), M.& H. Schaper, Alfeld, Hannover (2002), 51-58.
33. Evrard-Bras, M, M Coupe, JP Laroche, C Janbon: Manual lymphatic drainage. Rev. Prat. 50 (2000), 1199-1203.
34. Strößenreuther, R.H.K: Einsatzmöglichkeiten der ML/KPE in Chirurgie, Orthopädie und Sportmedizin. In: *Lehrbuch der Lymphologie*, 5. Aufl. Földi, M, S Kubik (Eds.),Urban & Fischer Verlag, München, Jena, (2000), 673-693.

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