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Impact of sex on osteoarthritis in horses

Diplomarbeit

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1. Introduction

Osteoarthritis (OA), or degenerative joint disease (DJD), is the most common musculoskeletal disorder in humans as well as in horses and is among the most important causes of pain, disability and health-related economic loss (Caron 2005, Mandl 2019). In horses, approximately 60 % of lameness are caused by OA (Frisbie 2005) with more than 50% of horses older than 15 years and 80 % to 90 % of horses older than 30 years suffering from the disease (van Weeren and Back 2016).

OA is a chronic disorder of synovial joints characterized by progressive degeneration of articular cartilage, sometimes resulting in a radiographic loss in joint space, intraarticular inflammation with synovitis and subchondral bone remodelling. (Goldring and Goldring 2007, Pool and Meagher 1990) Secondary to a variety of etiologic factors such as mechanical injury, genetics, ageing and obesity, a common molecular pathway linking biochemical and biomechanical processes leads to the typical pathological progression of OA with an imbalance of matrix synthesis and degradation and a vicious positive feedback loop involving cartilage breakdown and synovial inflammation (Loeser et al. 2012).

Clinically OA is characterized by varying degrees of lameness, the presence of synovial effusion, soft tissue swelling and heat, painful response to flexion and decreased range of motion (Jannson 1996, McIlwraith et al. 2012). To date, no treatment for OA is available, which disrupts this vicious circle and restores hyaline cartilage.

1.1. The intact joint

Osteoarthrosis is a complex disease affecting the entire joint. A synovial joint consists of two or more articulating bones, articular hyaline cartilage, the synovial fluid-filled joint cavity and the enclosing joint capsule (Clyne 1987).

Articular cartilage, an avascular, aneural tissue overlying the subchondral bone plate, consists of chondrocytes which are disseminated in a matrix of collagen type II fibres and an amorphous intercellular substance that consists of water, proteoglycans, glycosaminoglycans (for

example, hyaluronic acid) and non-collagenous proteins (Clyne 1987, Palmer and Bertone 1994). This matrix has a very high water-holding capacity, stability and elasticity (Clyne 1987). The chondrocytes, the only resident cells in the cartilage matrix, have low metabolic activity, survive under relatively hypoxic conditions and in the absence of vascular supply. They are responsible for cartilage metabolism and matrix synthesis but have only negligible regenerative capacity (Clyne 1987, Goldring and Goldring 2007). Hyaline cartilage is histologically and biochemically divided into four distinct layers that are named after the orientation of the chondrocyte population and the collagen fibre patterns of the matrix: the tangential, the transitional and the radial zones of uncalcified cartilage and the zone of calcified cartilage. The cells of the tangential zone form a thin layer, are immature and produce small strands of collagen; however, in the transitional zone, the cells are randomly arranged and synthesize proteins and proteoglycans. The chondrocytes of the deeper layers are arranged in small columns and are responsible for the enchondral ossification of the bone. The collagen fibres are arranged in two different ways: in the superficial layer, they are short, tightly packed, lie parallel to the surface and constitute the articular surface which withstands sheering processes; in the deeper layers, the fibres are collocated in an oblique criss-cross pattern to withstand compression (Clyne 1987).

The calcified cartilage forms, together with the immediately subjacent subchondral bone, the osteochondral unit. The subchondral bone plate consists of a cortical bone plate and cancellous bone, which serve as shock-absorber (Cucchiarini et al. 2016, Pool and Meagher 1990).

The joint capsule, which is, unlike the cartilage, well innervated and vascularised, consists of two different layers: a strong outer fibrous layer, which continues with the periosteum and contains the capsular ligament, and an inner layer, the synovial membrane, which produces the synovial fluid (Caron 1992, Clyne 1987, Pool and Meagher 1990).

The synovial fluid is a dialysate of plasma and is, among others, responsible for the nutrition of the articular cartilage. It contains hyaluronic acid, which is important for the lubrication of the joint, plasma electrolytes and plasma proteins (Clyne 1987).

1.2. Osteoarthritis – Epidemiology

OA is a multifactorial disorder, and there are many different possibilities to categorize it. Although none of them is fully satisfactory, each one contributes to the understanding of the disease.

Depending on the aetiology, primary and secondary OA can be differentiated. Primary OA has idiopathic, often age-related, causes which result in chronic deterioration of the normal joint structure and gradual loss of function. The lesions are often initially asymptomatic and may later result in painful, clinically apparent disease. In contrast, secondary OA develops in previously healthy joints after an injury. The injury may occur at any age and is followed by structural changes and clinical signs more or less rapidly depending on the nature and severity of the primary lesion. Causes of secondary OA include primary bone diseases such as osteochondrosis, osteochondritis or osteomalacia, poor conformation that results in abnormal loading, developmental disorders producing angular limb deformity, joint instability, acute inflammatory joint disease, joint infection and sudden or repetitive trauma (Clyne 1987, Pool and Meagher 1990).

Primary OA can be provoked or aggravated by so-called personal-level or systemic risk factors. Those include sociodemographic factors like age, race or sex, obesity, activity and genetics (Szoeke et al. 2006, Vina and Kwoh 2018). Whereas mechanical and heritable factors play a major role in the development of OA, the most important risk factor is increasing age. It results in softening of the articular surface and loss of the tensile strength and stiffness of the cartilage matrix (Goldring and Goldring 2007). Furthermore, as OA is not reversible, the prevalence of the disease increases with age (Symmons et al. 2006). Another important risk factor is obesity, which does not only affect the joints through increased mechanical load but also by causing systemic metabolic or inflammatory effects (Neogi and Zhang 2013). Genetics are also an important risk factor of OA – indeed, there is even a 40- 65% heritable component (in humans). However, not only the risk of developing OA but also the resulting pain may have genetic contributions (Cucchiarini et al. 2016, Neogi and Zhang 2013).

Secondary OA is generated by joint-level risk factors including bone and joint shape, muscle strength, joint malalignment, participation in certain sports, the structural integrity of the joint environment (like for example, meniscal damage), prior joint trauma or surgery and abnormal loading of the joints (Hunter and Felson 2006, Vina and Kwoh 2018).

Physical activity may have beneficial as well as adverse effects on the development and progression of OA. It may help by stabilizing the joints and strengthening periarticular muscles. Muscle weakness and atrophy can occur as a consequence of OA due to pain avoidance and following disuse of the joint, but it is not clear if it is also a risk factor in the formation or if it only facilitates the progression of the disease. On the other hand, activity may be a risk factor, particularly if it places an excessive load on the joint, especially if it is already vulnerable due to other risks or due to excessive physical activity at a young age (Neogi and Zhang 2013, Szoeke et al. 2006).

OA can affect both high and low motion joints: high-motion joints such as the fetlock joint where trauma occurs through repetitive overextension of the joint and low-motion, high-load joint such as the interphalangeal joints or the distal intertarsal joints, more precisely the tarsometatarsal joint (TMT) and the distal intertarsal joint (DIT), which are affected by bone spavin (Pool and Meagher 1990). The metacarpophalangeal joint (MCP) is the most commonly affected joint in racehorses, followed by the carpal joints (McIlwraith et al. 2012).

1.3. Aetiology and Pathogenesis

As described above, OA may be regarded as a multifactorial disease with several potential initiating causes, which may be divided into the effects of "abnormal" loading on previously healthy cartilage and "normal" loading on marred cartilage (Goldring and Goldring 2007). Examples of abnormal forces on a normal joint include intra-articular malalignment due to enchondral ossification disorders in foals; extra-articular malalignment because of valgus or varus deformities or ligamentous laxities in foals; increased weight bearing on a limb to evade pain on a contralateral limb; or increased workload placed on joints before being appropriately trained. In the second scenario, normal forces operate either on abnormal cartilage, priorly damaged by osteochondrosis, trauma or infection or on normal cartilage overlying subchondral bone that has been weakened by subchondral bone remodelling or osteochondrosis (Pool and Meagher 1990). All of them ultimately lead to degradative processes of the articular cartilage.

The pathology of OA is related to biochemical changes in synovial fluid and cartilage (Clyne 1987). Synovitis, but also changes in the equilibrium of catabolism and anabolism in the

extracellular matrix (ECM) and metabolism of the chondrocytes, lead to imbalances that affect the articular cartilage. And, although synovitis seems to play a major role in initiating and developing cartilage degeneration, the primary source of cartilage degrading enzymes seems to be the articular chondrocyte (Jannson 1996).

