A survey on Flemish veterinary practitioners' experience with stem cell therapy to treat equine tendon injuries

Een enquête peilend naar de ervaring van Vlaamse praktijkdierenartsen met stamceltherapie voor de behandeling van peesblessures bij paarden

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ABSTRACT

Horses in all equestrian disciplines are suffering from tendon injuries followed by several months of rehabilitation. Additionally, the scar tissue formed after injury is of inferior quality, resulting in high re-injury rates. Conventional treatments include rest, controlled exercise and anti-inflammatory drugs administration. In order to improve tissue regeneration and prevent re-injury, biological treatments, such as mesenchymal stem cells (MSCs) are gaining popularity. In this survey, the use of MSCs as treatment for tendon injuries by Flemish equine veterinary practitioners was evaluated. Although the respondents were in general unsatisfied with conventional therapy, it remains the preferred treatment. Approximately half of the survey population (47%) have already used MSCs therapy to treat tendon injuries. The other participants reported the high costs and complexity as major limitations. According to the respondents, the availability of strong scientific evidence of the mechanisms of action, accompanied by clear practical guidelines, could help to increase the practical application of MSC therapy.

SAMENVATTING

Peesblessures komen vaak voor bij sportpaarden van verschillende disciplines. Het herstel duurt lang en het gevormde littekenweefsel is van inferieure kwaliteit, wat resulteert in een hoog risico op herval. Klassieke behandelingen omvatten rust, progressieve revalidatie en het toedienen van niet-steroïdale ontstekingsremmers. Om het herstel te verbeteren en herval te voorkomen, groeit de belangstelling voor biologische behandelingen zoals mesenchymale stamcellen (MSCs). Een enquête werd rondgestuurd om het gebruik van MSCs voor de behandeling van peesblessures bij het paard in Vlaanderen in beeld te brengen. Hieruit blijkt dat de klassieke behandelingen de voorkeur genieten, ondanks de ontoereikende resultaten. Ongeveer de helft van de deelnemende praktijkdierenartsen (47%) heeft ervaring met MSC-therapie. De andere respondenten geven aan dat ze geen MSCs gebruiken omwille van de hoge kostprijs en complexiteit van de behandeling. Er werd door de respondenten aangegeven dat meer wetenschappelijk onderbouwde informatie in verband met het werkingsmechanisme en praktische richtlijnen het gebruik zou kunnen doen toenemen.

INTRODUCTION

Tendon injuries are commonly encountered in performance horses, with specific injuries being overrepresented depending on the discipline (Ortved, 2018). The superficial digital flexor tendon (SDFT) is often injured in racehorses, showjumpers and event horses, while the distal deep digital flexor tendon (DDFT) has an increased injury risk in showjumpers (Ribitisch et al., 2021), and the hindlimb suspensory ligament is most commonly injured in dressage horses (Birch et al., 2013).

Tendinopathy is classified as either an acute injury, due to excessive loading, or a chronic injury, as a result of the cumulative degenerative damage to tendons, which are already operating close to their functional limits (Shojaee and Parham, 2019). The presence of degenerative changes increases with age and exercise, due to a reduced collagen crimp and slower turn-over (Svensson et al., 2016). Risk factors associated with tendon injuries include poor foot conformation, uneven ground, high body weight, intense competition at high speed, and previous tendinopathy (Kummerle et al., 2019).

Tendon injuries often represent significant challenges as tendons are hypocellular structures, which contain little tendon cells (i.e. tenocytes and tendon stem cells). These cells are responsible for the production and maintenance of extracellular matrix (ECM). The ECM predominantly consists of collagen triple helices molecules, grouped progressively into (micro-)fibrils, fibers and fascicles. Between the fascicles, the proteoglycan-rich matrix is present, which determines the elasticity of the tendon, and each fascicle is surrounded by a connective tissue called the endotenon (Patel et al., 2012; Wang et al., 2018). The whole tendon is covered by the epitenon, which is a connective tissue sheath continuous with the endotenon and paratenon (Wang et al., 2018) (Figure 1).

Immediately after acute damage to the tendon, an inflammatory phase is observed, in which the level of pain experienced is not always in correlation to the extent of tendon damage (Voleti et al., 2012). Subsequently, the repair phase is initiated, characterized by fibroplasia and angiogenesis, followed by the remodeling phase. The latter is of great importance for tendon healing, as a much higher percentage of collagen III (20-30%) is formed during the repair phase when compared to normal, healthy tendon (1-3%), which is primarily composed of collagen type I (Kummerle et al., 2019). Therefore, collagen type III should be replaced in the final phase by collagen type I fibers. In adult horses, however, this final stage never gets completed, resulting in the presence of inferior scar tissue lacking the structural integrity and elasticity of the original tendon (Richardson et al., 2007; Adekanmbi et al., 2017; Khatibzadeh et al., 2019). The limited functionality of this repair tissue represents a substantial risk of reduced performance and/or reinjury (Dyson, 2004; Smith et al., 2013; Shojaee and Parham, 2019).

