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Original Article

Functional anatomy of the equine temporomandibular joint: Histological characteristics of the articular surfaces and underlining tissues

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ABSTRACT

It has been assumed that dental conditions cause disorders of the equine temporomandibular joint (TMJ), due to biomechanical overload or aberrant loading. However, the incidence of published TMJ disorders in horses is low and this leads to the question whether the equine TMJ is adapted well to its biomechanical requirements or is able to remodel its articular surfaces in response to modified loading conditions. The aim of this study was to determine the histological characteristics of healthy equine TMJs. The tissue components of the articular surfaces of 10 TMJs obtained from horses without any clinical history of dental or TMJ disorders were analysed. Apart from the mandibular fossa of the temporal bone, the osseous aspects of the TMJ exhibited a uniform zoning pattern. The articular surfaces were composed of three tissue layers: (1) a superficial cell-rich dense connective tissue layer; (2) a middle fibrocartilage layer; and (3) a deep hyaline-like cartilage layer. The articular disc was composed of an inner core of fibrocartilage and hyaline-like cartilage meshwork covered with both cell-rich dense connective tissue and fibrocartilage at its dorsal and ventral aspects. In contrast, the mandibular fossa was only covered by a dense connective tissue, frequently supplemented by a synovial membrane, suggesting low biomechanical stress. Glycosaminoglycans, which are indicative of compressive loads, were predominantly present within the rostral part of the articular tubercle and the retroarticular process, the dorsal part of articular disc and the entire mandibular head, but were absent within the mandibular fossa. The results of this study suggest the presence of different biomechanical demands in the dorsal and ventral compartment of the equine TMJ.

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Introduction

The macroscopic anatomy of the equine temporomandibular joint (TMJ) has been described in detail (May et al., 2001; Rodríguez et al., 2006, 2007, 2008, 2010) but, apart from an anecdotal report including a histopathological investigation (Smyth et al., 2017), no histological data have been reported for the equine TMJ. In a previous study, we elucidated the collagen fibre arrangement of the articular surfaces in the healthy equine TMJ (Adams et al., 2016). However, there was a need to supplement these data by further histological descriptions of the tissue components in regions underlying the articular surfaces.

The equine TMJ is a complex, incongruent, diarthrodial joint. Its temporal part is composed of the base of the zygomatic process of the temporal bone and consists of three portions: (1) the rostral articular tubercle; (2) the central mandibular fossa; and (3) the caudal retroarticular process. The ventral part of the TMJ is formed by the mandibular head on the condylar process. To compensate for the relative incongruity of the joint, a fibrocartilaginous articular disc is positioned between the bony components, dividing the TMJ into a dorsal discotemporal and a ventral discomandibular joint space (Rodríguez et al., 2006; Carmalt et al., 2016; Pereira et al., 2016).

The articular surfaces of the TMJ are covered with fibrocartilage rather than hyaline cartilage, as in other synovial joints of the human and equine locomotor apparatus (Milam, 2003; Wadhwa and Kapila, 2008; Carmalt et al., 2016). More detailed descriptions of the condylar cartilage in humans commonly emphasise the

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existence of four different tissue zones in the sagittal plane from dorsal to ventral (Wadhwa and Kapila, 2008; Singh and Detamore, 2009). The superficial fibrous (Singh and Detamore, 2009) or articular zone (Wadhwa and Kapila, 2008) is directly underlain by a cell-rich proliferative zone (Wadhwa and Kapila, 2008; Aryaei et al., 2016). The subjacent mature and hypertrophic zones both resemble hyaline-like cartilage, whereas the mature zone still contains proliferating cells (Wadhwa and Kapila, 2008; Singh and Detamore, 2009).

The articular disc has been subject of numerous studies in comparative (Kalpakci et al., 2011) and species-specific studies, including human (Detamore and Athanasiou, 2003a; Allen and Athanasiou, 2006; Stanković et al., 2013), porcine (Almarza et al., 2006; Detamore et al., 2006; Murphy et al., 2013; Vapniarsky et al., 2017) and bovine (Landesberg et al., 1996; Tanaka et al., 2001) investigations, but has not been studied in equids. It has been shown that the tissue characteristics of the articular disc depend on species-specific anatomy, as well as diet. Herbivorous mammals, which exhibit marked translatory joint movements (Herring, 2003; Kalpakci et al., 2011), express higher contents of glycosaminoglycans (GAG) than omnivorous mammals, which show a combination of both translatory and rotatory joint movements. The high GAG content in the articular disc of herbivorous mammals has been correlated with highly compressive stiffness throughout all zones of the articular disc (Kalpakci et al., 2011). The tensile characteristics of the articular disc are correlated more closely with the orientation of collagen fibres than with the total amount of collagen fibres (Detamore and Athanasiou, 2003b; Kalpakci et al., 2011; Vapniarsky et al., 2017).

A detailed knowledge of the ultrastructural features of the healthy TMJ in human beings and pigs has contributed to a thorough understanding of structure-function relationships and TMJ biomechanics in these species (Detamore and Athanasiou, 2003a; Detamore et al., 2005; Vapniarsky et al., 2017). The aim of the present study was to determine the histomorphological characteristics of the healthy equine TMJ in order to provide a basis for future investigations focused on pathological changes and regenerative capacities.

