Anatomia, Histologia, Embryologia

WILEY

ORIGINAL ARTICLE

Histological Study of the Normal Proximal Third Interosseus Muscle Enthesis in the Equine Pelvic Limb

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Received: 25 June 2025 | **Revised:** 14 July 2025 | **Accepted:** 16 July 2025

Keywords: enthesis organ | fibrocartilaginous | fibrochondrocyte | horse | ligament-to-bone insertion | proximal suspensory ligament

ABSTRACT

In human medicine, the enthesis, or tendon-to-bone insertion, is generally considered a linked entity or 'enthesis organ' and plays a crucial biomechanical role. This study aimed to histologically assess the equine proximal third interosseus muscle (suspensory ligament) enthesis in 10 pelvic limbs from 7 horses. The area of the proximal third interosseus muscle enthesis was divided into 6 compartments based on post-mortem computed tomography and prepared for histology using haematoxylin/eosin and trichrome staining. The proximal third interosseus muscle enthesis showed a fibrocartilaginous nature. All adult entheses exhibited a characteristic four-layered structure consisting of ligament, uncalcified fibrocartilage, calcified fibrocartilage and bone. Juvenile specimens lacked clear layering, suggesting ongoing endochondral ossification. One or more smooth, regular tidemarks separated the uncalcified and calcified fibrocartilage, while the margins separating the ligament and bone from the fibrocartilage layer showed pronounced irregular interdigitations. Mean cellularity, fibrocartilage thickness and interdigitation size and shape varied among specimens. The fibrocartilaginous nature of the proximal third interosseus muscle enthesis in the equine pelvic limb supports the role of a stress dissipation mechanism and may have implications in the biomechanics and pathology of proximal third interosseus muscle enthesis injuries.

1 | Introduction

The third interosseus muscle (suspensory ligament, TIOM) is a heterogeneous and complex ligament containing collagen bundles interspersed with fat, muscle, nerve and vascular tissues (Alsook et al. 2013; Guest et al. 2024; Souza et al. 2010; Schramme et al. 2012). The TIOM is a common site of injury in horses, affecting both thoracic and pelvic limbs regardless of activity (Dyson et al. 1995; Gruyaert et al. 2019; Guest et al. 2024; Murray et al. 2006), with certain disciplines and conformations – such as dressage and a larger tarsal angle – predisposing to disease in the pelvic limb (Dyson 1994; Murray et al. 2006; Routh et al. 2019). Proximal TIOM disease in the pelvic limb – more often chronic, involving bone and associated with a more guarded prognosis for return (Dyson 1994, 2023; Dyson et al. 1995; Routh et al. 2019) – remains a diagnostic and therapeutic challenge (Dyson 2003; Labens et al. 2010). Its variable clinical presentations (Dyson et al. 2017; Dyson 2006) and inconsistent responses to diagnostic anaesthesia (Contino et al. 2015; Hinnigan et al. 2014) are further complicated by individual variations in ligament and bone involvement, frequently necessitating multimodal imaging for accurate evaluation (Dyson et al. 2017, 2018; Dyson et al. 2006; Hinkle et al. 2023; Labens et al. 2010; Müller et al. 2023; Werpy and Denoix 2012) and patient-specific treatment (Bathe 2006; Dyson and Murray 2012; Horne and Redding 2025).

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In humans, the enthesis, or tendon-to-bone insertion, is indeed regarded as a unified structure known as the 'enthesis-organ'. This concept describes a collection of tissues adjacent to the enthesis that function together to dissipate stress (Benjamin, Moriggl, et al. 2004; Benjamin et al. 2006). Entheses are classified as either fibrous or fibrocartilaginous, depending on their composition (Apostolakos et al. 2014; Benjamin et al. 2002; Benjamin, Moriggl, et al. 2004; Benjamin et al. 2006; François et al. 2001; Kehl et al. 2016). Fibrous entheses are relatively sparse, broadly fanned out and attach directly to bone ('bony') or periosteum ('periosteal') via fibrous tissue, resembling ligamentous structures. They are characterised by perforating mineralised collagen fibres (Sharpey's fibres) and are typically found at the metaphysis or diaphysis of long bones where the insertion angle changes minimally during movement (Apostolakos et al. 2014; Benjamin et al. 2002). Fibrocartilaginous entheses are more common (Apostolakos et al. 2014; Benjamin et al. 2002), small and attach to bone through a fibrocartilage layer that serves as a transitional layer between tendon and bone. These fibrocartilaginous entheses consist of four distinct layers with a progressive mineralisation gradient and varying mechanical properties (tendon, uncalcified fibrocartilage, calcified fibrocartilage and bone) (Apostolakos et al. 2014; Benjamin et al. 2002). They are typically located at epiphyseal and apophyseal insertions, where a broad range of motion and significant variation in insertional angle are required (Apostolakos et al. 2014; Benjamin, Moriggl, et al. 2004; Benjamin et al. 2006; François et al. 2001; Kehl et al. 2016).