After an acute trauma, the joint often reacts with acute synovitis and capsulitis. Through the inflammatory response, mediators like kinin, histamine and complement factors are released into the synovial fluid, followed by leucocytes, prostaglandins, lysosomal enzymes, oxygen-derived free radicals (ODFR) and hyaluronidase, which results in the fluid becoming less viscous (Clyne 1987, Jannson 1996). Thus, nutrition of the cartilage cannot be adequately maintained.

The synovial membrane is also a source of several mediators known to activate the synthesis and release of enzymes, primarily cytokines like interleukin-1 (IL-1) and tumour necrosis factor (TNF), which have a catabolic function (Caron 1992). IL-1, for example, promotes the release of matrix proteoglycans, the synthesis of collagenase and other degradative enzymes and the suppression of cartilage matrix synthesis (Caron 1992). ODFR, on the other hand, are inflammatory products, either deriving from activated neutrophil leukocytes or from an ischemia-reperfusion circle, which directly react with cartilage components and thus are directly causing cartilage degradation (Jannson 1996).

Chondrocytes – as well as synoviocytes – react to the chemical stimuli released through the inflammation by releasing mediators, which cause cartilage breakdown (Caron 1992).

In the absence of disease, the chondrocytes maintain the cartilage with a low matrix turnover as it has limited capacity to regenerate the original matrix architecture (Goldring and Goldring 2007). In physiologic cartilage, matrix metalloproteinases (MMPs) and naturally occurring inhibitors of metalloproteinases (TMPs), which are produced by the chondrocytes and believed to control proteoglycan and collagen turnover in the matrix, are in a well-balanced equilibrium (Jannson 1996, Palmer and Bertone 1994). During OA, however, chondrocytes are stressed, become activated and react with cell proliferation and cluster formation and by upregulating the synthetic activity of matrix proteins and matrix-degrading enzymes including aggrecanases and collagenases which are members of the (MMP) family (Loeser et al. 2012). In addition, they increase the production of inflammatory cytokines, which intensifies the inflammation and changes in the ECM (Clyne 1987, Goldring and Goldring 2007), and downregulate processes essential for cartilage repair (Goldring and Goldring 2007).

If the inflammation continues, it may ultimately result in an imbalance of the catabolic and anabolic activities of the chondrocyte, or, in other words, an increase in the degradation of ECM with or without a concurring increase of matrix synthesis (Goldring and Goldring 2007, Palmer and Bertone 1994). The destruction of the ECM, more precisely the loss of proteoglycans and splitting of type II collagen, occurs initially at the cartilage surface, which results in an increase in water content and loss of tensile strength (Goldring and Goldring 2007). This process occurs through matrix metalloproteinases (MMPs), mainly MMP3 and MMP13, and aggrecanases (ADAMTSs), primarily ADAMTS5, whose expression is upregulated by proinflammatory cytokines and interleukin (IL)-1β (Son and Chun 2018). As articular cartilage matrix proteins are degraded, fragments of matrix proteins are produced and can stimulate further matrix degradation, a feedback mechanism emerges, and the process becomes chronic (Loeser et al. 2012).

At an early stage, chondrocytes still have the potential to synthesize new matrix molecules to replace those that have been degraded. At this point, the disease is potentially reversible. However, as OA progresses and the inflammation becomes chronic, the possibility of matrix molecule synthesis of the chondrocytes decreases, the collagen network degrades, the cartilage becomes thinner, and eventually, only subchondral bone is left (Palmer et al. 2013).

1.4. Impact of sex in Osteoarthritis

In humans, there are significant differences regarding the prevalence and clinical manifestation of OA between the sexes. Men are more likely to have OA before the age of 50, whereas women are more likely to have the disease after menopause and develop more severe symptoms, often in multiple joints. With age, the gap between the sexes increases and older women are much more likely to get more severe and painful OA in multiple joints than older men (Kinney et al. 2005, Ushiyama et al. 1999). The exact mechanisms of these proceedings are still not fully understood (Stevens-Lapsley and Kohrt 2010). However, oestrogen and testosterone, the so-called "sex hormones", seem, amongst other things, to play a major role in the scenario.

Currently, the exact mechanism of how oestrogen or its absence affects OA is unknown but different pathways are suggested to have either protective or detrimental influence on articular cartilage (Linn et al. 2012). Oestrogen may affect the cartilage and the subchondral bone or even the chondrocytes directly via oestrogen receptors (ER) (Sniekers et al. 2010, Ushiyama et al. 1999). Oestrogen depletion can directly increase cartilage damage and subchondral bone loss or may increase the susceptibility for additional triggers by altering the subchondral bone remodelling and subsequently the bone structure. This may in consequence lead to changes in load distribution followed by OA (Sniekers et al. 2010).

Although it has been assumed for a long time that articular tissues are unresponsive to oestrogen or its deficiency, ER have been found in articular cartilage, bone, synovial tissue and ligaments, through which oestrogen can have an influence on the joint (Roman-Blas et al. 2009, Sniekers et al. 2010, Ushiyama et al. 1999). A higher amount of ER has been found in men than in women – correlating with their ER gene expression – (Ushiyama et al. 1999); this might be a reason why women are more likely to develop OA after menopause as their oestrogen level decreases.

Three kinds of oestrogen receptors are known: ERα, ERβ and ERγ. ERα is mainly expressed in female sex organs or more precisely breast, uterus and ovaries, whereas ERβ is expressed broadly, interestingly more in females than in males (Chen et al. 2019). ERγ however is cartilage-specific (Son et al. 2017). Studies show that, in humans, all of them have an increased level in osteoarthritic joints but especially ERγ, however in various mouse models only an augmented amount of ERγ but not ERα or ERβ was found in cartilage samples. The overexpression of ERγ then upregulates directly the matrix metalloproteinases MMP-3 and MMP-13, which are known to play a critical role in the pathogenesis of OA (Son et al. 2017). If similar events are going on in the joints of horses has yet to be determined.

But oestrogen is not only able to act directly through OR but could affect OA indirectly through cytokines and growth factors, which have an important role in cartilage and bone metabolism and may modulate the inflammation process, which is responsible for OA (Nevitt and Felson 1996). IL-1 and TNF-a, for example, can be produced by joint tissue through the influence of oestrogens and may activate enzymes that degrade the cartilage matrix (Nevitt and Felson 1996). Estradiol, on the other hand, one of the oestrogens, has chondroprotective effects, it has anti-oxidative potential and can protect articular chondrocytes from ROS-induced damage

(Claassen et al. 2005). Decreased estradiol levels following menopause may therefore contribute to OA development (Claassen et al. 2005).

Other positive effects of oestrogens are that they may also lessen vascular defects in subchondral bone through the loss of peripheral vascular resistance and protect excessive joint loading by neuromuscular influence, more precisely through the anabolic effect on muscles and bones, which may act protective, and through their antioxidative potential (Nevitt and Felson 1996). And when oestrogen depletion occurs during menopause, all those positive effects dissolve.

Unlike oestrogen, testosterone seems to have only chondroprotective properties – in males and females. Different studies showed that higher serum testosterone levels are associated with less inflammation and pain and testosterone replacement has also similar effects (Freystaetter et al. 2020, Ganesan et al. 2008).

But there are also different other factors that may play a role in this bias. Weight, a factor strongly associated with OA, on average, increases significantly during menopause (Szoeke et al. 2006) and may therefore be a risk factor especially for women, but whether obesity is sex-related or not is still uncertain (Stevens-Lapsley and Kohrt 2010).

Also, the composition of the synovial fluid, chondrocytes and osteoblasts seems to differ between the sexes (Pan et al. 2016). Men for example have a higher level of MMPs and growth factors, whereas women have higher levels of inflammatory cytokines, which may explain that women are more likely to have more inflammation and pain when suffering from OA (Contartese et al. 2020, Pan et al. 2016). While MMPs have definitely a negative effect on OA, growth factors may support repair and remodelling processes. As women have fewer other protective factors, the loss of the protecting factor of oestrogen may increase their susceptibility to OA after menopause (Pan et al. 2016).

