Clinic for Horses, General Surgery and Radiology
Faculty of Veterinary Medicine
Freie Universität Berlin

Analysis of cartilage wear particles in equine joints and their relationship to several joint diseases

Thesis submitted for the fulfilment of a doctor degree in veterinary medicine at the Freie Universität Berlin

Submitted by
Ticiana Meireles Sousa
Veterinarian from Fortaleza, Brazil

Berlin 2008
Journal-Nr.: 3206

Aus der Klinik für Pferde, Allgemeine Chirurgie und Radiologie des
Fachbereichs Veterinärmedizin der
Freien Universität Berlin

Knorpelabriebprodukte in der Synovia beim Pferd und ihre Beziehung zu verschiedenen Gelenkerkrankungen.

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin an der
Freien Universität Berlin

vorgelegt von
Ticiana Meireles Sousa
Tierärztin aus Fortaleza, Brasilien

Berlin 2008
Journal-Nr.: 3206

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Leo Brunnberg
Erster Gutachter:	Univ.-Prof. Dr. Bodo-Wolfhard Hertsch
Zweiter Gutachter:	Univ.-Prof. Dr. Helmut Hartmann
Dritter Gutachter:	Univ.-Prof. Dr. Dr. Ralf Einspanier

Deskriptoren (nach CAB-Thesaurus):
Horses, horse diseases, joint diseases, cartilage, clinical aspects, diagnosis, joints(animal), lameness, osteoarthritis, osteochondritis, radiography, synovial fluid.

Tag der Promotion: 16.09.2008

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <http://dnb.ddb.de> abrufbar.

ISBN-13: 978-3-86664-470-0

Zugl.: Berlin, Freie Univ., Diss., 2008
D188

 Nordendstr. 75 - 13156 Berlin – 030-45494866
verlag@menschundbuch.de – www.menschundbuch.de

CONTENTS

ABBREVIATIONS

Abbreviaton	Meaning
AAEP	American association of equine practitioners
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
BAP	Bone-specific Alkaline phosphatase
BSP	Bone sialoprotein
COMP	Cartilage oligomeric matrix protein
CPII	Carboxypropeptide of type II collagen
CS	Chondroitin sulphate
CTX	Type I collagen C-telopeptides
DDFT	Deep digital flexor tendon
DPYR	Deoxypyridinoline
EDTA	Ethylenediaminetetraacetate
e.g.	*Exempli gratia* (Latin) - for example
et al.	*Et alii* (Latin) – and others
fL	femtoliter
g/dL	Grams per deciliter
GAG	Glycosaminoglycan
GOT	Glutamic oxaloacetic transaminase
HA	Hyaluronic acid; Hyaluronan
IA	Infectious arthritis
ICTP	Type I collagen nonhelical telopeptide
IL	Interleucin
KS	Keratan sulphate
LDH	Lactic dehydrogenase
mL	Mililiter
μL	Microliter
mm^3	Cubic millimeter

μm	Micrometer
MMP	Matrix metalloproteinases
MPQ	Mucinous precipitate quality
NS	Navicular syndrome
OA	Osteoarthritis
OC	Osteochondritis
OCD	Osteochondritis dissecans
PG	Proteoglycan
PGE	Prostaglandin E
PO	Podarthritis
RPM	Revolutions per minute (speed)
S.E.M.	Standard Error of Measurement
SF	Synovial fluid
TAA	Traumatic acute arthritis
TNFα	Tumor necrosis factor α
WA	Washes

FIGURES

TABLES

1 INTRODUCTION

Lameness is a major source of pain and distress to the horse and financial loss to the owner. A recently survey reported that 40% of all lameness causes had joint disease as a primary cause (Todhunter and Lust, 1990). Joint injury and joint disorders represent a major part of the workload of equine practitioners and, in the majority of cases cartilage failure has been identified as an important contributory factor (Trotter and McIlwraith, 1996).

Synovial joint allow movement and the transfer of loads between bones. The joints of the proximal limbs of the horse are surrounded by large muscle masses that contributes to joint stability and help dissipate the energy associated with movement. However, the joints in the distal limbs are solely dependent on the joint capsule, ligaments and osteocartilageneous contours of the joint for stability. Joints distribute and transfer varying loads, while maintaining the contact stresses on joint surfaces at a low level (Todhunter, 1996).

Although some of the energy of movement is dissipated in the bones of the distal limb joint, the lack of muscle results in a larger proportion being transmitted across the hyaline cartilage of the joint surface. Cartilage is subjected to different loading patterns, constant loads during weight bearing, intermittent loads during movement and sudden loads during training or competition. The selective breeding of the modern horse for speed and athletic performance has created long, elegant distal limbs; however, the negative consequence is increased stress on the joints and a greater chance of joint failure.

The inherent biomechanical characteristics of cartilage are strongly dependent on the prevailing mechanical stress to which the area is exposed. However, damage to cartilage can occur in athletic horses as a result of physical stresses of abnormal intensity, duration or frequency (Pool, 1996).

It was suggested that strenuous training could lead to a degenerative response at cartilage sites at which clinical lesions were commonly observed. Cartilage can, to some extent, adapt to high intensity training, but there is a very narrow margin between the adaptive and a degenerative response. Most joint lesions are caused by chronic fatigue of joint structure in which the rate of micro damage exceeds the rate of repair, which for cartilage is extraordinarily low (Pool, 1996).