Several studies in humans have examined the structure–function relationship of the enthesis organ and its biomechanical role in lesion development (Benjamin, Moriggl, et al. 2004; Benjamin et al. 2006; Kehl et al. 2016). Histology of the TIOM in the equine pelvic limb has mainly focused on the ligament itself (Alsook et al. 2013; Dyson et al. 2017, 2018; Schramme et al. 2012; Souza et al. 2010) and, except for a morphological imaging study on the proximal third metatarsal bone surface at the TIOM enthesis (Dancot et al. 2023), dedicated research is lacking. This study aimed to characterise the histological structure of the proximal TIOM enthesis in a series of isolated normal equine pelvic limbs.

2 | Materials and Methods

Ten pelvic limbs (5 right and 5 left) of 7 horses (6 adults and 1 foal) without a history of lameness were disarticulated at the tarsocrural joint. Prior to freezing, each limb was assessed using a standardised low-field magnetic resonance imaging (MRI; Hallmarq Veterinary Imaging Ltd., Guildford, Surrey, United Kingdom) protocol to verify the absence of detectable abnormalities. Normalcy was defined based on previously established MRI criteria for the proximal TIOM and third metatarsal bone enthesis (Bischofberger et al. 2006). Only limbs from client-owned animals, for which consent had been provided for the use of their bodies, euthanised for reasons unrelated to the study, were used, and ethics approval was not required.

Post-mortem computed tomographic (CT) examination (Siemens, Somatom Sensation 16, Erlangen, Germany) was used to divide the TIOM enthesis into six compartments (proximal and distal, each with lateral, sagittal and medial sections, Figure 1). To facilitate later histological slice positioning, the distance from each slice to the third–fourth tarsal/metatarsal bone interface – used as the entry point for a needle inserted into the tarsometatarsal joint via a plantarolateral approach on the isolated limbs – was measured for each pelvic limb.

On the frozen limb, the TIOM enthesis was then sectioned accordingly into six specimens using two horizontal slices, each divided into three equal parts (lateral, sagittal and medial). Respective cutting lines were determined by needle localisation of the tarsometatarsal joint via the plantarolateral approach and a tape measure, first for transverse and subsequently for sagittal cuts. Each specimen contained the macroscopically visible TIOM enthesis and measured approximately 15 × 10 mm (height × width).

FIGURE 1 | Dorsal and sagittal multiplanar reconstruction of the proximal aspect of the third metatarsal bone. Cutting lines for histological specimens (left), determined based on CT measurements, are indicated by double arrows. The insert (right) provides a reference for histological slice orientation.

Specimens were fixed and stored in 10% neutral buffered formalin for 24h, underwent decalcification in 10% formic acid containing 0.5% formalin for 72h, followed by 7days of immersion in the same solution with the addition of 5% phenol prior to paraffin embedding. Microtome sections were then prepared (approximately 5μm) and stained with haematoxylin–eosin (HE). After initial microscopic assessment (Olympus BX51, Evident, Tokyo, Japan and Toupcam camera with Toup-Lite software, Touptek, Hangzhou, China) of slice quality, six additional specimens were obtained from 6 limbs (4 adult horses and 1 foal) and prepared for trichrome (TC) staining. All microscopic examinations were conducted by a board-certified specialist of the European College of Veterinary Diagnostic Imaging (ZJ) and reviewed by a board-certified specialist of the European College of Veterinary Pathology (MMG).

The 96 specimens (60 HE and 36 TC slices) were histologically evaluated for their structural characteristics (Benjamin et al. 2002; Claudepierre and Voisin 2005; François et al. 2001; Shaw 2007), such as cell type (fibrocytes, fibrochondrocytes, osteocytes), cellularity and cellular organisation (disorganised, columnar), ligament fibre orientation (parallel, anisotropic), content of adipomuscular bundles and presence of Sharpey's fibres. The presence, number, shape and size of both ligamentous and bony interdigitations and tidemarks were recorded.