Current treatments include rest and a strict rehabilitation program, supported by anti-inflammatory drugs. The influence of inflammation and the use of anti-inflammatory drugs are however controversial. The early inflammation phase is of critical importance in (sub-) acute lesions; however, prolonged duration causes fibrosis and impairs the healing response (Dakin et al., 2014). Additionally, systemic corticosteroids are only recommended during the first 24-48 hours after the injury, as later administration is known to negatively affect tendon healing (Kümmerle et al., 2019). Cold therapy using ice-packs or cold water is

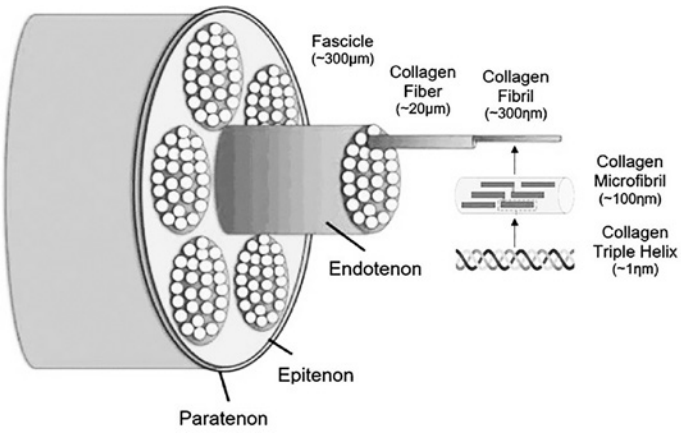

Figure 1. Illustration of the hierarchical tendon structure. The smallest tendon unit is a collagen triple helices molecule, which is progressively organized into (micro-) fibrils, bounded by intermolecular crosslinks, collagen fibers and fascicles. Each fascicle is surrounded by endotenon and the whole tendon is covered by the epitenon and paratenon (From: Meeremans et al. 2021).

an important element of treatment during the acute phase as it works analgesically and anti-inflammatory. After the acute inflammation phase, progressive rehabilitation should be started. Gradual increase in exercise is of major importance to support the realignment of the tendon fibers and the replacement of collagen III into collagen I, and is generally started from 90–120 days post injury (Gillis, 1997).

In the past, counterirritation using e.g. hot or cold firing and blistering, was used in equine patients immediately after the inflammation phase to obtain better-quality collagen (Ellis and Dey, 2011). However, the mechanism of action is unclear and at this moment, this is only performed rarely to protect animal welfare. The poor success of historical and conventional therapy supported the need to search for novel treatments, such as laser, ultrasonic, shock wave and biological therapies (Bonilla-Gutiérrez et al., 2019). The goal of biological medicine is to restore the functionality and regenerate a tissue as close to the initial structure of the tendon as possible in order to allow horses to perform at previous athletic levels with a reduced (re-)injury risk. Via recruitment of endogenous cells or exogenous delivery of cells and biomolecules, biological therapies have the potential to promote self-healing (Ortved, 2018). Platelet-rich plasma (PRP), autologous conditioned serum (ACS) and mesenchymal stem cell (MSC) therapy are the main products currently used to treat equine musculoskeletal injuries. MSCs are multipotent adult stem cells that are capable of differentiating into various cell types of mesodermal origin, such as osteoblasts, chondroblasts, adipocytes and tenocytes. Initially, the use of MSCs for primary tissue regeneration was advocated based on their ability to colonize the injury site and differentiate into various appropriate cell types. However, terminal differentiation and engraftment do not seem to be the principal mechanisms of

action. Indeed, when technetium-99m-labeled MSCs are injected intralesionally in horses with a naturally occurring tendon injury, it has been observed that only 24% of the injected cells remained in place after 24 hours (Colbath et al., 2017). Nowadays, MSCs are rather considered as 'medicinal cell factories' secreting a variety of bioactive molecules with immunomodulatory, anti-inflammatory, ECM modeling, trophic and anti-apoptotic effects. The complete set of secreted growth factors and cytokines is called 'the secretome' (Mocchi et al., 2020).

