**OARSI Abstract**

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THE ROLE OF MICRORNAS IN TENDON DYSFUNCTION

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**Purpose:** Tendinopathies are a significant cause of morbidity in both human and equine species, accounting for up to 50% of musculoskeletal injuries presented for medical (Littlewood et al 2013) or veterinary attention (Williams et al 2001). Tendinopathies are painful, mobility limiting, affect quality of life and carry a substantial financial cost associated with treatment, rehabilitation and lost productivity. A range of tendons are commonly affected in humans, such as Achilles, posterior tibial and rotator cuff tendons, whilst in horses, the superficial digital flexor tendon (SDFT) is over represented. It is increasingly recognised that tendon failure is the end result of a chronic, asymptomatic process of deterioration at the cellular and molecular level and once this occurs, mechanical integrity is never fully restored. MicroRNAs (miRs) are robust regulators of cellular pathways and physiological processes, modulating multiple gene expression. Altered miR expression has been demonstrated during ageing (Peffers et al 2015) and mechanical loading (Mendias et al 2012) of tendon. Identification of miRs dysregulated during ageing and disease offers the potential to more fully understand the disease process through identifying miR-regulated pathways which may be targeted to develop effective miR-based therapeutics. Equine SDFT is an ideal model to study human tendinopathy, being an elastic energy storing tendon sharing functional similarity with commonly injured human tendons. The longevity and use of horses makes them ideal for studying age and exercise related impacts on tendon integrity. This study aimed to identify miRs differentially expressed in equine SDFT during ageing and with naturally occurring tendinopathy.

**Methods:** Total RNA was extracted from equine SDFT collected from a commercial abattoir. Thirteen horses aged 3-25 years were included, eight samples were classified as healthy with no gross evidence of tendinopathy and five as diseased. Samples of both healthy and diseased tendon tissue were collected from 2 horses. Following spectrophotometric determination of nucleic acid concentration and purity, 100ng RNA template was converted into cDNA. Expression of miR-: 29a, 34a, 34b, 34c, 181a, 181b, 181c, 181d, 199a, 199b, let-7b and let-7f , selected by us based on predicted and validated target genes known, or predicted, to be associated with tendinopathy, was measured by quantitative real time PCR, using 2.5ng cDNA template. Relative expression was calculated relative to Snord61. For analysis purposes, individuals were classed as young (3-9 years, n=9) or old (19-25 years, n=4). Statistical analysis was performed using GraphPad Prism 6.

**Results:** Expression of miR-29a, previously shown to be associated with tendinopathy (Millar et al 2015), was significantly decreased in old versus young and diseased versus normal equine tendon. The expression of miR-34a was reduced in old horses, whilst 34b was upregulated. MiR-34a and 34b demonstrated higher expression in diseased than healthy tendons at the population level. The expression of miR-181b was significantly increased in older animals, and reduced in diseased tissue. The expression of miRs: miR- 181a, -181c and -181d was unchanged with age or disease. Expression of miR-199a and 199b was reduced with age and disease. Let-7f was expressed at much lower levels than Let-7b; neither showed altered expression with age or disease.

**Conclusions:** In equine SDFT we have identified significantly altered expression with age and disease of miRs associated with collagen synthesis, inflammation, apoptosis and cell cycle regulation. Expansion of the data set will address confounding effects of wide inter-individual variation. Gain- and loss-of-function experiments in equine and human primary tenocytes will further investigate the effects of these changes and the potential of miRNA-based therapies to ameliorate tendinopathy.