3 | Results

3.1 | Horses

The horses (5 warmbloods and 2 arabians) comprised 3 stallions, 3 mares and 1 gelding. There were 6 adults with a median age of 4years (3 to 18years) and median weight of 520kg (386 to 635kg), along with 1 foal (3days old, approximately 40kg in weight).

3.2 | Histological Specimens

In HE slices, the ligament-to-bone insertion was identifiable in 49 of 60 slices. The 11 slices lacking the ligament-to-bone enthesis were either too distal (2/11), located at the level of the adipomuscular bundle (7/11), or affected by preparation artefacts (2/11). Of the 49 HE specimens containing the enthesis, 40 were from 6 adult horses and 9 from the foal. In the TC slices, the ligament-to-bone insertion was available in 33 of 36 slices. The 3 slices lacking the TIOM enthesis (2 from adults, 1 from the foal) were affected by preparation artefacts. Of the 33 TC specimens containing the enthesis, 28 were from 4 adult horses and 5 from the foal.

3.3 | Histological Organisation of the Adult Specimens

In the adult specimens (40/49 HE slices and 28/33 TC slices), the ligament-to-bone entheses exhibited a consistently organised 4-layered structure comprising ligamentous collagen fibres, uncalcified fibrocartilage, calcified fibrocartilage and compact bone (Figure 2a,b).

3.3.1 | Ligament

The TIOM was generally hypocellular with ligament fibres oriented parallel or relatively parallel to the plantar third metatarsal cortex in 28 of 40 HE slices. In 12 of 40 HE slices, collagen fibres showed anisotropy, with some fibres inserting at a more oblique or perpendicular angle to the cortex (Figure 3d). The dorsal ligamentous outlines were mostly irregular, with variable interdigitations where continuous collagen fibres connected superficially or deeply to the collagen matrix of bone (Figure 3g–i). Apart from ligamentous collagen tissue, vascular structures, adipocytes and in some cases nerves and muscle tissue were observed within adipomuscular bundles. In 9/40 slices, both the ligamentous and adipomuscular entheses were visible. In regions where an adipomuscular bundle, rather than ligament fibres, contacted the bone, the fibrocartilaginous layer was absent (Figure 3f). Instead, although often detached during preparation, a thin periosteum-like layer was visible, with occasional fibroblasts, suggesting a more fibrous enthesis. In 6 out of these 9 specimens, multiple tidemarks were visible in the ligamentous part of the enthesis (4/6 triple and 2/6 double tidemarks).

3.3.2 | Fibrocartilage

In all 40 HE slices, the fibrocartilage layer had multiple aligned columns of chondrocyte-like cells, presumably (fibro)chondrocytes (Figure 2c,d), with a clear basophilic tidemark (or ossification front) demarcating the calcified and uncalcified fibrocartilage layers. (Fibro)chondrocytes appeared as round to oval cells with dark nuclei embedded in lacunae within the uncalcified fibrocartilage layer, gradually increasing in hypertrophy and developing more open nuclei toward the calcified fibrocartilage layer. Both thickness and cellularity of the uncalcified and calcified fibrocartilage layers varied among compartments and individuals (Figure 3a–c).

The tidemark was smooth, minimally curved and thin in most specimens (38/40), with only 2 specimens (2/40) exhibiting a more irregular and thickened tidemark. A single tidemark was observed in 22/40 slices, while 2 (5/18) or 3 (13/18) relatively parallel tidemarks were present in the remaining 18/40 slices (Figure 3d–f). Triple tidemark multiplication was consistently seen in all older specimens ($\geq$8years, 7/13), though both double (5/5) and triple (6/13) tidemarks were also identified in some younger subjects ($<$8years). Tidemark multiplication predominantly occurred in the distal half of the enthesis (11/18). No vessels or nerves were seen crossing the fibrocartilage or tidemark.

3.3.3 | Bone

In all the 40 HE slices, the plantar aspect of the third metatarsal bone at the enthesis consisted of lamellar compact bone and deeper trabecular bone. In 13 of 40 HE slices, a discrete thin interrupted basophilic cement line separated the calcified fibrocartilage from the underlying lamellar bone (fig3i). No bundle bone and/or Sharpey's fibres were observed. The plantar cortical outline was irregular with multiple interdigitations. The depth, width and number of these bony interdigitations varied among and within individuals (Figure 3g–i).