Although the first therapeutic use of equine MSCs was reported in 2003 and the clinical application of MSCs has been exploding since then (De Schauwer et al., 2013), many questions remain unanswered regarding the clinical use of MSCs in equine veterinary medicine, such as the efficacy of treatment, the optimal MSC dose, the most appropriate MSC source, the frequency of administration, and the use of autologous versus allogenic MSCs. Bone marrow (BM) and adipose tissue (AT) are two traditional MSC sources in horses with BM-MSCs being first used in equine SDFT patients (Smith et al., 2003). However, probably due to the superior differentiation capacities of BM-MSCs, ectopic bone formation has been reported after administration into a SDFT (1/1500 cases) (Alves et al., 2011). AT-MSCs on the other hand have a superior cell yield, a less invasive isolation procedure and superior anti-inflammatory and immunomodulating properties. Additionally, Burk et al. (2014) reported the highest tenogenic gene expression in AT-MSCs when compared to other tissue sources; therefore, these authors consider AT-MSCs as the most promising MSC source for tendon regeneration. According to Ortved (2018), 10 to $20x10^6$ MSCs should be administered within one month after injury as they might decrease fibrosis during the proliferation phase. Godwin et al. (2012) showed that increasing re-injury rates are associated with longer time-to-treatment interval. Using autologous MSCs, it might be challenging to achieve sufficient cell yield within time, since two-three weeks of culture are needed to obtain a sufficient number of cells. Recently, the use of commercially available allogenic peripheral blood derived-MSCs (PB) has gained popularity since it is an off-the-shelf product. Disadvantages of allogenic cells include a higher risk of immunological adverse reactions and less cell survival following injection. Advantages include the ability to bank cells and reduce the time to treatment, to collect MSCs from younger donors and the ability to induced differentiation towards the desired lineage prior to administration (Colbath et al., 2020).

Regarding the route of administration, local injection is the preferred method when treating musculoskeletal diseases. Alternatively, MSCs can be injected in tendon sheet or intravenously, when intra-lesional injection is hampered by the limited amount of space or in case of multifocal injuries (Mocchi et al., 2020). In clinical cases, MSCs can be combined with scaffolds that mimic the ECM and retain the cells intralesionally, and growth factors or other biological products such as PRP to improve tendon regeneration (Bonilla-Gutiérrez et al., 2019). However, the mechanism of action of these factors to induce tendon healing remains unclear indicating that current treatment protocols require further optimization. Despite the many promising in vitro studies, the therapeutic use of MSCs is still limited due to these many gaps in knowledge.

In this study, Flemish veterinary practitioners were questioned on the use of biological treatments, MSCs in particular, for equine tendinopathy patients and whether the knowledge gaps described previously hamper their use in practice. The goal of this study was to gain insight into the preferred treatments of practitioners to treat tendinopathy, and to investigate whether they consider the use of biological treatments (MSCs, ACS, PRP) and why (not). Finally, in case biological treatments are used, information was gathered regarding their preferred protocol including route of administration, MSC source and common indications for use.

MATERIALS AND METHODS

The survey was prepared as an electronic Google survey using multiple choice questions and was distributed by e-mail via the "Orde der Dierenartsen" and through social media (personal LinkedIn and Facebook accounts of the different authors) between November 2021 and March 2022. The target population included Flemish veterinary practitioners and responses were obtained from small and large animal veterinarians who treat tendon injuries at least at a yearly basis. The full questionnaire (in Dutch) is available as an annex to this article. All data were processed anonymously.

First, six questions were included regarding respondent experience (years in practice, practice type), frequency of treating equine tendinopathy patients, observed re-injury rate, preferred therapy and indications for MSC therapy. Following these general questions, participants were directed to different questionnaires, depending on whether or not MSCs were used. The following information was asked to the respondents familiar with MSC therapy: number of patients already treated, indication of use (tissue and injury type), MSC type (allogenic versus autologous and MSC source), and observed efficacy. Veterinarians not familiar with MSC therapy at the moment of the questionnaire, were asked about their opinion on MSCs, the reason why they did not use it, how likely it was they would use MSC therapy in the future, and what additional information should be provided in order to consider using MSC therapy.

After closing the survey and collecting all data, the answers were processed manually, and the summary statistics and graphical presentation of data were obtained using Microsoft Excel (version 16.16.27).

Table 1. Overview of the study population and proportions of veterinarians seeing tendinopathy cases on a daily, weekly, monthly or yearly basis.

		Daily	Weekly	Monthly	Yearly	Total
Small and large animal veterinarians		n = 0 (0%)	n = 1 (14%)	n = 4 57%)	n = 2 (29%)	n = 7 (21%)
Equine veterinarians – all disciplines		n = 1 (8%)	n = 2 (15%)	n = 10 (77%)	n = 0 (0%)	n = 13 (39%)
Equine veterinarians – focus orthopedics		n = 7 (54%)	n = 6 (46%)	n = 0 (0%)	n = 0 (0%)	n = 13 (39%)
	Total	n = 8 (24%)	n = 9 (27%)	n = 14 (42%)	n = 2 (7%)	**n = 33**
		Experienced clinicians		**General practitioners**		

RESULTS

Thirty-three Flemish veterinarians participated in the survey. Based on a more detailed description of their activities, the following categories were identified: (i) veterinarians treating both small and large animals, (ii) veterinarians treating horses only, and (iii) veterinarians treating horses only with a clear focus on orthopedics. The clinical experience with tendon injuries was estimated based on the frequency of treating equine tendinopathy patients. A small number of respondents were veterinarians treating both small and large animals (21%) and the majority (86%) of them were treating tendon injuries in horses only monthly or yearly. More than one third of the respondents were veterinarians treating horses only (39%), of whom 77% was treating tendon injuries monthly and 23% even daily to weekly (Table 1). Finally, an equally large group of respondents were equine clinicians with focus on orthopedics (39%) treating tendon injuries daily to weekly. As such, half of the respondents were treating horses with tendon injuries on a weekly or daily basis, and were therefore considered as experienced clinicians. The other half (49%) of the respondents were considered as general practitioners as they were treating tendon injuries in horses only on a monthly or yearly basis. Both groups, experienced clinicians versus general practitioners, were used to further evaluate the responses.

