1. Introduction

Flexural limb deformities represent a prevalent and well-documented orthopaedic condition, particularly in young foals. This pathology manifests as a sagittal plane deviation caused by abnormal hyperflexion or hyperextension of the appendicular joints. Hyperflexion most commonly affects the metacarpophalangeal, distal interphalangeal, and carpal joints and can be further classified as congenital or acquired (Kidd, 2019). First-line therapy is conservative and includes pain relief, reduced and controlled exercise, limb immobilisation, corrective hoof trimming or glue-on rubber shoes in older foals and intravenous administration of oxytetracycline (OTC). Surgical interventions are indicated in severe cases or cases not responding to conservative treatment and include desmotomy of the accessory ligament of the superficial digital flexor tendon, SDFT (superior check ligament) and of the accessory ligament of the deep digital flexor tendon, DDFT (inferior check ligament), as well as tenotomy of the DDFT in severe cases.

Oxytetracycline is a first-generation tetracycline used widely for its antimicrobial properties. Its usefulness for the treatment of flexural limb deformities was discovered incidentally in the 1970s and has since been objectively reported in clinical, radiographic and kinematic *in-vivo*-studies (Lokai and Meyer, 1985; Madison et al, 1994; Kasper et al., 1995).

Proposed mechanisms of action of OTC on muscle-tendon-units of affected foals include the inhibition of calcium-mediated muscle contraction through a calcium chelation (McIlwraith, 1987) or a neuromuscular blockage (Pittinger and Adamson, 1972). Alternatively, an interaction with collagen cross-linking has been suggested (Engesaeter et al., 2001; Wintz et al., 2002). Moreover, tetracyclines have shown inhibitory properties on matrix metalloproteinases (MMPs), which have found applications in the fields of dentistry and oncology (Golub et al., 1991; Acharya et al., 2004). Based on this knowledge, the currently favoured hypothesis is that inhibition of MMPs impairs myofibroblast-mediated collagen remodelling and tractional structuring, making tendons more vulnerable to time-dependent strain (Arnoczky et al., 2004).

Although widely used with success, the antimicrobial properties of OTC, its high dosage for this indication (Madison et al., 1994) and potential side effects (Ellero et al., 2020) raise questions about its use in a context of increasing antimicrobial resistance and growing awareness of prudent antimicrobial use guidelines (Bowen, 2013). Consequently, there is a need to investigate substances with therapeutic potential for flexural limb deformities but without antimicrobial properties. Candidates include substances with MMP-inhibitor properties, such as ilomastat, the chemically modified tetracycline (CMT) incyclinide, aprotinin or pentoxifylline, as well as those interacting with collagen cross-linking, such as β-aminopropionitrile fumarate (BAPN).

The aims of this doctoral thesis were to assess the influence of the five mentioned substances (1) on the viability and proliferation of juvenile tendon and ligament myofibroblasts, and (2) on collagen gel contraction by myofibroblasts *in vitro*.

2. Review of literature

2.1 Flexural limb deformities of the metacarpo-/metatarsophalangeal and distal interphalangeal joints in foals

Flexural limb deformities are a common and well-described orthopaedic problem, especially in young foals (Kidd, 2019). In this entity, the limb shows an axial deviation in the sagittal plane, which may be caused by either a hyperextension or a hyperflexion of the appendicular joints. This current work will concentrate on hyperflexion deformities. Contrary to its common use, the term “contracted tendons” is inadequate in most cases as the disorder is usually not caused by scar tissue contraction within the tendon but by a discrepancy in the longitudinal growth of bones and tendons (Nemeth, 1976; Litzke & Dietz, 1999; Kidd, 2019).

Flexural limb deformities can be congenital, that is, present since birth, or acquired, developing in the first weeks to months of life (Kidd, 2019). The most commonly affected joints in congenital deformities are the metacarpo-/metatarsophalangeal (MCP/MTP) joint and the carpus, while in acquired deformities the distal interphalangeal (DIP) joint and the MCP/MTP joints are the most commonly affected (Kidd & Barr, 2002).

The *etiopathogenesis of flexural limb deformities* in foals remains incompletely understood (McIlwraith, 1987; Kidd, 2019). Various causes have been advanced, but some are speculative, and most are based on anecdotal evidence. *In-utero*-malposition is often cited as a cause of congenital deformities (Badame, 1963; Rooney, 1966; McIlwraith, 1987; Kasper et al., 1995; Litzke & Dietz, 1999). It has also been suggested that genetic predispositions exist (Myers & Gordon, 1975, Litzke & Dietz, 1999). However, in a study comprising 80 foals with lineage, no evidence of heritability was found (Hertsch, 1990). Authors have also described poor nutrition, including deficiencies in certain minerals such as calcium, phosphorus, and copper, and in vitamins A, D, and E, as a potential cause of congenital flexural deformities. Finally, exposure to teratogens (McIlwraith, 1987) through ingestion of locoweed (McIlwraith and James, 1982) and Sudan grass (Pritchard and Voss, 1967) by the mare during gesta-

tion or diseases of the mare such an influenza (Fessler, 1977) have been mentioned as potential causes. Other examples found in the literature include diseases of the foal such as goitre (McLaughlin & Doige, 1981), neuromuscular disorders (Mayhew, 1984), defects in cross-linking of elastin and collagen caused by lathyrism (Keeler and James, 1971), and more recently a glycogen branching enzyme deficiency (Valberg et al., 2001).