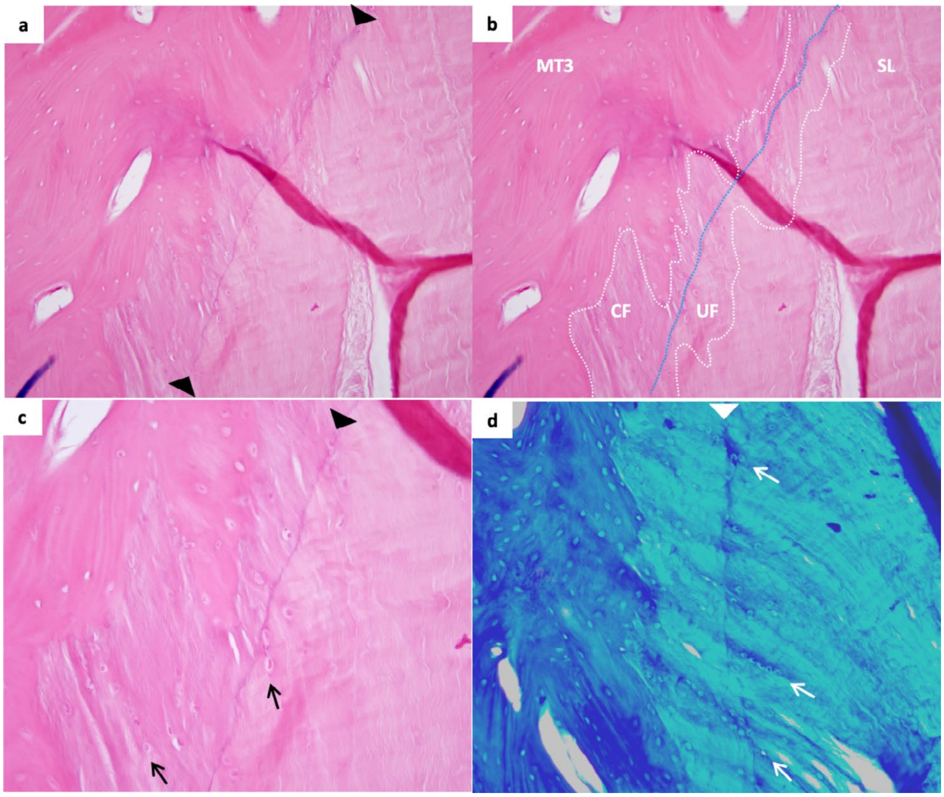

FIGURE 2 | Typical 4-layered appearance of fibrocartilaginous entheses of the proximal TIOM enthesis in the equine pelvic limb. (a) Native histological section and (b) corresponding illustrated detail of the enthesis (HE, 200×) showing the plantar aspect of the third metatarsal bone (MT3), calcified fibrocartilage (CF), uncalcified fibrocartilage (UF) and ligament fibres (SL). The tidemark (a: arrowheads, b: blue line) separates the CF and UF and is generally smooth. The cortical and/or ligamentous outline (b: white lines) show multiple variable interdigitations. (c and d) Higher magnification of the fibrocartilage layer (c: HE 400×; d: TC 200×) highlighting multiple aligned columns of fibrochondrocytes (arrows).

3.4 | Histological Evaluation of Juvenile Specimens

In the specimens from the foal (9/49 HE slices and 5/33 TC slices), a markedly different morphology was observed (Figure 4a). Cell density of all tissues was higher, and layer demarcation was indistinct. The fibrocartilage layer was wide and contained multiple randomly organised chondrocytes without a clear columnar orientation. Gradual ossification of the fibrocartilage was absent, with no calcified fibrocartilage layer or tidemark. At the bony side of the enthesis, multiple vascular structures were present, surrounded by ring-like homogeneous eosinophilic bone matrix deposits (Figure 4b). Ligament fibre orientation was absent and ligament-to-bone interdigitations were not visible.

4 | Discussion

This study demonstrates that, in horses, the proximal enthesis of the pelvic TIOM is fibrocartilaginous in nature. This finding aligns with a previous single-subject animal study conducted as part of a human medicine thesis (Shaw 2007). Fibrocartilaginous entheses are typically found in biomechanically complex structures, where their heterogeneous morphology, characterised by gradual ossification and progressive transition in tissue type, is considered to play a key role in stress dissipation from tendon to bone (Shaw 2007; Spalazzi et al. 2013). The concentration of stress at the hard–soft tissue interface makes entheses particularly vulnerable to acute or overload sports injuries, especially in sports with high ground impact forces (Benjamin, Moriggl, et al. 2004; Benjamin et al. 2006; Kehl et al. 2016). Given that the equine pelvic TIOM withstands significant mechanical loading during movements involving marked fetlock extension – such as extended gaits in dressage, takeoff and landing in jumping, and high-speed galloping in racing – its fibrocartilaginous organisation is thus unsurprising (Barone and Simoens 2010a, 2010b; Budras et al. 2009; Denoix 1994; Murray et al. 2006; Schramme et al. 2012; Walker et al. 2017). Fibrocartilaginous entheses are commonly located near epiphyses or on rugged bony ridges, particularly where a variable insertional angle is required (Apostolakos et al. 2014; Benjamin, Moriggl, et al. 2004). A previous study demonstrated that, even in normal horses, the plantaroproximal aspect of the third metatarsal bone exhibits an uneven surface, characterised by several macroscopic longitudinal ridges visible on both computed tomography and radiography (Dancot et al. 2023).