Regarding re-injury, about half (53%) of the experienced clinicians reported occasionally (10-50%) re-injury. Another large part (41%) of the experienced clinicals reported frequently (>50%) re-injury. General practitioners reported lower recurrence rates, with 19% of them having never observed a relapse.

As initial treatment for (sub)acute tendon injuries, all respondents preferred conventional therapy, consisting of rest, non-steroidal anti-inflammatory drugs (NSAIDs) and progressive rehabilitation. Additionally, corrective shoeing might be prescribed. The majority of the experienced clinicians (67%) recommended biological therapies as a second treatment option, with PRP, ACS and bone marrow aspirate being the most popular; only 13% recommended MSC treatment. 'On request of the owners' was indicated as main reason to use MSCs to treat tendon injuries (61%). General practitioners, on the other hand, preferred laser, ultrasound and shockwave therapy as a second treatment option.

Forty-seven percent (47%) of the experienced clinicians have used MSC therapy in practice of whom 75% have already treated more than 25 cases (Figure 2A). Since most MSCs research focusses on the treatment of musculoskeletal diseases, 90% of the experienced clinicians have used MSCs for orthopedic applications such as joint diseases and tendon injuries, of which mostly acute, tendon core lesions. Ten percent of the experienced clinicians additionally reported the use of MSCs for ocular therapy. Most popular was the allogenic use of MSCs (76%), with PB- MSCs mentioned as commonly used (70%) tissue source.

The observed efficacy after MSC therapy was rather variable. One third (33%) reported no improvement compared to patients treated with conventional therapy, while 67% reported a significant improvement, with lower re-injury rates in chronic cases as main observed finding (Figure 3).

Seventy percent (70%) of the respondents had never used MSCs at the moment of the survey, with price (74%) and complexity (61%) indicated as most limiting factors.

Half (52%) of the veterinarians who had never used MSCs, reported a positive impression of MSC therapy; they think it is a promising treatment or are convinced of its efficacy. In contrast, the other half (48%) reported a rather negative point of view regarding MSC therapy, they questioned its efficacy or were not familiar with MSCs (Figure 4). Moreover, 22% answered that they would probably never use MSC therapy. Allegedly, in order to boost the use of MSC therapy in practice, the therapy should be cheaper and more scientific data regarding the optimal