Women are also suggested to have thinner cartilage or an accelerated rate of cartilage volume loss (Neogi and Zhang 2013) and they are more likely to self-report OA, which may exaggerate the sex differences that are definitely present also in purely radiographic studies (Srikanth et al. 2005).

There are not only differences between the sexes themselves but also between the sites where OA occurs and the severity: knee, hand, and hip OA are more likely to occur in women, on the other hand, OA in the spine is more likely found in men. Knee OA is also more severe in

women, however, in the severity of hand and hip OA no significant sex differences have been found (Srikanth et al. 2005). If there is also a difference in the severity or site of OA in horses, will still have to be determined.

In animals, a connection between ovarian function and OA has been found as well. Several studies in animal models (e.g. monkeys, rats) suggest that ovariectomy leads to osteoarthritic changes and oestrogen replacement therapy has an OA-protective effect (Ham et al. 2002, Høegh-Andersen et al. 2004, Sniekers et al. 2008).

1.5. Osteoarthritis – Symptoms and Diagnosis

Clinically, OA is characterized by varying levels of lameness, induced by joint pain and stiffness, occasional synovial effusion, soft tissue swelling, and response to flexion in the absence of systemic features, such as fever (Hunter and Felson 2006, McIlwraith et al. 2012, Symmons et al. 2006). Pain is the most prominent clinical symptom of OA, and the major cause of disability and reduced quality of life (Goldring and Goldring 2007). The joint pain usually gets worse with exercise and improves with rest (Hunter and Felson 2006). The severity of the symptoms depends on the level of joint damage but also the individual and the joint site, but they are usually getting worse with time (Symmons et al. 2006).

However, the severity of the symptoms is often not matching the degree of cartilage defect found on the radiographs and structural joint changes are not always accompanied by pain (Martel-Pelletier et al. 2016).

The diagnosis of OA is usually made by a combination of clinical symptoms, confirmed by radiography. The physical examination should include an assessment of body weight, range of motion of the affected joints, the location of tenderness, muscle strength and ligament stability (Hunter and Felson 2006). To identify the affected joint nerve blocks and intrasynovial analgesia may be needed. Once the problem is localized, radiographs of the involved joints should be done (Jannson 1996).

Radiological findings in osteoarthritic joints include osteophytes, subchondral bone sclerosis and cyst formation, and end-stage joint space reduction (McIlwraith et al. 2012).

Radiography is the most common tool to diagnose the disease and is – unlike other methods – inexpensive and readily available (Glyn-Jones et al. 2015). However, as it is impossible to visualize cartilage on radiographs and it is very important to already find early and small changes to prevent a progression of the disease, research has been done to develop more precise methods (McIlwraith et al. 2012). The identification of early OA requires a technique sufficiently sensitive to detect and localize small changes in cartilage biochemistry and morphology before irreversible defects evolve (Palmer et al. 2013).

Therefore, MRI is also a very established method in OA diagnosis, allowing visualisation of cartilage and identification of early degenerative changes such as surface fibrillation or fissuring of the superficial zone. In addition, quantitative measurements including cartilage thickness, volume, surface area and roughness can be done (Palmer et al. 2013).

Other diagnostic possibilities include arthroscopy, which is the gold standard to define the degree of osteoarthritic changes and thermography, which can reveal vascular changes due to joint inflammation but not specific signs of osteoarthrosis (Jannson 1996, McIlwraith et al. 2012).

Research is also done on biomarkers as diagnostic tools, as pathophysiological changes are first present in synovial fluid, but further investigation is needed as none of the biomarkers is yet properly validated for use in clinical practice (Glyn-Jones et al. 2015, Kolhe et al. 2017).

1.6. Treatment

As OA is a multifactorial disease and results in numerous pathological processes, the choice and efficacy of the treatment depend on the severity of symptoms and pathologic findings. Treatment should be initiated at an early stage of OA, preferably before cartilage degeneration has become irreversible. Therefore, special attention should be given to the treatment of synovitis and its causes, as it will minimize the degenerative effects on the cartilage (Jannson 1996).

Once an irreversible point of cartilage degeneration has been reached, according to the current state of knowledge, only palliative treatment is a realistic option. At this stage, the therapeutic

management can consist of three different methods: nonpharmacological treatment, drugs, and surgery (Hunter and Felson 2006).

Nonpharmacological treatment includes weight loss, adequate exercise, appropriate housing and, if possible, orthopaedic shoeing (van Weeren and Back 2016). Studies have shown that in humans and dogs, weight loss results in a significant decrease of forces on the joints, less lameness; longer lifespans and similar benefits would be expected in horses. So weight loss in overweight horses would be an important therapeutic and prophylactic measurement which is unfortunately often overlooked (Contino 2018). Exercise should be consistent and regular, rather than intense and infrequent, but regular exercise is often unintendedly but fortunately done in routine work (Contino 2018). If the inflammation is acute, the horse should rest until the inflammation is completely halted because even low-grade synovitis may have major negative effects on the development of the disease (Palmer and Bertone 1994). However, prolonged immobilization has shown to be counterproductive for the maintenance of joint function, thus, hand walking as well as passive flexion routines are suggested (McIlwraith and Vachon 1988). Since horses with OA tend to be stiff and to warm up slowly, especially in cold weather conditions and with limited free moving space, adequate shelter and regular possibilities to move should be given (Contino 2018).

There are two possibilities to administer drugs for the treatment of OA: systemically or intra articular (IA).

Systemically, mostly nonsteroidal anti-inflammatory drugs (NSAIDs) are used. They reduce pain and inflammation, mostly of the soft tissues, but have no positive effects on existing cartilage degeneration and are even discussed to have a negative effect on cartilage metabolism (Jannson 1996, McIlwraith and Vachon 1988). The most commonly used NSAIDs in horses are phenylbutazone, flunixin meglumine and firocoxib (Contino 2018).

The following drugs can be administered intraarticular: corticosteroids, sodium hyaluronate (HA), polysulfated glycosaminoglycan (PSGAG) and biologics like interleukin-1 receptor antagonist protein (IRAP), platelet-rich plasma (PRP) or mesenchymal stem cells (MSCs) (Contino 2018). The most commonly used corticosteroids are betamethasone, methylprednisolone acetate (MPA) and triamcinolone acetate (TCA). Corticosteroids are administered to reduce pain and inflammation. TCA may be chondroprotective when injected at low doses and durations into an inflamed environment but, like other corticosteroids, it is chondrotoxic and has detrimental effects on cartilage metabolism at higher doses and

treatment durations or when injected into physiological joints (Céleste et al. 2005). The therapy is highly potent, effective and inexpensive but has many contraindications: corticosteroids should not be administered at the acute phase of the disease, because they delay soft tissue healing; they should not be administered in metabolic horses because of the high risk of laminitis (Contino 2018). HA can be administered alone or with corticosteroids but its efficacy is equivocal. HA can be used to treat synovitis and has shown to be chondroprotective, however, no beneficial effects on already existing cartilage defects could be established (Contino 2018, Jannson 1996). Biologics, especially IRAP, are increasingly used for OA treatment, substituting IA corticosteroids because they have no negative effects on metabolic diseases and promote soft tissue healing (Contino 2018). PSGAG has shown to have chondroprotective effects and may reduce effects of OA like increased synovial membrane vascularity or cartilage fibrillation and lameness (Contino 2018, McIlwraith et al. 2012, McIlwraith and Vachon 1988).