The type of enthesis is related to the mechanism of bone development (endochondral or intramembranous ossification) (François et al. 2001; Shaw and Benjamin 2007). Fibrous entheses are more common in bones developing from a fibrous primordium (membranous bone, as seen in diaphyseal circumferential growth), whereas fibrocartilaginous entheses are associated with bones formed from a chondroid precursor (endochondral bone, as seen in metaphyseal and epiphyseal length growth) (François et al. 2001; Shaw and Benjamin 2007).

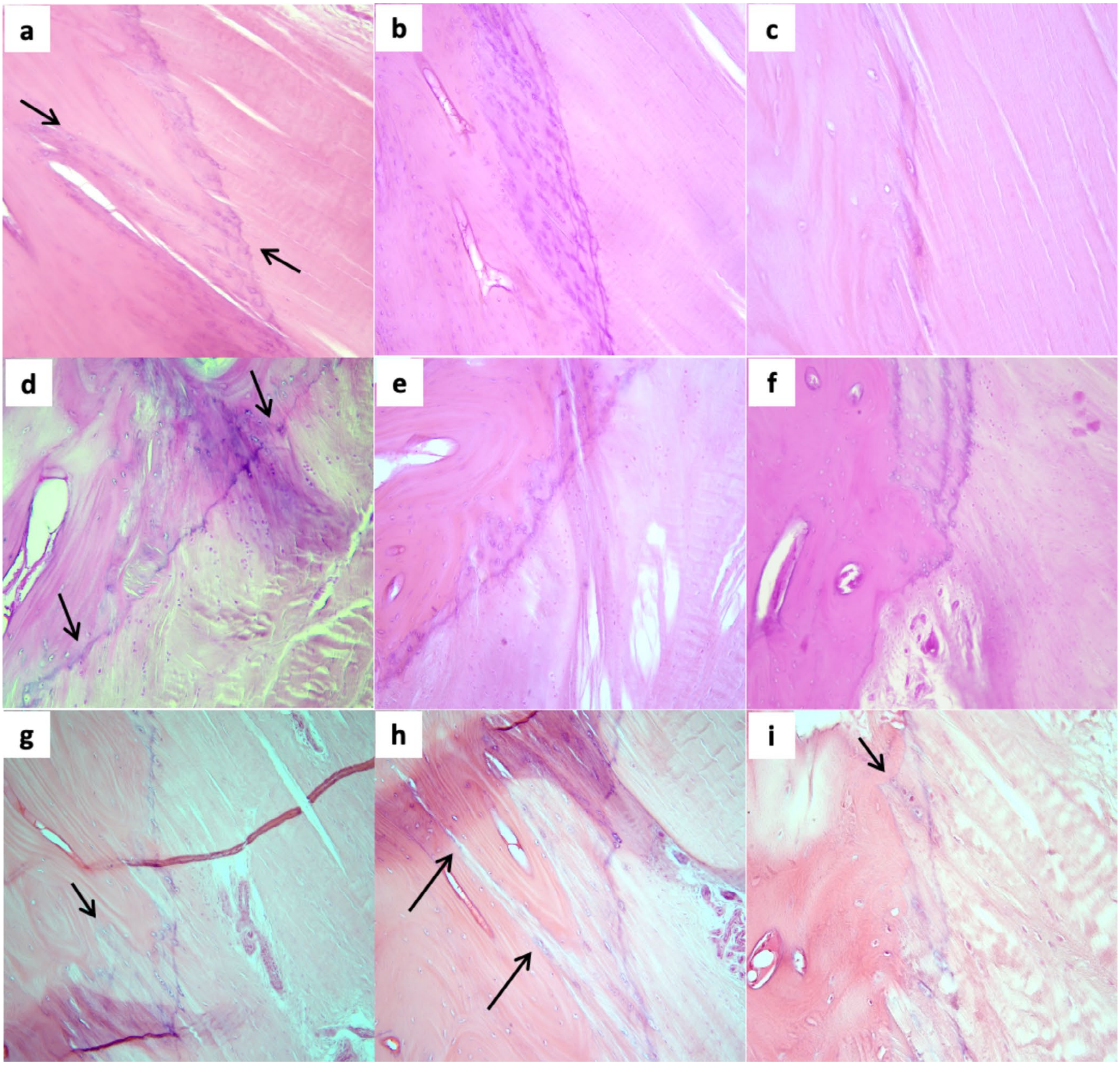

FIGURE 3 | Histological features of the equine pelvic TIOM enthesis in adult specimens (HE, a–h: 200×, i: 400×). (a–c) Significant variation in fibrocartilage layer cellularity and thickness was found among specimens and within individuals, ranging from strongly cellular and thick (b) to nearly completely acellular and barely noticeable (c). Note the distinct columnar orientation of the fibrochondrocytes (a, arrows). (d–f) In most specimens, a single tidemark was visible (d, arrows), though a subset exhibited 2 or more largely parallel tidemarks (e and f). Even when multiple tidemarks were present, they remained generally straight. TIOM ligament fibre anisotropism was common; note the parallel insertional angle of the ligament fibres in the distal half of (d), compared to the more perpendicular orientation in the proximal half. In regions where the adipomuscular bundle contacted the third metatarsal bone (f), a fibrocartilaginous layer was absent. The plantar cortical outline was delineated by a thin periosteum, with occasional fibroblasts suggestive of a fibrous enthesis. (g–i) Both