Early detection of osteoarthritis (OA) in horses represents a challenge for equine practitioners (Jouglin et al., 2000). Indeed, early diagnosis of OA is a major problem, both in human and veterinary medicine. It is a frequent sequela of other joint diseases, especially with severe injuries or if attempts at therapy are unsuccessful. OA is defined as the inability of the articular cartilage to be repaired at the rate it is damaged, resulting in irreversible degeneration of the articular cartilage (McIlwraith, 1996; Pool, 1996). The study of equine joints complements comparative studies on human and other animal cartilages, allowing similarities and differences between species to be assessed.

With radiology only, it is not possible to have an accurate prediction of the status of articular cartilage, as osteoarthritic deterioration begins long before a radiological diagnosis can be made. Arthroscopy still seems to be the most sensitive technique in the evaluation of osteoarthritis. A precise prognosis without surgical intervention is especially difficult (Chan et al., 1991; Fife et al., 1991; Ike, 1993; Blackburn et al., 1994). Development of effective methods that make possible an early detection of cartilage injury or degeneration in an affected joint is therefore of paramount importance.

Several researchers have suggested that the principles of wear particle analysis may provide a means of assessing the functional integrity of articular surfaces, as well as identifying lesions or deterioration of the articular cartilage (Mears et al., 1978; Evans et al., 1980). The pathologic changes associated with osteoarthritis and a number of diverse joint diseases of both humans and animals have been shown to produce significant debris within the joint capsule (van Pelt, 1974; Yehia and Duncan, 1975). The presence and morphology of such fragments has been suggested as a possible indicator of the extent of articular damage (Muirden, 1970; Tew, 1980).

The aim of this study was not only to prove if the number and size of cartilage fragments correlate with the different disease, but also to develop a very simple method that could eventually be used for diagnosis. Using this technique, improved diagnosis of arthritis may be possible. In addition, it may lead to a deeper understanding of the aetiology and pathogenesis of degenerative arthritis and other destructive joint diseases. Costly or time-consuming analytic methods have been avoided to make the overall analysis practical for routine use.

2 LITERATURE

2.1 Important aspects of the articular anatomy

Equine diarthrodial joint anatomy consists of opposing, congruent articular cartilage-covered osseous structures secured by a joint capsule and ligaments, containing synovial fluid. The joint capsule, ligaments, and menisci consist mostly of connective tissue and are of low cellularity. The extracellular matrix of these tissues is predominantly collagen, proteoglycans, noncollagenous proteins, and water (Amiel et al., 1984).

The joint capsule consists of thick fibrous portion, which is lined by a thin subsynovium (lamina propria) and the synovium (synovial membrane), which is in contact with the synovial fluid. Synovium contains synoviocytes, a continuum of cells that have both secretory and phagocytic functions. Type A synoviocytes are synovial lining cells that can phagocytose and pinocytose. Type B synovial lining cells synthesize hyaluronan (hyaluronic acid; HA), which is secreted into synovial fluid at the plasma membrane, as well as the other components of the extracellular matrix of the synovium. Lubricin, a glycoprotein involved in the boundary lubrication of cartilage, is probably also synthesized by the synovium (Henderson and Pettipher, 1985; McIlwraith and Trotter, 1996).

Articular cartilage is translucent and has a glasslike (hyaline) appearance, that is due primarily to its high water content (70% by weight in mature healthy cartilage and approaching 80% in neonatal cartilage) and the very fine structure of its collagen (most of it type II) fibril network. It is composed by chondrocytes and an extracellular matrix. In addition to collagen, that forms a 3-dimensional meshwork (Miller, 1980; Mayne, 1989), in cartilage matrix can also be found proteoglycans, as hyaluronan and aggrecan (Poole, 1993; McIlwraith and Trotter, 1996; McIlwraith, 2002).

The articular cartilage is subdivided into three unmineralized zones (I to III), which are delineated from the calcified cartilage (zone IV) by the tidemark (the junction between noncalcified and calcified cartilage observable on histologic sections. The lower boundary of the calcified cartilage in the cement line formed during endochondral ossification of the articular epiphyseal growth plate at maturity. In adult animals, zone I (also called superficial

or tangential zone) has the highest cell density. The condrocytes are relatively small and flat, and oriented with the long axes parallel to the surface. Zone II (transitional zone) shows larger and rounded cellular profiles. In zone III (radiate zone), the cells are larger and arranged with their long axes perpendicular to the surface (Schenk et al., 1986).

The geometric organization and alignment of the fibrils vary according to anatomic site and depth within the tissue. In the superficial zone, the collagen fibrils are mostly tangential to the articular surface. In the middle zone, fibrils are arranged in an intricate three-dimensional network, with many of the larger fibrils perpendicular to the surface, whereas the small fibrils fan out like branches on a tree. In the deep and calcified zones, the fibrils are larger, are predominantly perpendicular to the surface, and form a more rigid mesh, which may be impregnated by crystals of hydroxyapatite in the calcified cartilage (McIlwraith and Trotter, 1996).

The superficial layer appears to form a wear-resistant protective diaphragm that can withstand tension in the plane of the articular surface. In contrast, the fibrils in the middle and deed zones are organized to provide increased resilience to compressive loading because of their tendency to perpendicular alignment to the tidemark (McIlwraith and Trotter, 1996).

Thirteen types of collagen have been isolated from the articular cartilage matrix including types II, V, VI, IX, X and XI collagen (Mayne and Irwin 1986; Mayne 1989). Type II is the primary of equine articular cartilage comprising 85-90% of the total collagen content (Vachon et al., 1990). The function of type II collagen is to provide tensile stiffness to the cartilage. Although all of the fibrillar collagen types (Type II, V and XI) follow a similar pattern of synthesis and formation, differences among these types exist in transcriptional splicing events and post-secretional enzymatic events to give rise to collagen isotypes and variations in the number and locations of collagenous and non-collagenous regions within the final fibril (Eyre et al., 1992).