So far, no available therapy is able to reverse or stop the structural defects created by the disease. But there are recent approaches to the thematic, which may, in the future, be able to achieve these goals. These include disease-modifying OA drugs (DMOADs), which may influence the catabolism and anabolism of the cartilage, and regenerative therapeutic approaches using stem cells and their components (Grässel and Muschter 2020). DMOADs with anabolic function include sprifermin, which triggers chondrocyte proliferation and cartilage matrix production and BMP-7 which may induce cartilage repair and reduce pain (Grässel and Muschter 2020). Regenerative surgical strategies using stem cells include osteochondral autograft transfer systems (OATS), microfracture, autologous chondrocyte implantation (ACI), matrix-induced ACI (MACI) and different versions of mesenchymal stem cell (MSC) transplantation (Roelofs et al. 2013). OATS use osteochondral plugs from other, non-weight-bearing regions of the same joint, which then get transplanted to repair damaged cartilage regions, whereas in the procedure of microfracture a surgical communication between the joint space and the subchondral bone marrow is formed, with the aim, that MSCs from the subchondral bone marrow would form a repair cartilage tissue (Roelofs et al. 2013). In ACI and MACI however, a cartilage biopsy is being taken from a healthy area, which is then isolated and culture-expanded, or in the case of MACI cultured on a three-dimensional, biocompatible scaffold, and later implanted within the cartilage defect (Roelofs et al. 2013). MSCs can be administered in different ways, surgically or via IA injection, they then tend to disappear relatively fast but continue to work via their chondroprotective and

immunomodulatory effects and have shown to have the potential to promote articular cartilage repair and may decrease the development of secondary OA (Grässel and Muschter 2020, Roelofs et al. 2013).

In end-stage OA patients with chronic pain and functional disability, arthroplasty remains the ultimate possibility (Roelofs et al. 2013). But in horses, the surgical possibilities are limited and it is doubtful if the benefits outweigh the costs in horses (van Weeren and Back 2016). In low motion joints, however, surgical arthrodesis can be performed and can lead to athletic soundness (Jannson 1996).

Lots of different feed additives and nutraceuticals are frequently used and many of them claim to have a beneficial effect on chronic joint disease. However, their benefit is questionable (van Weeren and Back 2016).

1.7. Hypothesis

As female horses do not experience menopause and are usually not ovariectomized, which would also lead to a decrease of the oestrogen level, I hypothesize that there are no sex differences in OA prevalence in horses.

2. Material and Methods

This is a retrospective survey. The data of all horses presented to the University Equine Hospital Vienna between 1.1.2009 – 31.12.2018 were extracted from the hospital patient database (TIS). The patient records of all horses that received an "intrasynovial injection" plus radiographs (n=1507) were evaluated and horses that were diagnosed with osteoarthritis (keywords "Arthrose", "OA", "DJD", "Arthritis" (excluding septic arthritis) and "Osteoarthritis") were included after removal of duplicate entries for repeated visits of the same horses (n= 623, osteoarthritis group). The horses' gender, age, body weight, breed, number and localization of the affected joints and limbs, OA severity of the most severely affected joint and the equestrian discipline were extracted out of the TIS and included in the data analysis. The total hospital population presented to the University Equine Hospital in the same period of time served as the control group (n= 10814 after removal of duplicate entries due to repeat visits). For the control group, the horse's gender, age, body weight and breed were extracted from the hospital records. To facilitate comparison, horses were classified into different breed groups: warmblood horses (including Hanoverian horse, Oldenburger, Trakehner, Irish Sport Horse, Holsteiner, Westphalian horse etc.), draft horses (including Noriker and other draft breeds), pony, Arabian, western breeds (including Quarter Horse, Paint Horse, Appaloosa, Pinto), robust breeds (including Haflinger, Fjord horse, Icelandic horse), racehorses (including trotters, thoroughbreds) and other horses (including Mustang, Gypsi horse, PRE, Lippizan, Friesian horse, Kladruber and mixed breeds).

Data were entered into GraphPad Prism (Version 9). Baseline characteristics were analyzed by descriptive statistics and were compared between males and females using unpaired *t*-tests for continuous and Chi-square tests for categorical data. Continuous variables were expressed as mean ± standard deviation (s.d.), and categorical variables were expressed as percentages. Spearman correlation between the different ordinal variables was calculated. A t-test was used to assess differences for continuous variables and a chi square test for categorical variables. A p-value < 0.05 was considered significant.

3. Results

Of the 623 horses included in the OA-group of the study 240 (38.52 %) of the horses were mares, 354 (55.38 %) were geldings and 38 (6.10 %) were stallions. Those were compared to the control group, the total hospital population, which included 10814 horses: 4367 (40.38 %) mares, 5038 (46.58 %) geldings, 1407 (13.01 %) stallions and two ovariectomized mares (0.018 %). In the OA-group a total of 1034 limbs and 1530 joints were affected by the disease. 210 (20.31 %) of the horses had osteoarthritis in their left foreleg and just as many were affected on their right foreleg. 294 (28.43 %) of the horses had osteoarthritis in their left hind leg and 320 (30.95 %) in their right hind leg. 131 horses (21.03 %) were affected on one and 63 horses (10.11 %) on both forelegs, 193 horses (30.98 %) were affected on one hind leg and 122 (19.58 %) on both. 28 horses (4.49 %) were affected on one foreleg and one hind leg, 16 (2.57 %) on two forelegs and one hind leg, 29 (4.65 %) on one foreleg and two hind legs and 37 (5.94 %) were affected on all four legs. Four horses (0.64 %) were affected on their vertebrae.