cortical and ligamentous outlines showed high variability; yet all displayed interdigitations ranging from thick and short (g) to thin and long (h). In some specimens, a faint basophilic cement line was visible, separating the calcified fibrocartilage layer from the underlying cortical bone (i, arrow).

Fibrocartilaginous entheses share structural similarities with the attachment of hyaline articular cartilage and are sometimes regarded as arrested growth plates with the potential for re-activation throughout life (Benjamin et al. 2000). They have a characteristic 4-layered architecture with a progressive mineralisation gradient from ligament to bone. Within this structure, chondrocytes (often referred to as fibrocartilage cells or fibrochondrocytes) are arranged in columns that extend from the uncalcified to the calcified fibrocartilage layer (Benjamin et al. 1986; Benjamin et al. 2006). However, this columnar organisation is not continuously present throughout the enthesis; rather, it represents remnants of the fibroblast orientation from which the fibrocartilage cells differentiate through metaplasia (Benjamin et al. 2000, 2006; Gao et al. 1996). This distinct

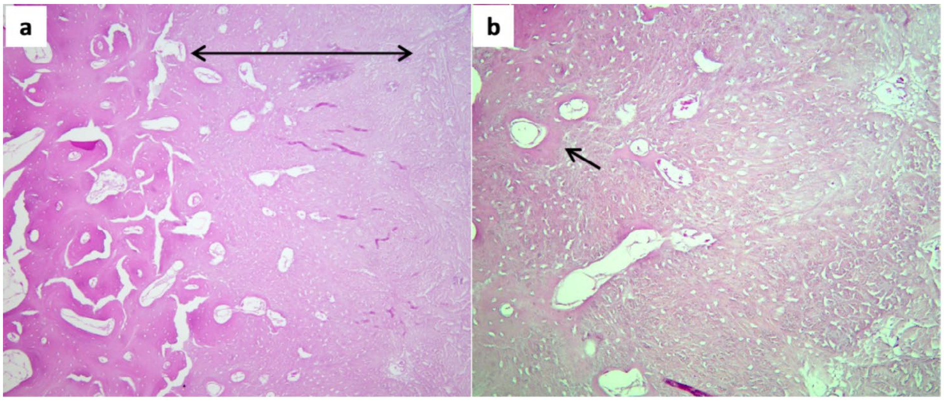

FIGURE 4 | Histological features of the equine pelvic TIOM enthesis in a juvenile specimen (HE, a: 200×, b: 400×). (a) The fibrocartilage layer or cartilage precursor (double arrow) is notably wide, with diffusely dispersed chondrocytes and a general lack of stress-oriented organisation. (b) Bone matrix deposition surrounds the vascular structures, indicating ongoing ossification. A calcified fibrocartilage layer and tidemark are not observed.