The rate of complete collagen fibril turnover is considered extremely slow with values in dogs and humans approaching 300 and 1800 days, respectively (Treadwell and Mankin, 1986). Normal collagen turnover is mediated through activated specific collagenases secreted by chondrocytes. The activity of cartilage collagenases is controlled by proteolytic enzymes

which cleave inactive procollagenase precursors and by specific inhibitors of colllagenase present within the articular cartilage matrix.

The widely dispersed and heterogenous population of proteoglycans and glycosaminoglycan present within the articular cartilage ground substance provides compressive stiffness to the articular cartilage. The most recognised of the proteoglycans species is the high molecular weight aggrecan, so named for its ability to bind to the hyaluronic acid (hyaluronan, HA) component portion of the articular cartilage. Aggrecan is among the most studied proteoglycans and contains chondroitin sulphate and, in most species, keratin sulphate. The non-covalent association of the proteoglycan core protein with HA is stabilised by the additional non-covalent association of a link protein, resulting in the aggregate stable form of proteoglycan. The lack of link protein does not necessarily imply lack of the aggregating ability, but does decrease the ability of the proteoglycans to remain aggregated to HA (Rosenberg and Buckwalter, 1986; Hardingham and Bayliss, 1990, McIlwraith and Trotter, 1996).

The presence of highly charged, polyanionic sulphate and carboxy groups on the glycosaminoglycans, chondroitin sulphate and keratan sulphate, combined with the high molecular weight of the proteoglycan aggrecan, is responsible for the large osmotic pressure created within the articular cartilage. The effect of the glycosaminoglycan is to draw water into the tissue and to expand the collagen matrix. The resistance of the tightly packed collagen fibrils to this expansion provides the articular cartilage with a great capacity to resist compressive forces (Greenwald et al., 1978). Alterations in the structure or quantity of the glycosaminoglycan will result in a change of compressive stiffness of the articulate cartilage.

The most important glycosaminoglycans in articular cartilage are chondroitin-4-sulphate, chondroitin-6-sulphate, keratan sulphate and hyaluronic acid. Dermatan sulphate has been detected in low quantities in articular cartilage, but is not considered to play a major role in its biomechanics (Rosenburg, 1992). Glycosaminoglycan synthesis initiation and elongation are late events in the cellular processing of the proteoglycans, occurring within the Golgi apparatus of the chondrocyte. Its attachment to the proteoglycans core protein is not a requirement for proteoglycans cellular translocation, secretion or aggregating ability to hyaluronan (hyaluronic acid).

Hyaluronan (HA) is unique among the proteoglycans in that it is not synthesized attached to a core protein. It is the only glycosaminoglycan that is not sulphated. Much of it is associated with aggrecan, in the form of large aggregates of as many as 100 aggrecan monomers associated with a single hyaluronan molecule (Hardingham and Bayliss, 1990). In contrast to the other glycosaminoglycans, HA sythesis is initiated along the intracellular surface of the plasma membrane (Prehm, 1986).

In normal articular cartilage the proteoglycans are actively turned over, resulting in a balance between synthesis by chondrocytes and breakdown by extracellular proteinases (McIlwraith, 2005). A proportion of proteoglycans molecules will undergo proteolytic cleavage by chondrocyte-derived stromelysin. Stromelysin is synthesised at low levels in normal circumstances and requires enzymatic activation. Enzymatic cleavage of proteoglycans by stromelysin generates large chondroitin-sulphate rich fragments (Pelletier et al., 1988). These fragments may diffuse out of the articular cartilage, but some may be endocytosed by nearby chondrocytes and further degraded within chondrocytic lysossomes. The reported half-lives of radiolabelled proteoglycans have ranged from as little as 3.5 to 20 days, probably reflecting the presence of different proteoglycan synthetic pools (Makin and Lippiello, 1969; Treadwell and Mankin 1986).

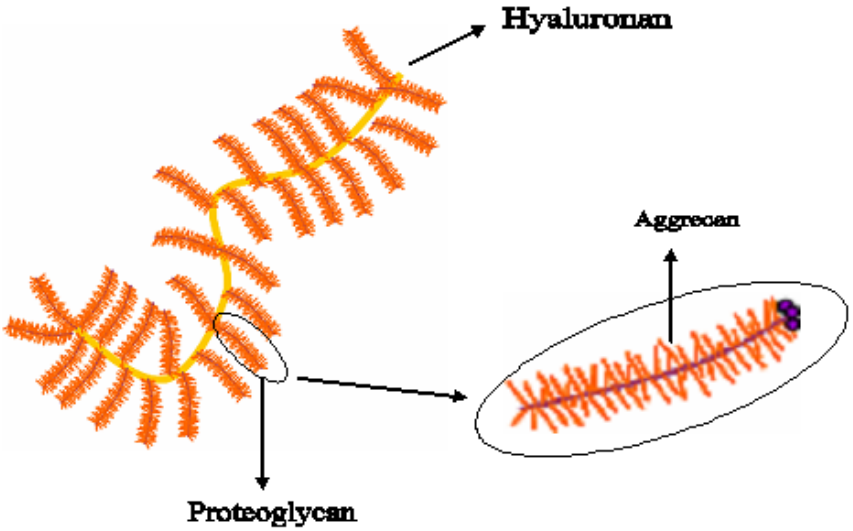

Figure 1. Aggregate of proteoglycans and hyaluronan, which provide the articular cartilage with compressive stiffness.