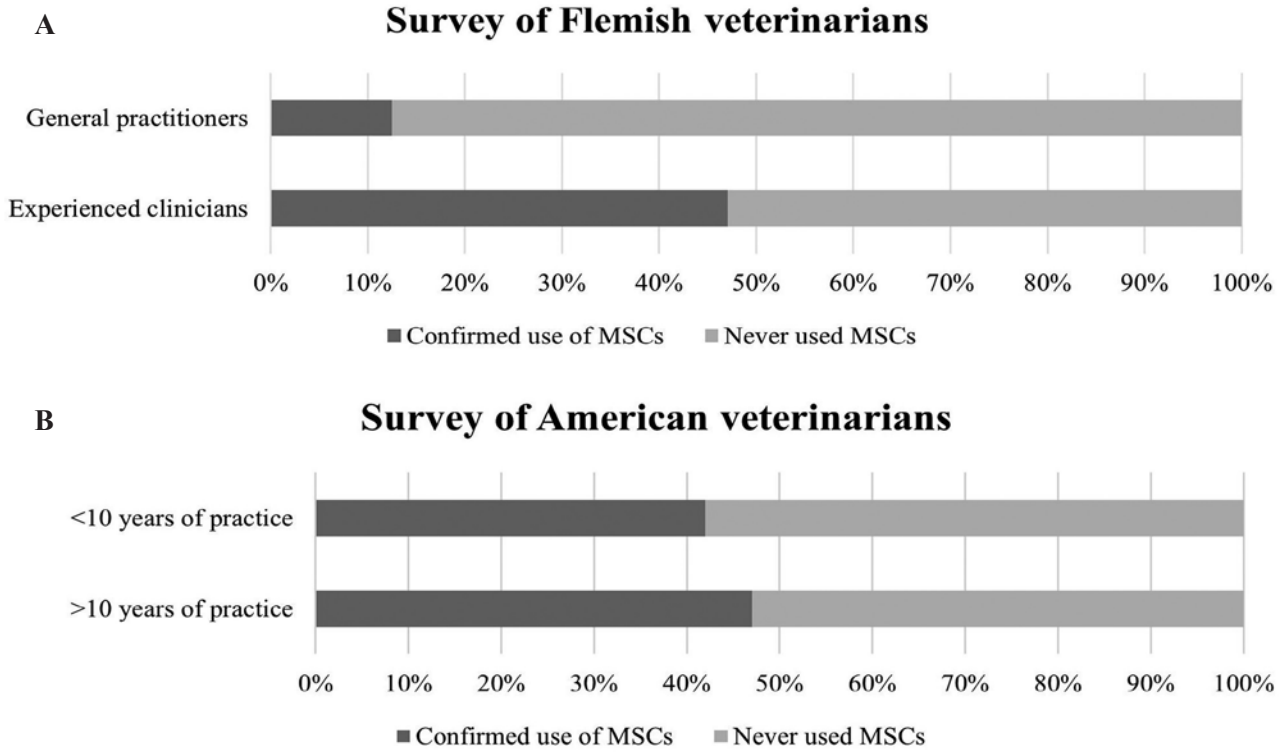

Figure 2. Schematic overview of the use of stem cells in horses by A. Flemish veterinarians in this survey and B. American veterinarians from the survey of Knott et al. (2022).

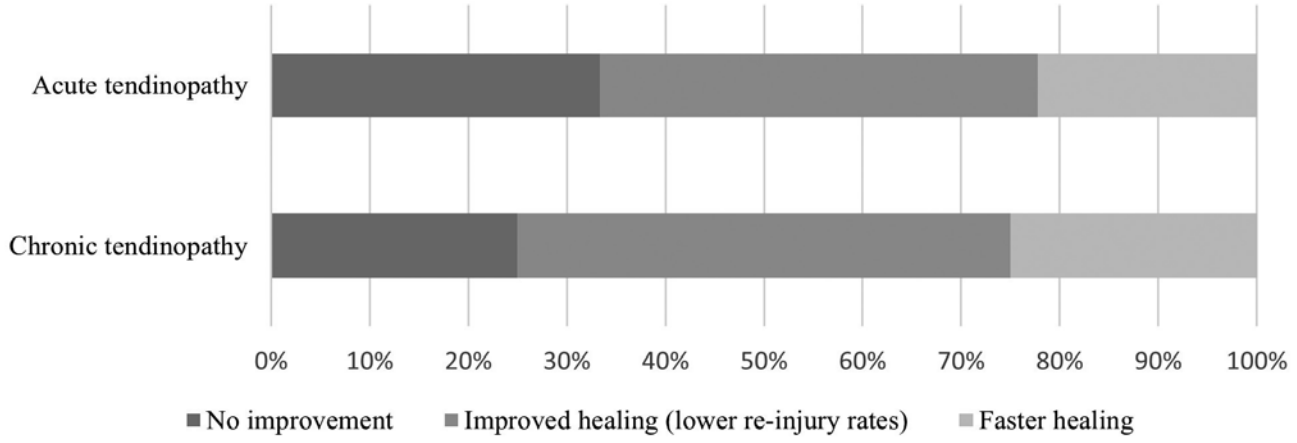

Figure 3. Overview of the perceived added value of MSC therapy to treat acute versus chronic tendinopathies as observed by the respondents of this study.

indication(s), the mechanism of action and treatment efficacy become available.

DISCUSSION

This survey was performed to gain insight into the use of biological treatments, MSCs in particular, to treat equine tendinopathy patients by Flemish veterinarians.

First, some general information was requested. In this survey, all experienced clinicians reported a recurrence rate between 10% and more than 50% after tendon injury, which is in agreement with a recurrence rate of 23-67% within two years post-injury reported in the literature after conservative treatment (Thorpe et al., 2010). The high re-injury rate is indicative for an unsatisfying treatment outcome; factors affecting the re-injury rate include size of the lesion, location, other risk factors (such as hoof conformation) and activity type (Kummerle, 2019). In contrast, one fifth of the general practitioners (19%) had never observed any relapse. This might be explained by the fact that either minor injuries are potentially missed by the less

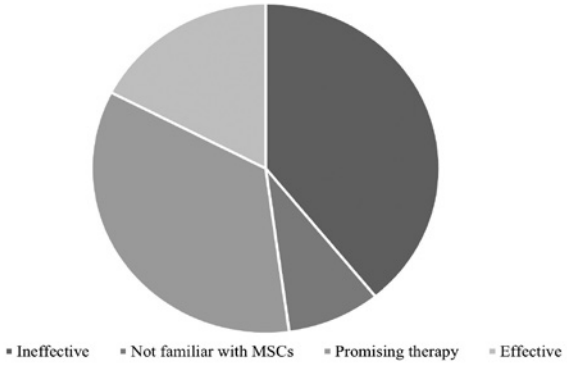

Figure 4. Schematic overview of the perception on MSC therapy by veterinarians not using MSCs.

experienced veterinarians, or patients with severe tendon injuries are send to referral centers, or that owners immediately consult an experienced clinician when symptoms reoccur.