fibrocartilaginous structure was observed at all sites of TIOM insertion on the third metatarsal bone in our adult specimens. Although intra-ligamentous chondroid metaplasia is well documented in previous histological studies of pathological TIOM in the pelvic limb (Dyson 2010; Dyson et al. 2017, 2018), areas of chondroid metaplasia (pelvic limbs; Dyson 2006) or chondroid clusters (thoracic limbs; Nagy and Dyson 2012) at the bone-ligament interface of the normal equine TIOM have only been sporadically mentioned in transverse slices, but were not described in detail. In contrast, fibrocartilaginous entheses have been histologically detailed in horses at the distal insertion of the deep digital flexor tendon (Wulfen and Bowker 2002; Blunden et al. 2006a, 2006b) and on the proximal sesamoid bones (Okudaira et al. 2024). Similar to the present study, the deep digital flexor tendon fibrocartilaginous enthesis in adult horses transitions from dense connective tissue to uncalcified fibrocartilage, then to calcified fibrocartilage as it flares to attach to the distal phalanx (Wulfen and Bowker 2002; Blunden et al. 2006a, 2006b). Recently, the podotrochlear apparatus, considered as a functional unit, has been conceptualised as a premier 'enthesis organ' that modulates mechanical stress across the various structures of the foot (Osborn et al. 2021). Fibrocartilaginous entheses have also been described in foals at the bone-ligament interfaces of the proximal sesamoid bones, with differences in maturation and morphology among the apical, flexor and basilar ossification fronts (Okudaira et al. 2024). Notably, the flexor enthesis differs from the apical and basilar entheses, suggesting that the mechanical environment may influence the developmental process. While fibrocartilage cells were visible by 2 days of age and a typical 4-layered structure by 240 days in the apical and basilar entheses, fibrocartilage cells in the flexor enthesis only appeared by 240 days and remained immature at 540 days of age.

Similar to the findings of Okudaira et al. (2024), a layered fibrocartilage architecture was entirely absent in the specimens obtained from the 3-day-old foal in the present study. During prenatal development, tendon fibres initially connect directly to the cartilaginous skeleton. The cartilage precursor is then progressively replaced by bone through centrifugal endochondral ossification following cartilage erosion and lacunar blood vessel invasion (Benjamin et al. 2000; François et al. 2001; Shaw and Benjamin 2007). In the foal of the present study, the wide uncalcified fibrocartilage-like layer likely corresponds, at least partially, to this cartilage precursor, with perivascular ossification fronts on the bone side suggesting ongoing ossification (Figure 4), similar to fibrocartilaginous enthesis formation observed in immature rats (Benjamin et al. 2000). For the fibrocartilaginous enthesis to form, some cartilage must persist as a transitional layer of uncalcified fibrocartilage, maintained by fibrochondrocytes on the tendon side. Fibrochondrocytes deeper within the fibrocartilaginous enthesis will gradually lose cell–cell contact, hypertrophy and calcify over time, with this calcified fibrocartilage ultimately providing a strong attachment to bone (Benjamin et al. 1986, 2000, 2006; François et al. 2001; Shaw and Benjamin 2007). The absence of a distinct 4-layered fibrocartilage structure in our juvenile specimens supports the hypothesis that biomechanical stress influences fibrochondrocyte orientation and, more broadly, ligament-to-bone insertion. In mice, studies have demonstrated that limb loading is necessary for functional enthesis development, with the absence of mechanical load resulting in altered fibrocartilage progenitor cell differentiation with reduced fibrocartilage ossification and overall biomechanical strength (Schwartz et al. 2013; Schwartz et al. 2014).

The biomechanical strength of a fibrocartilaginous enthesis is partially attributed to the 'irregular interlocking shape' of ligament fibres extending between bony lacunae, as well as to a denser cement line connecting the calcified fibrocartilage to the underlying compact bone (François et al. 2001; Shaw and Benjamin 2007). The ligament and bone insertions in the specimens of the present study exhibited marked irregularity, with pronounced (so-called) interlocking, interweaving or interdigitations. During the development of the fibrocartilaginous enthesis of the Achilles tendon of rats, this interlocking pattern of ligament fibres and bone spurs is formed as blood vessels invade and tunnel through the columnar fibrocartilage cells, around which bone is subsequently deposited, leading to the formation of a bone spur (Benjamin et al. 2000). This interlocking aspect is believed to increase the cross-sectional area and overall strength of the enthesis, like how tree roots anchor in the ground (Benjamin et al. 2000, 2006; François et al. 2001). A similar interlocking or interdigitating pattern has also been noticed in the distal fibrocartilaginous enthesis of the deep digital flexor tendon and distal sesamoidean impar ligament in the horse

(Wulfen and Bowker 2002). An interrupted cement line at the calcified fibrocartilage-bone interface was observed in a subset of our specimens (Figure 3i), but was not universally present.