Materials and methods

Source of specimens

Ten TMJs (five left, five right) were obtained from five adult warmblood horses without any history of TMJ disorders or dental diseases. The horses had been euthanased for reasons unrelated to this study. The articular surfaces of the temporal bone (articular tubercle, mandibular fossa and retroarticular process), the condylar process of the mandible (mandibular head, caudomedial aspect of the mandibular head) and the articular disc were removed from each specimen for analysis. Dental arcades were examined and specimens showing dental disorders (missing teeth, displaced teeth, fractured teeth, widened interdental spaces, infundibular caries etc.) were excluded from the study. All TMJs were confirmed to be free of gross signs of degenerative joint disease (i.e. chondral lesions, ulcerations, hyperplasia, pannus and osteophytes) and were further processed for histological investigations, as described by Adams et al. (2016).

Histological examination

Histological specimens were sectioned by use of a diamond blade band saw with a water cooling system (Micro Bandsaw MBS 240/E, Proxxon). Three representative samples were dissected out of each mandibular head and each articular tubercle (Fig. 1a, b). Furthermore, from each TMJ, two samples were collected from the mandibular fossa (Fig. 1a) as well as one sample of the retroarticular process (Fig. 1a) and the caudomedial aspect of the mandibular head (Fig. 1b). Twelve histological specimens were obtained from each articular disc (Fig. 1c). Each specimen was decalcified in buffered ethylene diamine tetra-acetate (EDTA, pH 8.0) at room-temperature for 4–6 weeks, embedded in paraffin and sectioned at 5 μm in a rostrocaudal direction. The first section from each block was stained with safranin O, and the second section was stained by the elastica van Gieson method.

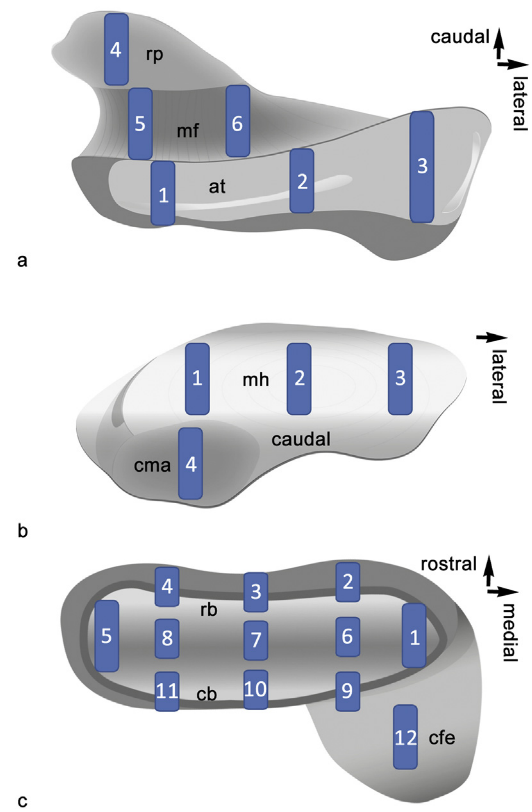

Fig. 1. Sampling pattern of the temporal part of the equine temporomandibular joint (TMJ). The numbered rectangles indicate the number and location of samples for histological investigations. (a) Ventral view: six specimens were collected from the temporal part of the TMJ; three specimens were collected from the articular tubercle (at), two specimens were collected from the mandibular fossa (mf) and one specimen was collected from the retroarticular process (rp). (b) Mandibular head and its caudomedial aspect from a dorsal view: three specimens were collected from the mandibular head (mh) and one specimen was collected from its caudomedial aspect (cma). (c) Articular disc from a ventral view: three specimens were collected from the rostral border (rb), three specimens were collected from the caudal border (cb), five specimens were collected from the central part and one specimen was collected from the caudomedial fibrous expansion (cfe).

Sections stained with safranin O (Figs. 2 a, c and 3 a, c, e) were analysed according to a scoring system using nominal and ordinal parameters. In the nominal scale, the presence of different tissues was documented according to their specific histological characteristics (fibrous tissue, fibrocartilaginous tissue, hyaline-like cartilage and lamellar bone) and attributed to specific zones (fibrous zone, fibrocartilaginous zone, hyaline-like cartilage zone and lamellar bone). In a second step, the GAG content at each zone was analysed using a scoring system derived from protocols suggested by Rosenberg (1971), Reifenrath (2005) and Redöhl (2009). In the ordinal scale, the GAG content was classified according to staining intensity as absent (no colour), present (pale red) and abundant (dark red). Additionally, the cell density (sparsely distributed and densely packed; nominal scale) and fibre orientation (rostrocaudal, dorsoventral, oblique and varying; nominal scale) were recorded. The histological sections of the bony components of the TMJ were divided equally into rostral, intermediate and caudal parts on the basis of GAG content.

Sections stained with elastica van Gieson (Figs. 2 b, d and 3 b, d, f) were analysed for the occurrence of elastic fibres (present and absent; nominal scale) and the orientation of elastic fibres (parallel to collagen fibres and non-parallel to collagen fibres; nominal scale). Additionally, the presence of blood vessels and nerve fibres within each zone was documented (nominal scale).

Statistical analysis

Statistical analyses were conducted using StatXact version 9.0.0 (Cytel). The absolute and relative frequencies of the outcomes of each criterion within equivalent specimens of the same sampling location were determined. The data were compared amongst specimens of the same bony components (sampling positions 1–4 of the mandibular head and its caudomedial aspects, Fig. 1b; sampling positions 1–4 of the articular tubercle and retroarticular process of the temporal part of the TMJ, Fig. 1a; sampling positions 5–6 of the mandibular fossa of the

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