Potential *reasons for acquired flexural limb deformities* include nutritional imbalances, rapid growth, trauma, pain and lateral grazing behaviours (Kidd, 2019; van Heel et al., 2006). It consequently appears that the aetiopathogenesis of flexural limb deformities is multifactorial (Kidd, 2019). The exact prevalence of flexural limb deformity is not known, but Crowe and Swerczek (1985) reported a prevalence of 33.2% for the “contracted foal syndrome”, i.e. a bilateral static flexion (“contraction”) of the appendicular fore- or hindlimb joints or both. Moreover, they reported a prevalence of 20% for “miscellaneous limb contracture” in a sample of 608 foetuses and newborn foals submitted for *post-mortem*-examination. Another study conducted on a cohort of thoroughbred foals in New Zealand found that 28% were affected by hyperflexion (“contracture”) and 43% by hyperextension (“laxity”) (Shotton et al., 2015).

The *diagnosis of flexural deformities* is made by clinical examination, and radiographic examination is rarely needed (Kidd and Barr, 2002). In acquired flexural deformities, radiographs can, however, help identify secondary changes that affect prognosis (Wagner et al., 1982). Severe congenital flexural deformities can cause dystocia (Juzwiak et al., 1990), and the inability to stand can be an early and vital clinical finding in foals with pronounced deformities. While congenital deformities are present from birth, acquired deformities usually appear between 1 and 4 months after birth in the DIP joint and between 1 and 6 months after birth, and up to 18, months in the metacarpophalangeal joints (Kidd, 2019). Several gradings have been developed for acquired deformities of the DIP joint and MCP/MTP joints. Deformities of the DIP joint can be classified into stage I and II. Foals with stage I deformities have a dorsal hoof wall with an angle between the dorsal hoof wall and the sole (ground) of 60-90°. In stage II deformities, the dorsal hoof wall is beyond the vertical plane, forming an angle with the ground of greater than 90°. Deformities of the MCP/MTP joints are divided into 3 grades (Wagner et al., 1985): In mild deformities (grade 1),

the dorsal joint angle is smaller than 180°. In moderate deformities (grade 2), the dorsal joint angle exceeds 180°, with the MCP/MTP joint positioned dorsally relative to the digit. When walking, however, the MCP/MTP may extend caudally and assume a position palmar/plantar relative to the digit. In severe deformities (grade 3), the dorsal joint angle is greater than 180° under all circumstances.

The *treatment of flexural deformities* associated with hyperflexion is multimodal and, in the first line, conservative. It includes reduced exercise, analgesia, limb immobilisation with splints or casts, corrective hoof trimming or glue-on rubber shoes in older foals, and the use of intravenous OTC. In acquired cases, nutrition should also be considered by reducing the energy content and meeting calcium and phosphorus needs (Kidd and Barr, 2002). Surgical options exist for severe cases or cases which do not respond to conservative therapy. For deformities of the DIP joint, surgical options include desmotomy of the ALDDFT under general anaesthesia (Kidd, 2019), ultrasound-guided desmotomy of this ligament in the standing horse (White, 1995) or tenotomy of the DDFT for stage II deformities (Stick et al., 1992). For deformities of the MCP/MTP joints, palpation can determine whether the superficial or deep flexor system is most under tension, but it is not always straightforward. For mild deformities, desmotomy of the ALDDFT or/and of the accessory ligament of the superficial digital flexor tendon (ALSDFT) has been successful (Blackwell, 1980). Several techniques have been described for desmotomy of the ALSDFT, including standard approaches either cranial to the flexor carpi radialis muscle (Hunt, 2011; Jann et al., 1986) or through the flexor carpi radialis tendon sheath (Bramlage, 1987). A tenoscopic approach has also been developed (Southwood et al., 1999). For severe deformities both desmotomies can be performed in conjunction with a tenotomy of the SDFT (Adams and Santchi, 2000) with the goal to return to a dorsal joint angle below 180°.

The prognosis for flexural deformities with hyperflexion of the MCP/MTP and DIP joints depends on the severity of the deformity (Kidd, 2019). The prognosis for correction of mild to moderate congenital flexural deformities is good with conservative therapy (Kidd, 2019). Acquired stage I deformities of the DIP joint have a better prognosis than those classified as stage II (McIlwraith and Fessler, 1978). Earlier initiation of the treatment seems associated with a better