A similar survey study has recently been performed in the United States, in which diplomates of the American College of Veterinary Surgery (ACVS), and American College of Veterinary Sport Medicine and Rehabilitation (ACVSMR) were questioned about their current use of biological therapies for musculoskeletal disease (Knott et al., 2022). The biological therapies evaluated in this survey included MSCs, PRP and ACS. Questions focused on frequency of use, indications of use, route of administration, injection protocols, limitations for use and adverse effects. The survey population was further divided into two groups based on years in practice: >10 years (n = 123) and <10 years (n = 30). Since only diplomates were questioned in this survey, all participants can be considered as specialists regardless of their years in practice, which is different from the survey population of the present study, containing both experienced clinicians (51%) and general practitioners (49%). Similar to the present survey, 47% of the American diplomates used MSC therapy in practice (Figure 2B). They also mentioned costs as the most limiting factor to use MSC therapy (74% in USA versus 74% in Flanders). Fifty-six percent (86/153) of the American specialists preferred the use of biological products, based on the scientific literature and data showing the efficacy of the product, while 20% (30/153) mentioned personal experience as main reason. Ten percent (15/153) mentioned lack of efficacy of previous treatments as their main reason, and 5% (8/153) client request. The latter is in contrast with the outcome of the present study, in which Flemish veterinarians report specific request of the owner as main (61%) indication to use MSC therapy to treat tendon injuries.

It must be mentioned that this study had some limitations. First, the title of the survey was rather specific, resulting in a group of respondents mainly specialized in equine patients and even with a clear focus on orthopedics. Out of the 33 respondents, 47% had already treated equine patients with MSCs. Due to the narrow respondent population, this percentage might not be representative for all veterinarians in Flanders. Moreover, due to the clear orthopedic focus of the respondents, the use of MSCs for ocular therapy might be an underestimation of the actual use of MSCs for this application. Additionally, the study group was defined as all "veterinary practitioners", but veterinarians working in more specialized referral centers (including many EBVS recognized diplomates) might have felt not properly addressed. A more general title and a more detailed description of the study group might have resulted in more representative answers. Secondly, only multiple choice questions were asked without open space for additional comments in order to have straightforward answers, limiting the amount of information obtained. Thirdly, some outcome parameters such as clinical improvement upon treatment were not clarified in the questions. Therefore, the responses are subjective based on the impression and assumptions of the treating veterinarians, rather than on a direct comparison between conventional therapy and MSC injection in a single case. Finally, in contrast to the American survey, no questions were asked whether or not horses were treated only once or multiple times. In future research, treatment frequency should be questioned as well. Since reportedly, owner motivation was the main reason for considering MSC therapy in Flemish equine patients, it would equally be interesting to question horse owners on their experience with MSC therapy.

CONCLUSION

In this survey, it is shown that Flemish veterinary practitioners currently prefer conventional treatment (rest, NSAIDs and rehabilitation) for tendon injuries, although the results are often unsatisfactory. Regardless of the explicit need for effective treatments to induce tendon regeneration and increasing the scientific literature on biological therapies, veterinarians were reported to be hesitating to use MSCs in practice. General practitioners were less familiar with tendinopathy patients and reported the high costs of MSC treatment and the associated complexity as the main limiting factors. More experienced clinicians examining tendinopathy patients daily or weekly, were more likely to use MSCs, although their main motivation was owner persistence. Strong scientific evidence of the mechanisms of action and success rate, accompanied by clear practical guidelines (when, how many, where) might increase the therapeutic use of MSCs.

REFERENCES

Adekanmbi, I., Zargar, N., Hulley, P. (2017). An in vitro scratch tendon tissue injury model: effects of high frequency low magnitude loading. *Connective Tissue Research 58(2)*, 162-171.

Alves, E.G.L., Serakides, R., Rosado, I.R., Paez, O.L.A., Varon, J.A.C., Machado, F.N. Fukushima, F.B., Góes,

A.M., Rezende, C.M.F (2017). Osteoprogenitor cells can enhance early bone formation in critical bone defects. *Ciência Rural 47(7)*, 1-8.

Birch, H.L., Sinclair, C., Goodship, A.E., Smith, R.K.W. (2013). Tendon and ligament physiology. In: Hinchcliff K. W., Kaneps A. J., Geor R. J. (editors). *Equine Sports Medicine and Surgery*. Second Edition. Elsevier Ltd. St. Louis, MO, USA, 167-185.

Bonilla-Gutiérrez, A.F., López, C., Carmona, J.U. (2019). Regenerative therapies for the treatment of tenodesmic injuries in horses. *Journal of Equine Veterinary Science 73*, 139-147.

Burk, J. (2019). Mechanisms of action of multipotent mesenchymal stromal cells in tendon disease, in tendons. *IntechOpen*, 73-83.

Colbath, A.C., Frisbie, D.D., Dow, S.W., Kisiday, J.D., McIlwraith, C.W., Goodrich, L.R. (2017). Equine Models for the Investigation of Mesenchymal Stem Cell Therapies in Orthopaedic Disease. *Operative Techniques in Sports Medicine 25*, 41-49.