Proteoglycans are not always aggregated. There are also nonaggregating proteoglycans: chondroitin sulphate – dermatan sulphate proteoglycans and keratin sulphate proteoglycans (Hardingham and Bayliss, 1990). Some other noncollagenous and nonproteoglycan glycoproteins have also been identified in the extracellular matrix (Fife and Brandt, 1993).

2.2 Joint disease in the horse

Joint injuries often arise as a consequence of physical disruption of tissues due to mechanical stress. This may occur when the joint is exposed to loads that exceed physiological limits. Loss of articular cartilage is the major cause of joint dysfunction and disability in joint disease. Damage to cartilage can occur in athletic horses as a result of physical stresses of abnormal intensity (excessive magnitude), duration (excessive number of cycles) or frequency (Pool, 1996; Riggs, 2006).

Damage to joints can occur when normal joints are submitted to abnormal loads, but also when abnormal joints are submitted to normal loads. Articular tissues compromised by existing disease are more prone to physical damage due to a structural weakened matrix and/or impaired ability to initiate reparative processes due to cell death or dysfunction (McIlwraith, 1996).

Horses with impropriate conformation of the limbs can also develop joint problems when submitted to normal training program due to an abnormal direction of the load. Conformational abnormalities, including poor foot balance, and other factors likely to affect the rate of loading of the limbs, such as track to surface and shoeing, can also be expected to influence the incidence of joint injury (Turner and Anderson, 1996).

Repetitive high-speed exercise resulting in cyclical impact loading of a joint is most likely to result in maximum injury. After each cycle of load there is a lag phase as the cartilage regains its relaxed dimensions, which is associated with redistribution of fluid within the matrix and a high rate of cyclical loading may interfere with full function of this mechanism (Mow and Hung, 2003). There are reparative mechanisms that take days or weeks to complete. Repeated impact loading on a daily basis is more likely to overwhelm such mechanisms, leading to cumulative damage (Burr, 2003; Hunziker and Tyler, 2003).

2.2.1 Osteoarthritis (OA)

The term arthritis (or arthrosis) can be used for any affliction of the joint. It is an all-encompassing term that covers a great number of entities in the horse. Etiologic factors may be divided in three groups: traumatic, developmental (osteochondrosis), and infectious (septic). As the severity of any individual condition increases, or treatment methods are unsuccessful, the likelihood of the development of osteoarthritis increases. Osteoarthritis is characterised by progressive and permanent disease of articular cartilage and associated bone and soft tissues of the joint. The term is specific and represents one end-stage of a series of pathological processes, which may be initiated by joint trauma (McIlwraith and Vachon, 1988; Riggs, 2006).

In the beginning it was thought that osteoarthritis involved primary degradation in the articular cartilage and that all other events were secondary, but it is now clear that this old concept was wrong. The sequence of events is variable but synovial membrane, fibrous joint capsule, articular cartilage, subchondral bone and intra-articular ligaments can all be involved in primary and/or secondary roles (McIlwrath, 2002). Indeed, McIlwraith and van Sicke (1981) demonstrated the ability of primary synovitis to produce early degradation of articular cartilage in the horse, even without any instability or traumatic injury.

Injury to articular cartilage may arise as a consequence of any one or a combination of the following pathological processes:

* Direct mechanical damage to matrix (Thiabault et al., 2002): disruption of the fibrillar collagen network occurs following joint trauma (Pool, 1996). High strain rates associated with impact loads can cause fluid pressures within the cartilage matrix that exceed the restraining capacity of the collagen network, causing its tensile failure (Morrel and Quinn, 2004). This may facilitate the action of collagenases by providing more favourable access to their target substrates (Patwai et al., 2003).

* Destruction of matrix through effects of locally produced catabolic enzymes (McIlwraith, 1996): up-regulation of catabolic processes is a fundamental aspect of cartilage injury and disease. Chondrocytes and synoviocytes are a rich source of inflammatory mediators and enzymes capable of degrading the extracellular matrix. Under physiological conditions, chondrocytes regulate a dynamic metabolic steady state in which anabolic and catabolic

processes are balanced; there is a steady turnover of molecules, which is essential for maintenance of a healthy matrix (Kuettner and Thonar, 2001).

* Damage to chondrocytes resulting in death (due to necrosis or apoptosis); altered synthesis of selective matrix molecules (up and down-regulation) and of destructive enzymes and inflammatory mediators; phenotype modulation, associated with an overall altered gene expression profile (Aigner et al., 2002): chondrocytes are sensitive to excessive mechanical deformation and even solitary loads of sufficient magnitude will severely impair chondrocyte metabolism or cause cell death. The consequences of loss of chondrocytes are compounded by the fact that proliferative activity of remaining chondrocytes id very poor (Aigner et al., 2001) and the cell clusters which do form are unlikely to add significantly to matrix anabolism (Aigner et al., 1997). Various mediators of apoptosis are under investigation. Nitrous oxide, a putative regulator of apoptosis in a number of cell systems (Chung et al., 2001) is elevated in diseased cartilage (von Rechenberg et al., 2000; Clegg and Mobasheri, 2003). Interleukin-I and TNF-α are potential inducers of cell death, although their role in this capacity in cartilage is still to be elucidated (Aigner et al., 2002). Several studies using electron microscopy, flow cytometry, and the DNA fragmentation assay have shown that apoptosis is the major pathway of the chondrocytic death in the articular cartilage of human osteoarthritis (Blanco et al., 1998; Hashimoto et al.,1998a/b/c; Lotz et al., 1999).