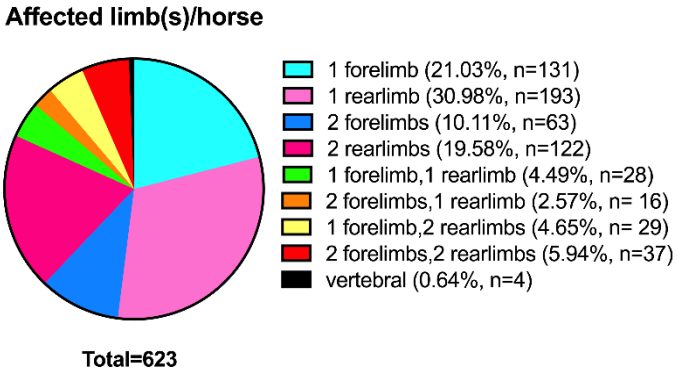


Figure 1: Distribution of the affected limbs per horse

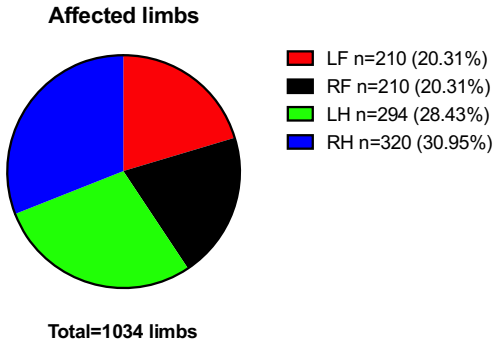


Figure 2: Distribution of the affected limbs

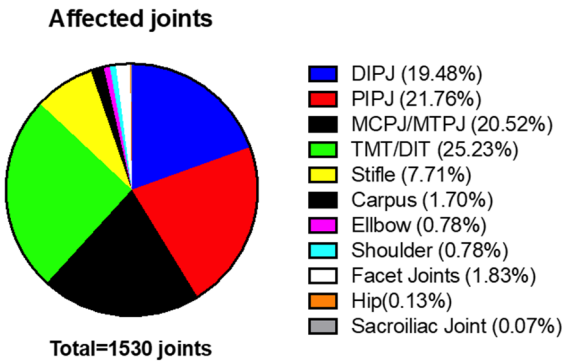


Figure 3: Distribution of the affected joints of the OA-patients

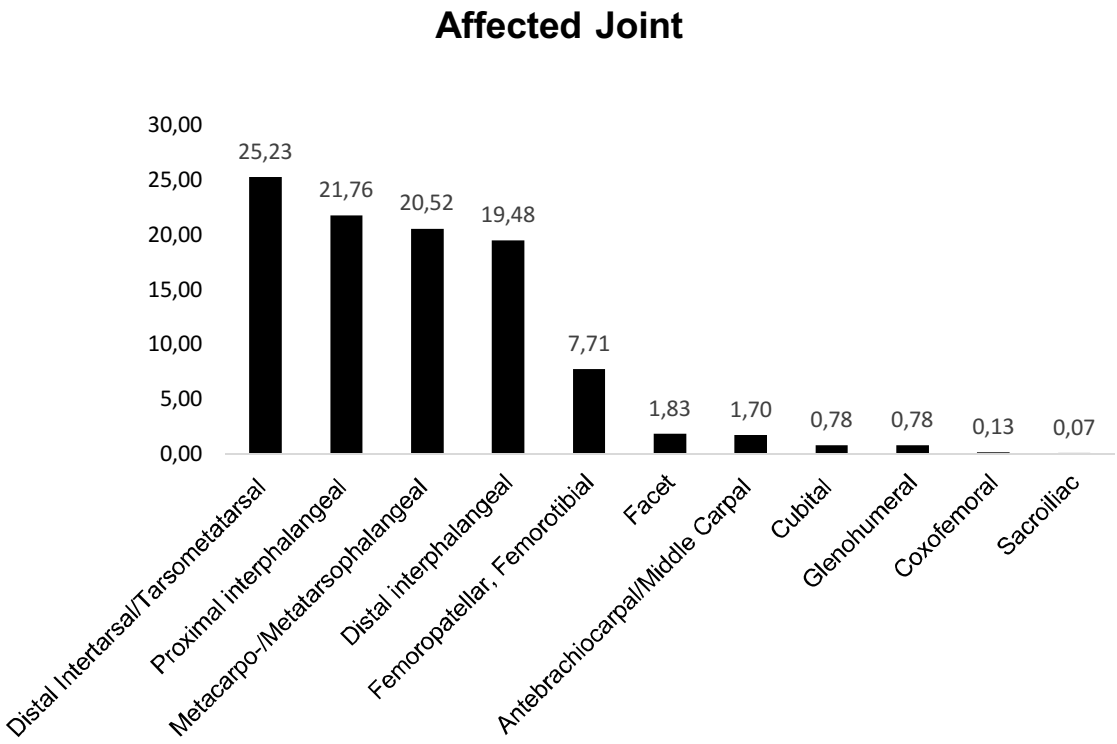


Figure 4: Distribution of the affected joints of the OA-patients.

The tarsometatarsal (TMT) joint and the distal intertarsal joint (DIT) that together form the spavin joints are, with 25.23 %, the joints that are most affected by OA. They are followed by the pastern joint or proximal interphalangeal joint (PIPJ) with 21.76 %, the fetlock joint or metacarpophalangeal joint (MCPJ) and metatarsophalangeal joint (MTPJ) with 20.52 %, the hoof joint or distal interphalangeal joint (DIPJ) with 19.48 % and the stifle with 7.71 %. Less affected joints were carpus (1.70 %), elbow (0.78 %), shoulder (0.78 %), facet joints (1.83 %), hip (0.13 %) and sacroiliac joint (0.07 %).

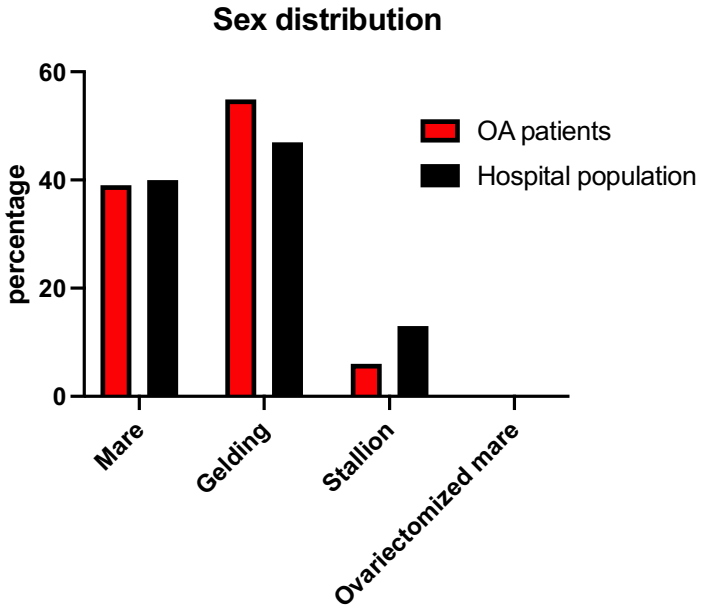


Figure 5:sex distribution of the horses with and without OA

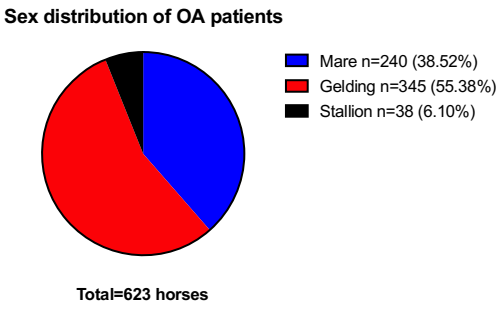


Figure 6: sex distribution of OA-patients

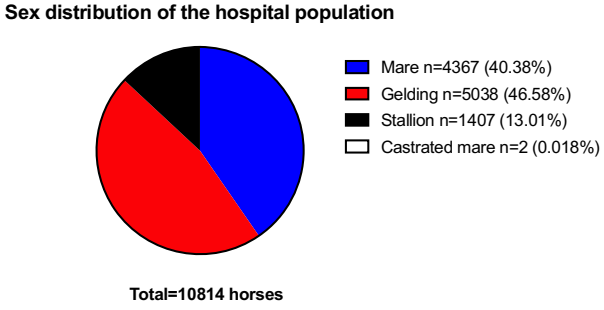


Figure 7: sex distribution of the control group

The sex distribution of OA patients differed significantly from the hospital population ($p<0.0001$, Chi-square = 33.85, DF = 3). Geldings, which constituted 55.38 % of the OA group compared to 46.59 % of the hospital population, seem to be more at risk to develop OA, whereas stallions, with 7.38 % of the OA group, versus 10.50 % of the hospital population, seemed slightly less at risk. Mares also seemed to be very slightly less susceptible, with 38.52 % in the OA group compared to 40.38 % in the hospital population.

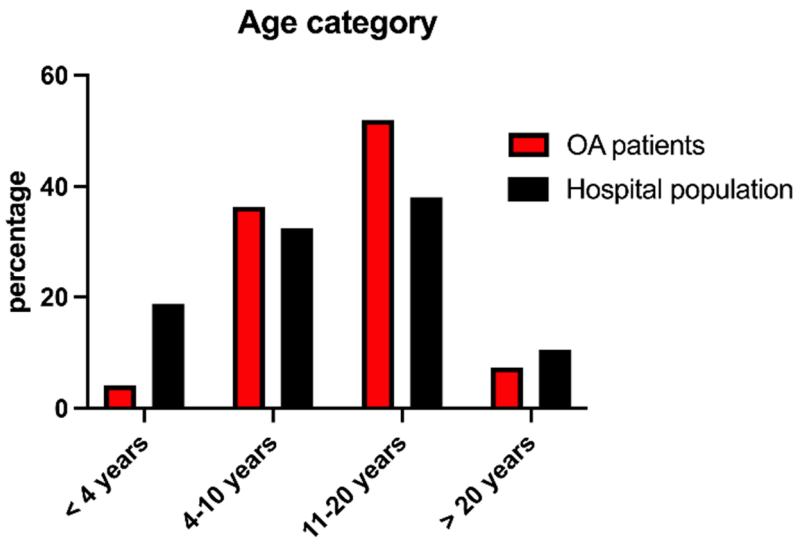


Figure 8: Age category of horses with and without OA

Age was significantly different between the total hospital population (mean 11.07, s.d. 6.887) and the OA patients (mean = 12.18, s.d. = 5.408) ($p<0.0001$, $t = 3.957$, $df = 11435$ $F = 1.622$, $DFn = 10813$, $Dfd = 622$). In addition, horses were classified into four different age categories: under four years, four to ten years, ten to twenty years and over twenty years. There was a significant difference in the distribution between age categories of OA horses compared to the total hospital population ($p<0.0001$, Chi-square = 111.5, DF = 3). Horses were most likely to be presented at the hospital for OA between ten and twenty years. 26 (4.17 %) of the horses presented for OA were less than four years old, 227 (36.44 %) were four to ten years, 324 (52.01 %) were ten to twenty years, and 46 (7.38 %) were older than 20. In contrast, in the hospital population, 2037 (18.84 %) of the horses were under the age of 4, 3524 (32.59 %) were four to ten years old, 4117 (38.07 %) were ten to twenty years old, and 1136 (10.50 %) were over 20 years old when they were presented at the clinic.