Colbath, A.C., Dow, S.W., McIlwraith, C.W., Goodrich, L.R. (2020). Mesenchymal stem cells for treatment of musculoskeletal disease in horses: Relative merits of allogeneic versus autologous stem cells. *Equine Veterinary Journal 52(2)*, 654-663.

Dakin, S. G., Dudhia, J., Smith, R. K. W. (2014). Resolving an inflammatory concept: The importance of inflammation and resolution in tendinopathy. *Veterinary Immunology and Immunopathology 158*, 121-127.

De Schauwer, C., Van de Walle, G.R., Van Soom, A., Meyer, E. (2013). Mesenchymal stem cell therapy in horses: useful beyond orthopedic injuries? *Veterinary Quarterly 33(4)*, 234-241.

Dyson, S. (2004). Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992-2000). *Equine veterinary journal 36(5)*, 415-419.

Ellis, D. R., Dey, S. P. (2011). Counterirritation. In: Ross, M.W., Dyson, S.J. (editors). *Diagnosis and Management of Lameness in the Horse*. Second edition, W.B. Saunders, 867-869.

Gillis, C.L. (1997). Rehabilitation of tendon and ligament injuries. *The American Association of Equine Practioners 43*, 306-309.

Godwin, E.E., Young, N.J., Dudhia, J., Beamish, I.C., Smith, R.K.W. (2012). Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. *Equine Veterinary Journal 44*, 25-32.

Khatibzadeh, S.M., Menarim, B.C., Nichols, A.E.C., Werre, S.R., Dahlgren, L.A. (2019). Urinary bladder matrix does not improve tenogenesis in an in vitro equine model. *Journal of Orthopaedic Research, 37(8)*, 1848-1859.

Knott, L.E., Fonseca-Martinez, A.B., O'Connor, A.M., Goodrich, L.R., McIlwraith, C.W., Colbath, A.C. (2022). Current use of biologic therapies for musculoskeletaldisease: A survey of board-certified equine specialists. *Veterinary Surgery 51*, 557–567.

Kümmerle, J.M., Theiss, F., Smith, R.K.W. (2019). Diagnosis and management of tendon and ligament disorders. In: *Equine Surgery*. Fifth Edition, Elsevier Inc. St. Louis, MO, USA, 1411-1445.

Meeremans, M., Van de Walle, G.R., Van Vlierberghe, S., De Schauwer, C. (2021). The lack of a representative tendinopathy model hampers fundamental mesenchymal stem cell research. *Frontier in Cell and Developmental Biology. 9*, 1051.

Mocchi, M., Dotti, S., Bue, M. Del, Villa, R., Bari, E., Perteghella, S., Torre, M.L., Grolli, S. (2020). Veterinary regenerative medicine for musculoskeletal disorders: can mesenchymal stem/stromal cells and their secretome be the new frontier? *Cells 9*, 1–23.

Ortved, K. (2018). Regenerative medicine and rehabilitation for tendinous and ligamentous injuries in sport horses. *Veterinary Clinics of North America - Equine Practice 34*, 359-373.

Patel, D., Sharma, S., Bryant, S.J., Screen, H.R.C. (2017). Recapitulating the micromechanical behavior of tension and shear in a biomimetic hydrogel for controlling tenocyte response. *Advanced healthcare materials 6(4)*, 160-195.

Ribitsch, I., Oreff, G.L., Jenner, F. (2021). Regenerative medicine for equine musculoskeletal diseases. *Animals 11*, 1-30.

Richardson, L.E., Dudhia, J., Clegg, P.D., Smith, R. (2007). Stem cells in veterinary medicine–attempts at regenerating equine tendon after injury. *Trends in Biotechnology 25(9)*, 409-416.

Shojaee, A., Parham, A. (2019). Strategies of tenogenic differentiation of equine stem cells for tendon repair: Current status and challenges. *Stem Cell Research and Therapy 10*, 1-13.

Smith, R., Korda, M., Blunn, G.W., Goodship, A.E. (2003). Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. *Equine Veterinary Journal 35(1)*, 99-102.

Smith, R.K.W., Werling, N.J., Dakin, S.G., Alam, R., Goodship, A.E., Dudhia, J. (2013). Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. *PLoS One 8*, 1-14.

Svensson, R.B., Heinemeier, K.M., Couppé, C., Kjaer, M., Magnusson, S.P. (2016). Effect of aging and exercise on the tendon. *Journal of Applied Physiology 121*, 1353-1362.

Voleti, P.B., Buckley, M.R., Soslowsky, L.J. (2012). Tendon healing: Repair and regeneration. *Annual Review of Biomedical Engineering 14*, 47–71.

Wang, T., Chen, P., Zheng, M., Wang, A., Lloyd, D., Leys, T., Zheng, Q., Zheng, M.H. (2018). In vitro loading models for tendon mechanobiology. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society 36(2)*, 566-575.