* Increased risk/rate of cartilage damage through change in mechanical environment (subchondral bone sclerosis; Radin and Rose, 1986).

Articular cartilage degeneration is always present in osteoarthritis. It is recognized grossly or arthroscopically by the presence of fibrillation, erosion (partial and full thickness), and wear lines in the articular cartilage (McIlwraith, 1982). This may be accompanied by subchondral bone sclerosis, subchondral cystic lesions, penetrating of the tidemark by blood vessels, focal osteonecrosis, and periarticular osteophyte formation. Biochemically, there is reduction in the proteoglycan content of the articular cartilage as well as changes in the structure of the proteoglycans (alterations in their size and degree of extractability), changes in their degree or aggregation, an increased water content, alterations in collagen structure and breakdown of collagen framework (McDevitt and Muir, 1976; Sweet et al., 1977; Vasan, 1980, Dodge and Poole, 1989).

One of the more common abnormalities identified in equine joints, particularly metacarpo-/tarsophalangeal joints of performance horses, is the presence of wear lines. These gross pathological features are associated with chondrocyte death, loss of proteoglycans from the matrix and disruption of collagen fibres in the superficial cartilage layer (Pool, 1996).

It has been proposed that enzymatic degradation of articular cartilage precedes morphologic breakdown and plays a central role in equine joint disease (McIlwraith and Sickle, 1981; McIlwraith, 1982). This biochemical degradation is considered to represent an imbalance of the normal homeostatic processes within the matrix of the articular cartilage (McIlwraith and Vachon, 1988). Homeostasis of normal cartilage represents a delicate balance between degradation and synthesis of extracellular matrix components to maintain the functional integrity of the joint (Howell and Pelletier, 1993). Proteoglycans are fairly rapidly exchanged, whereas the turnover of type II collagen is slow. Matrix-degrading enzymes are a focal part of the degradation of articular cartilage matrix and many of the proteinases are released by cytokines.

Metalloproteinases are considered to play a major role in the degradation of the extracellular matrix (Poole, 1993; Nagase and Woessner, 1993). They are characterized by a requirement for zinc in their active site. Calcium is also required for the expression of proteinase activity but does not reside in the active site (Sapolsky et al., 1974, 1976; Werb, 1992). Collagen is mostly degraded by tissue collagenase (MMP-1) and gelatinases (MMP-2 and MMP-9). Stromelysin 1 (MMP-3) degrades aggrecan, fibronectin, types IX and XI collagen, link protein, decorin and elastin, and it activates procollagenase (Gadher et al., 1988; Werb, 1989). Other proteinases like serine, cysteine and aspartatic has also been identified (Werb, 1989; Treadwell et al., 1991).

A useful classification of osteoarthritis (OA) in horses recognizes four main categories of the disease based on joint characteristics and predisposing factors (McIlwraith, 1982 and 1996):

* OA of high motion joints (having a high range of movement, such as fetlock joint), which is thought to be induced by trauma to the joint capsule after repetitive overextension of the joint;

* OA of low-motion, high-load joints, which occurs in the proximal interphalangeal joint affected by ring-bone and in a distal intertarsal joint developing bone spavin;

* OA developing secondary to predisposing disorders such as osteochondrosis, articular fractures, infectious arthritis and the like;

* Nonprogressive, idiophatic cartilage erosions, observed as incidental findings in arthroscopic and necropsy examinations.

Studies in which a layer of bone was left attached to the cartilage or not (Torzilli et al., 1999; Patwai et al., 2001; Lewis et al., 2003) showed that sudchondral bone plays a protective role, which be partly accounted for by it acting as a shock absorber (Radin, 1999) and partly by it physically anchoring the deeper layers of cartilage, thereby limiting load (Lewis et al., 2003).

2.2.2 Osteochondritis dissecans (OCD)

Osteochondrosis is a multifocal disease of epiphyseal cartilage affecting both the articular epiphyseal cartilage complex, immature joint cartilage covering the ends of growing long bones, and the growth (physeal) plate of bones. It is a disturbance in the process of endochondral ossification of the articular epiphyseal complex. This disturbance leads to irregularities in the ossification front and the subsequent formation of thick cartilage plugs that may become necrotic and detach, forming osteochondral fragments or joint mice. In the veterinary literature the term “osteochondrosis” (OC) is used for all stages and presentations of the disease, “osteochondritis dissecans” (OCD) is used when loose or semi-loose intra-articular fragments are present. Clinically, animals will mostly present with moderate to severe joint effusion. Lameness may be present, but often is not, and lesions may even remain silent for years. Similar conditions are seen in broilers, turkeys, dogs, cattle, pigs and man, but with varying prevalence (van Weeren, 2006).

This disease has become more attention just since the late 1960s and the early 1970s, although it was first described by Nilsson in 1947 (Birkeland and Haakenstad, 1968). Its great importance is due to the big prevalence and significance in athlete horses. The prevalence in Warmblood horses, Thoroughbreds and Standarbreds is about 25% (van Weeren, 2006).