The continuity of collagen fibres from ligament to bone is primarily evident at the tidemark separating uncalcified and calcified fibrocartilage layers (Benjamin et al. 2006). This tidemark, which becomes visible following decalcification, was clearly seen in our adult population as a generally smooth line between the uncalcified and calcified fibrocartilage layers as opposed to the marked irregular outline of the ligament and bone interdigitations (Benjamin et al. 1986, 2006; François et al. 2001). It is thought that the smooth outline of the tidemark forms during mineralisation to protect the soft tissues from mechanical damage at the hard and soft tissue interface (Apostolakos et al. 2014). It is also likely this tidemark that is seen in imaging of the normal equine TIOM enthesis as the generally smooth sloping plantar third metatarsal cortical outline between the longitudinal cortical ridges (Benjamin et al. 2002; Benjamin et al. 2008; Dancot et al. 2023). A double or triple tidemark was observed in several specimens. In osteoarthritis, tidemark multiplication has been proposed as an adaptive response to abruptly altered mechanical loading, wherein a former tidemark becomes buried and a new tidemark forms at a higher level (Roudier et al. 2013). However, tidemark multiplication has also been reported as a normal feature in multiple species and as a potential artefact due to slice obliquity during microscopy (Bonde et al. 2005).

Unlike the area of ligament enthesis, the regions where the adipomuscular bundle abutted the third metatarsal bone lacked a fibrocartilage layer. Although most adipomuscular bundles were separated from the bone due to the decalcification process (Mandal et al. 2025) – making interpretation challenging – a thin periosteum-like layer was visible, suggesting that the adipomuscular bundles may have a more fibrous periosteal enthesis (Benjamin et al. 2002). Such fibrous entheseal areas, void of fibrocartilage, are known to occur in fibrocartilaginous entheses (Benjamin et al. 1986; Gao et al. 1996), often near the most superficial or distal regions or near muscular or fatty tissue components, and likely reflect regional differences in tensile strength and loading (Benjamin, Redman, et al. 2004). Additionally, in most specimens where both the ligament and adipomuscular bundle entheses were visible, the fibrocartilaginous enthesis of the ligamentous part displayed multiple tidemarks forming a bone spur or ridge over the adjacent adipomuscular bundle (Figure 3f). This may represent an adaptive response to accommodate differences in entheseal strength (Benjamin, Redman, et al. 2004) and likely corresponds to the longitudinal third metatarsal bony ridges detected in normal horses facing the adipomuscular bundles (Dancot et al. 2023). This hypothesis is further supported by the presence of tidemark duplication in both young and older horses, with the highest prevalence in the distal part of the enthesis in this study population.

Finally, fibrocartilage thickness is known to adapt in response to biomechanical load and often varies within an individual enthesis (Benjamin and Ralphs 1998), a pattern also observed in our study population, where the fibrocartilage ranged from a single cell to several cell layers width. Notably, none of our specimens showed vascular structures crossing the fibrocartilage. This aligns with prior findings (François et al. 2001; Shaw and Benjamin 2007) indicating that the uncalcified fibrocartilage layer serves as a vascular and cellular barrier, reducing compressional wear and tear similar to articular cartilage. This avascularity also contributes to the overall poor healing capacity of the enthesis organ and may play a role in preventing bony spur formation, which often occurs along vascular in-growth tracts, to expand the load-bearing surface (Apostolakos et al. 2014; Benjamin et al. 2006).

Although this study provides a detailed description of the fibrocartilaginous nature of the enthesis, the small sample size remains a notable limitation – particularly due to the low number of individuals within each age and weight group and the inclusion of only one foal – making it difficult to draw definitive conclusions related to age and weight. Furthermore, the small sample size restricts the ability to assess the influence of sport, discipline and/or workload on enthesis development. Future research should aim to detail the developmental stages of the TIOM enthesis across different ages and explore its correlation with weight, limb conformation and biomechanics in horses exposed to varying training environments and workloads.

5 | Conclusion

The equine pelvic TIOM enthesis is fibrocartilaginous in nature. Since fibrocartilaginous entheses serve as complex stress dissipators, this finding is biomechanically plausible in a region exposed to high mechanical load, such as the proximal TIOM–a structure frequently affected by enthesopathy.

Acknowledgements

This research is part of a doctoral thesis at the Faculty of Veterinary Medicine, University of Liège, Belgium.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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