Full questionnaire (in Dutch)

1. Welke beschrijving sluit het best bij u aan?
   - o Praktijkdierenarts voor kleine en grote huisdieren
   - o Praktijkdierenarts voor paard – alle disciplines
   - o Praktijkdierenarts voor paard – focus op orthopedie

2. Hoe vaak ziet u paarden met peesblessures op consultatie?
   - o Nooit
   - o Dagelijks
   - o Wekelijks
   - o Maandelijks
   - o Jaarlijks

3. Gebaseerd op uw ervaring: hoeveel paarden hervallen na de behandeling van een peesblessure?
   - o Nog niet vastgesteld
   - o Zelden (<10%)
   - o Af en toe (10-50%)
   - o Regelmatig (>50%)

4. Welke therapie(ën) raadt u meestal aan bij een (sub)acute peesblessure?
   Meerdere opties mogelijk.
   - o Rust + niet-steroïdale ontstekingsremmers (NSAID's)
   - o Gecontroleerde revalidatie (trainingsschema)
   - o Stamcellen
   - o Laser-, ultrasone- of schokgolftherapie
   - o Beenmergaspiraat, plaatsjesrijk plasma (PRP), autoloog geconcentreerd serum (vb. IRAP)
   - o Fysiotherapie
   - o Aangepast hoefbeslag

5. Wat is de waarschijnlijkheid dat u deze therapieën aanraadt bij peesblessures? Rangschik in toenemende waarschijnlijkheid met 1 = minst waarschijnlijk en 7 = meest waarschijnlijk
   - o Rust + niet-steroïdale ontstekingsremmers (NSAID's)
   - o Gecontroleerde revalidatie (trainingsschema)
   - o Stamcellen
   - o Laser-, ultrasone- of schokgolftherapie
   - o Beenmergaspiraat, PRP, autoloog geconcentreerd serum etc.
   - o Fysiotherapie
   - o Aangepast hoefbeslag

6. Wanneer zou u stamceltherapie bij een peesblessure aanbevelen? Meerdere opties mogelijk.
   - o Nooit
   - o Bij een acuut peesletsel
   - o Bij een recidiverend peesletsel
   - o Bij symptomen van overbelasting zonder ruptuur
   - o Wanneer alle andere therapieën niet werken
   - o Wanneer een eigenaar hierop aandringt

7. Gebruikt u stamceltherapie in de behandeling van uw patiënten?
   - o Ja ➔ Vraag 8 – 1 3
   - o Nee ➔ Vraag 14 – 17

8. Hoeveel patiënten heeft u al behandeld met stamceltherapie?
   - 1-5
   - 6-10
   - 11-25
   - >25

9. Voor welke toepassing gebruikt u stamceltherapie?
   Meerdere opties mogelijk.
   - Peesblessures
   - Gewrichtsproblemen
   - Botaandoeningen
   - Huidwonden
   - Oogaandoeningen

10. Indien u stamceltherapie gebruikt bij peesblessures, bij welk type letsels gebruikt u dit dan? Meerdere opties mogelijk.
    - Volledige ruptuur
    - Longitudinale ruptuur
    - Centraal peesletsel (core lesion)
    - Diffuse tendinopathie
    - Ik gebruik geen stamceltherapie bij peesblessures

11. Welke stamcellen gebruikt u?
    Meerdere opties mogelijk.
    - Stamcellen van het paard zelf – afkomstig van beenmerg
    - Stamcellen van het paard zelf – afkomstig van vetweefsel
    - Stamcellen van het paard zelf – afkomstig van bloed
    - Stamcellen van een ander paard – afkomstig van vetweefsel
    - Stamcellen van een ander paard – afkomstig van bloed

12. Bent u tevreden over stamceltherapie?
    Schaal 1 tot en met 5
    1 = niet tevreden, 5 = heel tevreden

13. Ervaart u een verschil in de behandeling met stamceltherapie ten opzichte van de huidige conventionele therapieën?
    Meerdere opties mogelijk.
    - Nee, geen verschil
    - Ja, beter herstel (minder herval)
    - Ja, sneller herstel

14. Hoe denkt u over stamceltherapie?
    - Het werkt niet volgens mij
    - Ik ken het niet
    - Het is veelbelovend
    - Ik ben overtuigd dat het werkt

15. Waarom gebruikt u geen stamceltherapie?
    Meerdere opties mogelijk.
    - Geen invloed op de duur van herstel
    - Geen betere genezing (kwaliteit herstelweefsel)
    - Te complex voor in de praktijk
    - Te duur
    - Te weinig informatie beschikbaar

16. Hoe groot is de kans dat u in de toekomst wel stamceltherapie gaat gebruiken?
    Schaal 1 tot en met 5
    1 = zeer klein, 5 = zeer groot
17. Welk element is het meest noodzakelijk om u ervan te overtuigen om stamceltherapie te overwegen in de toekomst?
    - Bewijs van effectiviteit
    - Goedkopere methode
    - Eenvoudigere methode
    - Meer wetenschappelijk onderbouwde informatie (gebruik, werking en lange termijn resultaten)