Whereas OC initially was seen as a rather static condition, this view changed when more comprehensive longitudinal radiographic studies were undertaken that showed that small

lesions could grow larger in young animals (Dabareiner et al., 1993). Such a process of maturation and further aggravation of lesions is common to many pathological processes, but the observation that, apart from becoming more severe, small lesions could become less clear and eventually disappear (Carlsten et al., 1993) gave OC a special character. Lesions can be initiated by a variety of factors that include genetic predisposition, nutritional factors, biomechanical influences and conformation, which may, but need not, act in concert. The lesions trigger a reparative response that may result in the partial or complete healing of the lesions. Because of the fact that the effectiveness of this reparative response is related to the remodelling rate of the extracellular matrix of cartilage, which is known to decrease rapidly after birth (Fig. 1), the capacity to repair lesions diminishes quickly and, after a certain age, lesions cannot be repaired anymore (van Weeren and Brama, 2003). This explains the fact that, after an initial dynamic period in which lesions come and go, the situation stabilises after a certain age. This age may vary per joint and per affected site in a joint. In the tarsocrural joint lesions tend to stabilise after 5 months. In the femoropatellar joint lesions appear later and also stabilise later, after 8-9 months (Dik et al., 1999).

Osteochondrotic lesions may show up in a wide variety of clinical manifestations. The typical OC case is the yearling that is not lame but presents with severe effusion of the femoropatellar or tarsocrural joint and shows a radiographic OC lesion, with or without fragmentation and often bilaterally. However, OC may present differently as well. In some cases OC becomes already clinically manifest at foal age and may present as a severe lameness with a devastating radiographic lesion, mostly located on the lateral trochlear ridge of the femur. These cases cannot be treated. Alternatively, OC may not become clinically manifest until the age at which horses are put into training, which may be not until their 3rd or 4th year in case of Warmblood horses. In some cases, horses may even not present clinical signs of OC until much later.

Osteochondrosis is most commonly treated by arthroscopic surgery during which loose fragments are removed and/or other lesions are debrided. It is questionable, however, to what extent treatment is necessary when lesions are not too severe and lameness is not present. Although it has been stated that the presence of osteochondrotic lesions will affect overall performance (Stock, 2004), this may depend on the use of the horse and there are many studies that report no significant influences of OC on performance, especially in the racing breeds (Storgaard-Jørgensen et al. 1974; Kane et al. 2003; Langlois et al. 2006). The presence

of osteochondrotic lesions will, however, affect the economical value of animals, especially in Warmbloods, and this is an important reason to opt for treatment (van Weeren, 2006). Given the dynamic character of OC, it is advisable to wait with surgical intervention until an age of at least one year because no major changes in the appearance of lesions will take place after that age. In severe cases in foals, earlier intervention may be indicated.

Prevention measures are always more rewarding than therapeutic interventions. In recent years several large radiographic population screenings have been undertaken in order to better define the genetic background of OC and to identify possible genetic markers. Given the dualistic character of OC with its pathogenesis and repair process, it cannot be expected, however, that a single genetic marker will be discovered. Another promising track is the quest for biomarkers in either synovial fluid or serum. Billinghurst and colleagues (2004) could discriminate between severely and less severely affected foals by the analysis of anabolic and catabolic collagen markers in serum. Recently, osteocalcin levels at the very early age of 2 weeks were shown to have predictive potential with respect to the development of OC at a later age (Donabédian, 2006). Early identification of animals that are prone to develop osteochondrotic lesions would allow for the timely modification of environmental factors such as nutritional level on order to reduce the risk of the development of OC. Most estimations of heritability of OC are around 0.25 (Schougaard et al., 1990; Brendov 1997), so more effect can be expected from the manipulation of environmental factors than from genetic selection.

2.2.3 Infectious (septic) arthritis (IA)

Joint infection frequently produces the most exaggerated response that is possible in the articular environment. This response includes an acute inflammatory as well as a delayed immunologic component due to the presence of bacterial (or other) antigen. Many factor determine the degree of articular response (host, bacterial number, bacterial virulence), the degree of joint destruction (host age, host debilitation, degree of inflammation, duration of infection, bacterial virulence, pre-existence of joint disease), and the response to treatment (all the above factors plus therapy selection; McIlwraith, 1983; Bertone and McIlwraith, 1987; Ho, 1993).

The normal synovial membrane is capable of controlling an impressive number of bacteria and not permitting bacterial proliferation and the resultant inflammation. However, organisms can overcome these defenses if the numbers overwhelm the synovium, the organism is more virulent, or the synovium is compromised. Results from the equine studies show that the most common isolates from all equine joint infections were Enterobacteriaceae and *Escherichia coli*. Gram-positive organisms were isolated almost as frequently as gram-negative, and more frequently in adult horses with infection (Schneider et al., 1992).

The acute inflammatory response seen in most cases of infectious arthritis is stimulated by the host's recognizing the organism as foreign and initiating a rapid influx of inflammatory cells, mostly neutrophils, to eliminate the infection. Destructive enzymes such as lysozyme, elastase, cathepsin G, gelatinase, and collagenases can be released by the neutrophils, or are released upon its death. The activation of the enzymes occurs and is amplified by the activation of plasmin in the joint fluid. Many non-specific mediators of inflammation enter the joint cavity because of disruption of the blood.synovial barrier and amplify the activation of the kinin, coagulation, complement, and fibrinolytic systems and contribute to the cascade of events that amplify the inflammation and activate synoviocytes and chondrocytes. Chondrocyte responds with its own matrix metalloproteinases and decreased proteoglycans synthesis (Bertone et al., 1987; Palmer and Bertone, 1994).