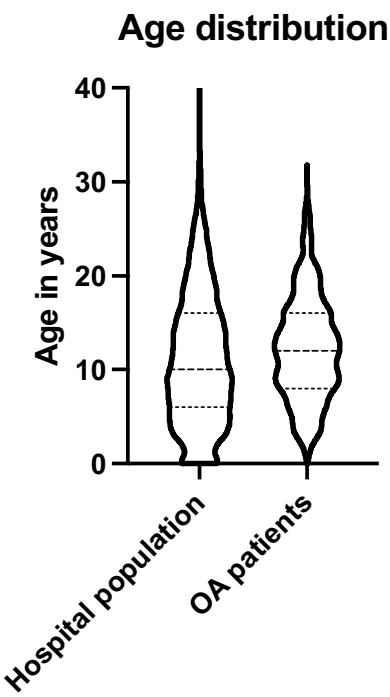


Figure 9: age distribution of Horses with and without OA; --- : median, ⋯ : quartile

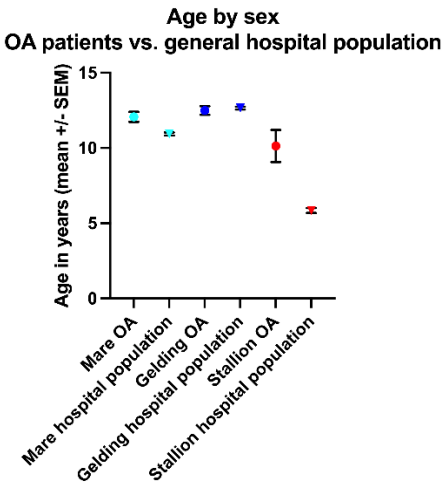


Figure 10: Age compared to sex in OA-patients and hospital population

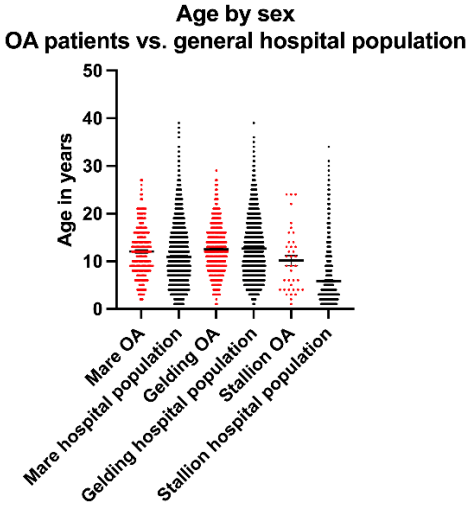
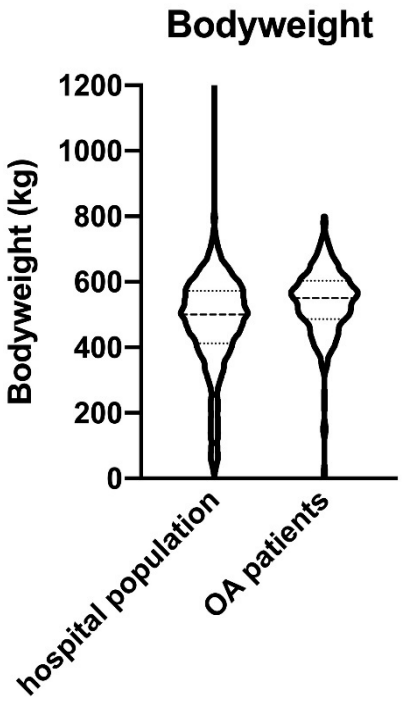


Figure 11: Age compared to sex in OA- patients and hospital population

Figures 10 and 11 try to show the relationship between age and sex of the OA-patients and general hospital population. Mares seem to be older when they get treated for OA than when they get treated for other symptoms for the first time. In geldings, the difference is much smaller; furthermore, geldings seem to get OA a little earlier than other issues. In stallions, it is very difficult to compare. Not only because of the effect of ageing on OA but also, because they usually get castrated at quite a young age. Thus, stallions that have been hospitalized at a young age for other issues than OA, may later fall in the gelding category, when they are castrated. Overall, it seems that stallions seem to get OA earlier, while geldings seem to get the disease at a higher age, but for more precise results on this topic, further research should be done.



Bodyweight played a major role in the development of OA: OA-patients had a mean weight of 538 kg (s.d. = 102 kg), the total hospital population however, only had a mean weight of 477,70 kg (s.d. = 179.6 kg) ($p<0.0001$, $t = 6.38$, $df = 11949$, $F = 3.098$, $DFn = 11585$, $Dfd = 364$).

Figure 12: distribution of bodyweight of horses with and without OA. --- : median,⋯ : quartile

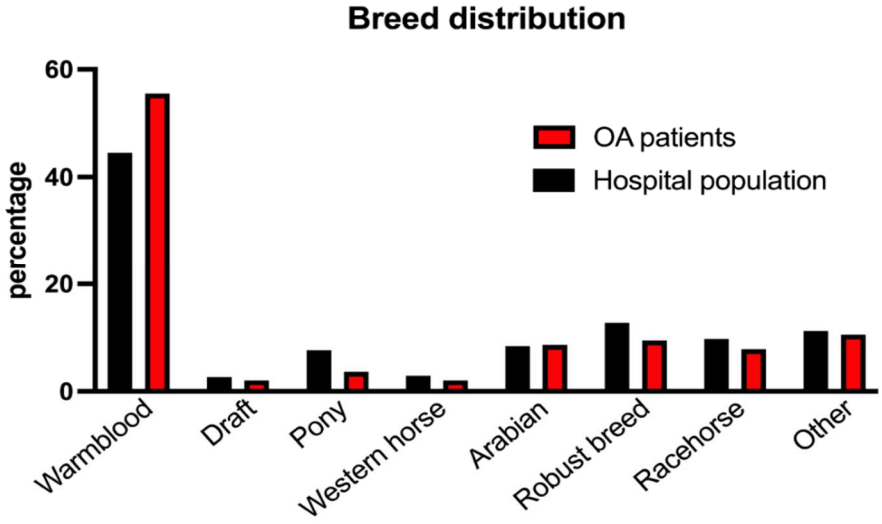


Figure 13: Breed distribution of the horses with and without OA

The breed distribution was significantly different between OA patients and the total hospital population ($p<0.0001$, Chi-square = 40.05, DF = 7). As the breed showed no correlation with the equestrian discipline ($p = 0.422$), the breed bias does not seem to be associated with the work or equestrian discipline they are engaged in. Warmblood horses seemed to be most susceptible to OA, as 55.54 % of the OA-Patients but only 44.51 % of the total hospital population were warmbloods. Ponies and robust breeds seemed to be less susceptible to OA, as only 3.69 % of the OA patients but 7.66 % of the total hospital population were ponies. Robust breeds constituted only 9.47 % of the OA-Patients but 12.75 % of the hospital population. Also, racehorses didn't seem more susceptible to OA as they make only 7.87 % of the OA patients but 9.76 % of the hospital population, but they may be presented at a younger age since they are more closely observed at that point, which may confound the results. The other breeds and breed groups were similarly distributed between OA patients and the total hospital population: Western horses constituted 8.67 % of the OA-Patients and 8.42 % of the hospital population; draft breeds 2.09 % of the OA-patients and 2.68 % of the hospital population; Arabians 2.09 % of the OA and 2.94 % of the total patients, and other breeds 10.59 % of the OA-and 11.29 % of the total patients.