Joint infection produces the classic cardinal signs of inflammation centred around the joint involved. Heat, swelling (oedema and joint effusion), pain in palpation and flexion, erythema of surrounding skin, and a severe lameness. The presence of these clinical signs, particularly with a history of a recent joint infection, recent surgery, or a recent wound, warrants immediate diagnostic tests and treatment (Bertone et al., 1992; Schneider et al., 1992).

It is well recognized in medicine that therapy selection for infectious arthritis should be immediate and include a combination of antimicrobial and joint drainage protocols, but each individual case requires the selection of treatment options to maximize efficacy, minimize morbidity and toxicity, minimize patient distress, and address cost restraints (McIlwraith, 1983; Bertone and McIlwraith, 1987; Ho, 1993).

2.2.4 Navicular syndrome (NS) and Podarthritis (PO)

Navicular syndrome is a chronic forelimb lameness associated with pain arising from the distal sesamoid or navicular bone. It is characterised by degenerative changes in structure, composition and mechanical function of the cartilage, subchondral bone and surrounding soft tissues, like the deep digital flexor tendon (DDFT), impar ligament, collateral ligaments, podotrochlear (navicular) bursa and distal interphalangeal joint (Rijkenhuizen, 2006).

Due to a variety of clinical presentations, it is likely that there are a number of different clinical conditions, of different aetiologies, that give rise to pain in the navicular apparatus. According to several studies, Prof. Dr. Bodo Hertsch has described three different clinical presentations (Hertsch et al., 1982; Hertsch and Höppner, 1999; Zuther und Hertsch, 2004):

"Podothrochlose" – degeneration of the Facies Flexoria of the sesamoid bone. At the last stage, there is a fracture in the central part of this bone, without deformities in sesamoid canals. It occurs often in combination with alterations in the deep digital flexor tendon and the podothrochlear bursa.

"Insertion desmopathy" – alterations in the insertion of the navicular ligaments. It can be recognized in radiography as an exostosis along the insertion of the sesamoid ligaments.

"Navicular disease with involvement of the distal interphalangeal joint" – it appears radiographic as canals of different shapes at the distal border of the navicular bone, which can be visualized in Oxpring exposure (1935).

It is well recognized that in association with advanced navicular disease, fibrillation of the dorsal aspect of the DDFT, with or without adhesion formation between the tendon and the navicular bone, are common features (Dyson et al., 2006). Clinical studies using magnetic resonance imaging (Dyson et al., 2005) and *post mortem* studies (Blunden et al., 2006a and b) have demonstrated that there may also be abnormalities of closely related structures, including the collateral sesamoidean ligaments, distal sesamoidean impar ligament and navicular bursa.

It can result in an insidious onset , slowly progressive bilateral forelimb lameness, or an acute onset, relatively severe unilateral forelimb lameness, each with a variety of different

radiological manifestations. It can be seen in young horses, just commencing work or in mature riding horses. It is also seen in horses with vastly different distal limb conformation (Dyson et al., 2006).

Navicular disease has not been reproduced experimentally, therefore, all proposed aetiologies remain speculative. Earlier theories suggesting a vascular aetiology with arteriosclerosis (Rijikenhuizen et al., 1989), or thrombosis, resulting in ischemia within the navicular bone (Colles and Hickman, 1977), have been rejected due to failure to identify ischemia bone or thrombosis, failure to reproduce clinical signs or pathological changes by occluding blood supply to the bone and expanding evidence demonstrating increased bone modelling (Ostblom et al., 1982; MacGregor, 1984; Pool et al., 1989; Wright et al., 1998).

Podarthritis can be diagnosed by the manometry of the distal interphalangeal joint (Schött, 1989; Hertsch, 1991; Höppner, 1993. The high pressure of this joint can lead to alterations in the navicular bone and in navicular apparatus (Hertsch, 1983; Bowker and Van Wulfen, 1996).

2.2.5 Synovitis and capsulitis

Synovitis and capsulitis are common initial changes in the joints of athletic horses and have been presumed to be associated with repeated trauma. It is also presumed that synovitis occurs in conjunction with damage to the fibrous capsule, and severe injury to the latter can cause instability (McIlwraith and Sickle, 1981; McIlwraith, 1982). Synovial membrane itself is mechanical weak and has no known biomechanical role, but it is recognized that synovial injury may have consequences for the physiology and pathophysiology of the joint. It has been suggested that some of these injuries affect diffusion across the synovial membrane and others have a primary effect on the metabolism of the synoviocytes. Mechanically damaged synoviocytes may release degradative enzymes and cytokines. These situations could alter the intraarticular environment and affect articular cartilage (Evans, 1992).

High intraarticular pressures in injured joints could be sufficient to impair the flow of blood through the synovial capillaries. This would not only lower the oxygen tension in the joint but could potentially lead to reperfusion injury (Levick, 1990) and the production of oxygen-

derived free radicals could damage both cellular and matrix macromolecules (Allen et al., 1989). In addition to direct injury that may occur to the synovial membrane, the reaction of synovial membrane to articular cartilage damage or other mechanical destruction of intraarticular tissues is well recognized. The presence of cartilaginous wear particles increases the cellular production of prostaglandin E_2 (PGE_2), cytokines, and the neutral metalloproteinases (collagenases, stromelysin, and gelatinase). It has also been shown that proteoglycans released into synovial fluid cause synovitis (Evans et al., 1982; Boniface et al., 1988)

The condition of primary synovitis in the absence of gross articular is a common clinical entity in the carpal and fetlock joints of young, actively training racehorses (McIlwraith, 1987). Given current race training regimes and economic limitations, the rest period required to permit a good inflammatory response and recovery is often shortened or neglected. The result is often an excess or prolonged production of the mediators responsible for inflammation (the healing process) and the subsequent production of enzymes potentially deleterious to components of the articular cartilage matrix (Palmer and Bertone, 1994).