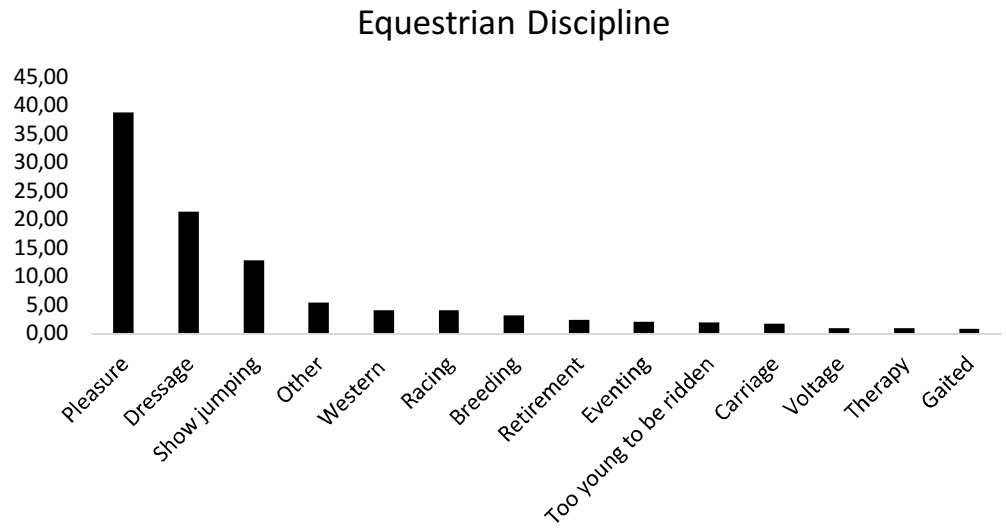


Figure 14: Equestrian discipline of the horses with OA of whom their discipline was known (540 horses)

Also, the equestrian discipline the OA-patients are performing was analyzed, but it couldn't be determined in 83 of the 623 horses. Therefore, the percentage of the equestrian discipline of the horses was calculated with the 540 remaining horses. Most of the horses (38.70 %) were

pleasure horses, followed by dressage (21.30 %) and show jumping (12.78 %). The other disciplines were less represented (0.74 % to 5.37 % incidence).

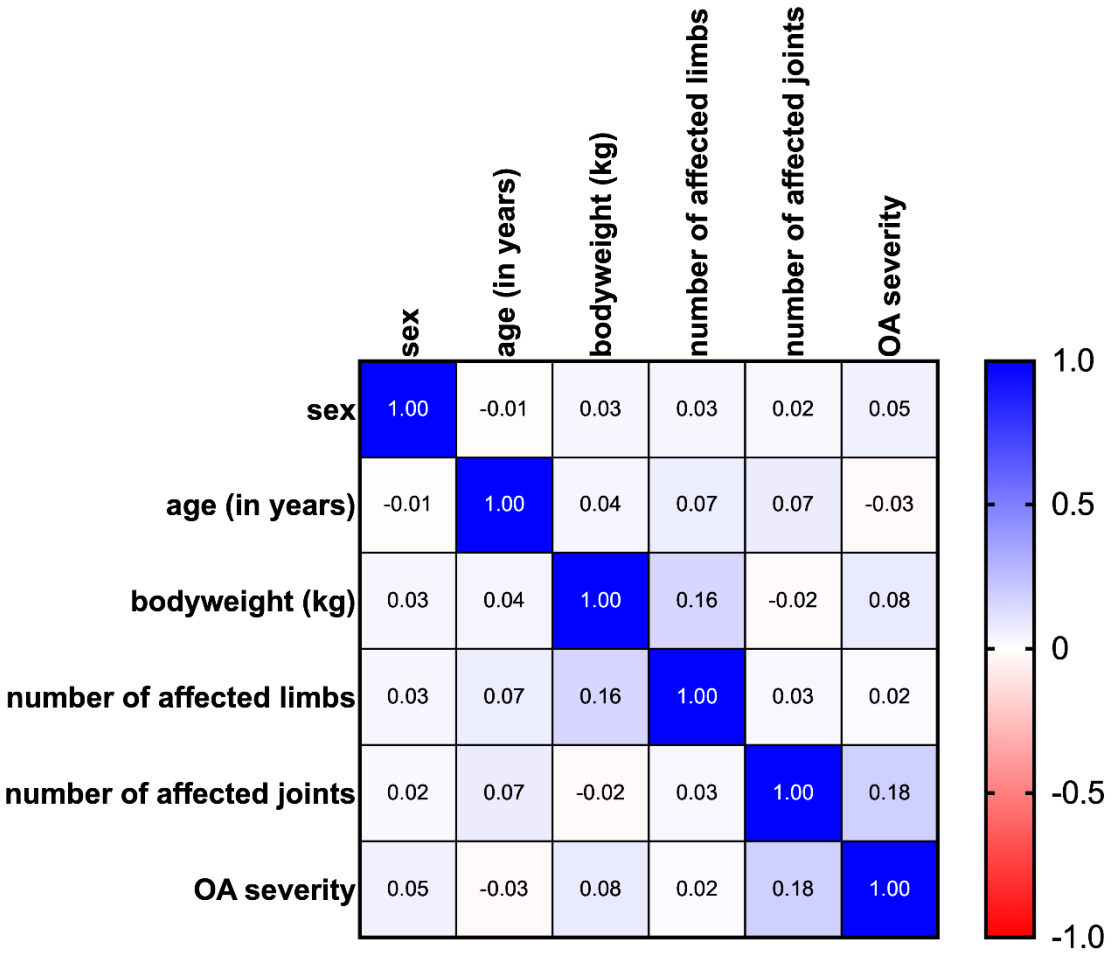


Figure 15: Correlation of the different susceptibility factors for OA

As we can see, bodyweight has a slight correlation with the number of affected limbs ($p = 0.003$, $r = 0.158$). Also, the number of affected joints has a correlation with the severity of OA ($p < 0.001$, $r = 0.181$).

4. Discussion

To my knowledge, this is the first study to research sex differences in OA prevalence in horses. Our study can reject the hypothesis that there are no sex differences in OA prevalence in horses. While we did not observe the higher prevalence in mares (38.52 % of the OA patients in our study were mares) that has been reported for human women, geldings were affected to a significantly higher percentage (55.38 %) than could be expected based on the total hospital population (46.59 %).

This can very likely be associated with the lack of testosterone, since, as described previously, testosterone and its effects are very likely to act chondroprotective. Without testicles, the testosterone level is low. Testosterone has a significant impact on the development of skeleton and muscles and if castration is done later when the horses have already achieved full sexual maturity, the loss of testosterone may trigger a reduction in muscle and bone mass and impact the physical function of muscles and bones (Horstman et al. 2012, Kessler et al. 2016). Intact and sexually mature stallions between four and 14 years of age have been shown to have a serum testosterone level of 2.00 ng/ml (s.d. 0.85) whereas geldings of the same age had only <0.1 ng/ml (Inoue et al. 1993). Studies have shown, that high testosterone levels are not only associated with higher muscle and bone mass, which may act protectively but also less pain and inflammation, which plays a major role in OA (Freystaetter et al. 2020, Ganesan et al. 2008). Similarly, high levels of androstenedione, a precursor of testosterone, oestrogen and antigens, are associated with a decreased risk of OA, especially in overweight and obese men (Hussain et al. 2016).

As testosterone clearly seems to have protecting effects against OA, different studies have also been done with castration and testosterone replacement, but their results differ. A study on mice suggests for example, that castration greatly reduces OA severity by 47% and the replacement of endogenous testosterone with dihydrotestosterone (DHT) reversed this effect and worsens OA (Ma et al. 2007). On the other hand, a study on rats showed exactly opposite results (Ganesan et al. 2008) which agrees with a study on mice that suggests that gonadectomy triggers cartilage damage in both sexes and hormone replacement reverses the effect (Da Silva et al. 1994). A study on humans however suggests that low levels of endogenous testosterone only had negative effects on females but not males (Jin et al. 2017). Another study on humans showed also that higher testosterone levels led to less pain in both

sexes, but only in women to less disability (Freystaetter et al. 2020). Another study suggests that low testosterone levels affect OA susceptibility only after a certain amount of time (Park et al. 2020). Anyway, testosterone replacement therapy should be considered wisely, as it may have not only positive but also severe negative effects (Tsametis and Isidori 2018).

Mares, in contrast to human females, don't seem to be more susceptible than males. But, also in contrast to women, mares don't undergo menopause and do therefore not have a severe loss of sexual hormones at a certain age. As previously described, oestrogens play a role in many proceedings in the body, which have positive but also negative effects. They do not only have protective effects on cartilage and subchondral bone but also increase sensitivity to inflammation and pain (Contartese et al. 2020, Xu et al. 2019). However, oestrogen deficiency results in increased damage to articular cartilage, subchondral bone resorption and therefore OA (Xu et al. 2019). Different studies show that replacement therapy seems to have a favourable effect on OA symptoms of females with a lack of oestrogens, but the effects don't seem to be as strong as they are with testosterone replacement (Chlebowski et al. 2013, Sniekers et al. 2010, Xu et al. 2019).

To get further information about the exact role of presence, or more precisely the absence, of certain sex hormones for the development of OA in horses, further clinical surveys will have to be done. Especially the role of testosterone requires further investigation because in horses, unlike other animals, ovariectomy is very rarely done and they don't undergo menopause, but orchidectomy is done frequently. Unfortunately, in this study, we could not determine if female or male horses have more severe OA or if there is a difference in the sites of OA like in humans but it would be interesting to do further research on this topic.

This study, agreeing with common knowledge, showed that age is one of the most prominent risk factors for OA. In this context, one of the limitations in the study is that the difference in the age of stallions in the OA group versus the hospital population cannot be attributed solely to the well-established increase of OA with age but also to stallions being castrated typically at a young age.

The exact mechanisms of how ageing influences the musculoskeletal apparatus to develop the disease are still not fully understood. There are different theories on how it may happen. Either, OA develops simply because of the continuous mechanical stress happening on the cartilage, or because of the modification of cartilage matrix components and the loss of viable cells over time, which makes the cartilage more susceptible to damage (T. Aigner et al. 2004).

On the other hand, ageing usually comes along with the loss of sex hormones, which may be responsible for loss of muscle and bone mass and their weakness and thereby for the disease (Horstman et al. 2012). Most probably, all of the theories play together to increase susceptibility to OA in older horses. And, because in horses, as well as in humans, the age of the population is increasing, the incidence of OA is as well (Ireland et al. 2012, Rahmati et al. 2017). To be more precise, 50 % of the horses that are older than 15 years suffer from OA and even 80 % to 90 % of the horses over 30, which is a clear sign that age has an enormous impact on OA (van Weeren and Back 2016).

The loss of sex hormones with age is well known in humans. In men, beginning around 40 years of age, testosterone levels decline by about 1% per year (Feldman et al. 2002). And in women, the oestradiol levels drop from between 10^{-9} to 10^{-11} in the menstrual cycle to $0.5 - 1.5x10^{-11}$ after menopause (Claassen et al. 2005). In horses, however, this doesn't seem to be the case. Stallions before the age of 15 have testosterone levels of 2.00 ng/ml (s.d. 0.85) whereas stallions older than 15 years have testosterone levels of 2.09 ng/ml (s.d. 0.90) (Inoue et al. 1993). Similarly, in mares, oestradiol levels do not drop significantly with age although the peak concentrations are slightly higher in young mares (5-6 years) with 6.9 ng/ml (s.d. 0. 5) than in mares older than 18 years with 5.7 ng/ml (s.d. 0.4) (Ginther et al. 2008).

Another risk factor for OA that is commonly known is increased body weight. Studies show that obesity is also very common in horses, with 26 % to 31 % of the horse population being overweight, and the prevalence is even increasing (Fowler et al. 2020, Ireland et al. 2012, Robin et al. 2015). Formerly, increased bodyweight was thought to impact the development of OA only mechanically, but recent findings show, that adipose tissue contributes to OA also through adipocytokines such as leptin, resistin and adiponectin, who act either through direct joint degradation or by controlling local inflammation processes (Sowers and Karvonen-Gutierrez 2010). While leptin, resistin and other adipocytokines were found to increase the inflammatory process, adiponectin is suggested to have a protecting function on the cartilage (Chen et al. 2006). And there is another mechanism that is contributing to OA through increased bodyweight: mechanoreceptors on the surfaces of chondrocytes detect the excessive weight and therefore trigger cascades of cytokines, growth factors and metalloproteinases, which boost the inflammation process (Sowers and Karvonen-Gutierrez 2010). Thus, obesity increases the risk for OA, but if there is a difference in obesity between

the sexes will still have to be determined (Stevens-Lapsley and Kohrt 2010), although one study looking at the prevalence of obesity in horses found no influence of age or gender on the likelihood that a horse would be overweight (Kosolofski et al. 2017).

Our study could unfortunately not exactly determine which connection exists between sex, equestrian discipline of the horse and OA. Another study, which investigated the relationship between different orthopaedic problems and the physical activities the horses participate in, showed, that 47% of the injured dressage horses had OA, 29% of the jumpers, 20% of the racing horses, 44% of the polo ponies, 37% of the working horses and 33% of the western horses. This led to the conclusion, that in physical activities that require longer training or in which animals are used for a longer period of time, OA was more present (Sousa et al. 2017).

5. Zusammenfassung Deutsch und Englisch

Arthrosen sind beim Pferd, genau wie beim Menschen, die häufigste muskuloskelettale Erkrankung und primäre Schmerzursache. Viele Studien beschäftigen sich damit, Ursachen und Zusammenhänge besser zu verstehen um der Krankheit besser vorbeugen, beziehungsweise frühzeitig mit der Therapie beginnen zu können. Beim Menschen und bei anderen Tieren ist bereits seit längerer Zeit bekannt, dass Sexualhormone einen großen Einfluss bei der Entstehung und dem Fortschreiten der Krankheit haben. Frauen haben nach dem Abfall der Östrogene durch die Menopause ein stark erhöhtes Arthrose Risiko. Dies ist meines Wissens nach, die erste Studie, die sich mit dem Einfluss des Geschlechts auf Osteoarthrosen beim Pferd beschäftigt. Wir haben herausgefunden, dass, anders als beim Menschen, nicht die Stuten vermehrt betroffen sind, die ja auch keine Menopause durchlaufen, sondern die Wallache. Diese retrospektive Studie umfasst 623 Pferde mit Arthrosen und eine Kontrollgruppe mit 10814 Pferden ohne bekannte Arthrosen. In der Arthrose Gruppe waren 55.38 % Wallache, 7.38 % Hengste und 38.52 % Stuten, verglichen mit 46.59 % Wallachen, 10.50 % Hengsten und 40.38 % Stuten in der Kontrollgruppe. Dieses Ergebnis könnte sich damit erklären, dass Testosteron, wie in einigen Studien gezeigt, durch verschiedene Mechanismen eine schützende Wirkung auf die Gelenke hat. Und da die Wallache einen im Vergleich stark erniedrigten Testosteronspiegel haben, wäre dies eine mögliche Erklärung für die erhöhte Anfälligkeit für Arthrose.

Osteoarthritis (OA) is the most common musculoskeletal disorder in humans as well as in horses and is among the most important causes of pain. Many surveys attempt to find causes and connections for a better understanding of the disease to find possibilities for prevention or early onset of treatment. In humans and other animals, it is commonly known, that sex hormones have a great influence on the onset and progression of the disease. Women are, after the decrease of oestrogens through menopause, at a much higher risk to develop OA. To my knowledge, this is the first survey, to investigate sex differences in OA prevalence in horses. We found out that, in contrast to humans, mares are not at higher risk, likely because they don't undergo menopause, but geldings are. This retrospective survey includes 623 horses with OA and a control group of 10814 horses without known OA. The OA group consisted of 55.38 % geldings, 7.38 % stallions and 38.52 % mares, which were compared to

46.59 % geldings, 10.50 % stallions and 40.38 % mares of the control group. This outcome could be because testosterone has been shown in many studies to be protective for joints through different mechanisms. And because geldings have, in comparison, a very low testosterone level, this could explain their higher OA-susceptibility.

6. Abbreviations

OA – Osteoarthritis

DJD – Degenerative Joint Disease

ECM – extracellular matrix

ER – oestrogen receptor

ODFR – oxygen-derived free radicals

IL-1 – Interleukin-1

TNF – tumour necrosis factor

MMP – matrix metalloproteinases

IMP – inhibitors of matrix metalloproteinases

IA – intraarticular

NSAID – nonsteroidal anti-inflammatory drug

HA – sodium hyaluronate

PSGAG – polysulfated glycosaminoglycan

IRAP – interleukin-1 receptor antagonist protein

PRP – platelet-rich plasma

MSCs – mesenchymal stem cells

DIT – distal intertarsal joint

TMT – tarsometatarsal joint

MCP – metacarpophalangeal joint

PIP – proximal interphalangeal joint

DIP(J) – distal interphalangeal joint

MCP(J) – metacarpophalangeal joint

MTP(J) – metatarsophalangeal joint

s.d. – standard deviation

DMOAD – disease-modifying OA drug

OATS – osteochondral autograft transfer systems

ACI – autologous chondrocyte implantation

MACI – matrix-induced ACI

MSC – mesenchymal stem cells

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8. Table of figures