All chronic joint inflammations may ultimately result in an imbalance of normal articular cartilage metabolism, specifically an increase in the destruction of matrix with or without a concurrent increase in matrix synthesis. Many of the current therapeutic regimes for acute synovitis may potentiate the original inflammation, cause possible contamination of the joint, or be deleterious to normal cartilage metabolism. The end result is an alteration in the biomechanical properties of articular cartilage, possibly contributing to the conditions of osteochondrosis dissecans, traumatic fracture and/or degenerative joint disease, thereby limiting the athletic capabilities of the animal (Palmer and Bertone, 1994).

2.3 Synovial fluid analysis

2.3.1 Synovial Fluid

The synovial fluid is an ultrafiltrate of plasma; most of its ions and molecules are present in plasma, with exception of hyaluronan (hyaluronic acid or HA), which is at a high concentration in synovial fluid. Large proteins are generally excluded from intrasynovial

space. Synovial fluid contains mononuclear cells (synovial lining cells, monocytes, and lymphocytes constitute 90% of the total, and remainder of the cells are polymorphonuclear leukocytes. But not more than 500 nucleated cells per μL (McIlwraith and Trotter, 1996).

The intima is the fine barrier between the intravascular and interstitial fluid of the subintima, and the synovial fluid. The absence of an intimal basement membrane and the proximity of the capillaries to the intimal surface facilitate the exchange of solutes. Fluid exchange between plasma and synovial fluid seems to be governed by Starling forces, i.e., a hydraulic or hydrostatic pressure difference and a colloid osmotic pressure difference between plasma and synovial fluid (Levick, 1984). An intermediate joint angle, a net imbalance of pressure has been shown to favour ultrafiltration of plasma into the joint (Knox et al., 1988). Synovial fluid volume balance in the long term depends on motion-driven lymph flow and the effect of the joint angle on synovial fluid pressure (Levick, 1987).

The endothelium prevents large molecules from leaving the synovial capillaries. The synovial permeability barrier to small molecules is maintained by the narrow space between synoviocytes and the composition of the extracellular matrix. Hyaluronan also functions as a barrier to small molecular exchange (Kerr and Warbuston, 1985).

2.3.2 Analysis

The collection and evaluation of synovial fluid was first used in veterinary medicine for the diagnosis of large animal lameness and joint disease (MacWilliams and Friedrichs, 2003). Synovial fluid analysis is of value to the equine practitioner as an aid in determining cause, type (i.e., non-inflammatory joint disease as opposed to inflammatory joint disease), and probable duration of joint disease in horses (Ropes, 1957; Hollander et al., 1961; Cohen, 1967). It provides useful information in the therapeutic management of the various joint disorders encountered in horses. Synovial fluid analysis is also recommended in disorders characterized by persistent or fluctuating fever of unknown origin, shifting leg lameness, or generalized malaise in which arthralgia is suspected (Willard, 1989). Careful evaluation and interpretation of results of this analysis can provide useful guidelines in rendering a prognosis (West et al., 1963; Van Pelt, 1971).

Pathologic synovial fluids reflect changes produced in synovial membranes and articular cartilages by disease processes (Ropes and Bauer, 1953). Its aspiration provides not only a means of obtaining samples for analysis, but also immediate symptomatic relief from the local discomfort created by increased intraarticular pressure and serves to remove pathologic synovial effusion, whether the effusion contains cartilage fibrils and fragments (degenerative joint disease, osteochondritis dissecans, and osteochondromatosis), blood (acute to subacute traumatic arthritis), or septic material (idiopathic septic arthritis and infectious arthritis caused by pyogenic microorganisms; Cohen, 1967).

Arthrocentesis and joint fluid analysis are integral to the clinical evaluation of not only primary joint disorders but systemic diseases in which joint effusion is part of the clinical picture. The samples should be collected as aseptically as possible, not only to prevent infection of the joint at the time of arthrocentesis, but to avoid contamination of the sample and any subsequent bacterial growth that could alter its physical, chemical, and cytologic properties. Cytologic detail of synovial fluid leukocytes is best preserved if a portion of the sample is transferred to a tube containing either sodium or tripotassium ethylenediaminetetraacetate (EDTA). Synovial fluid from non-inflammatory forms of joint disease do not clot, however, cellular detail is best preserved by the addition of EDTA. It should be remembered that the salts of EDTA inhibit ALP activity and cause minor depolymerization of the hyaluronic acid moiety of synovial fluid, with resultant reduction in viscosity and mucinous precipitate quality (Van Pelt, 1974).

The laboratory assessment of synovial fluid includes an evaluation of physical characteristics, chemical tests, cell counts, and differential count (Hardy and Wallace, 1974).

2.3.3 Physical features

Evaluation of physical features includes a visual examination of colour, turbidity, viscosity and volume.

* Appearance

Normal synovial fluid in the horse is pale yellow or yellow straw, clear and free of flocculent material.

In degenerative joint disease samples may vary from pale yellow and clear to pale yellow and opaque, with some flocculent material. Occasionally, in degenerative joint disease, osteochondromatosis, or osteochondritis dissecans, cartilage shreds give the joint sample a markedly flocculent appearance.

Red or red-tinged fluid indicates haemorrhage in the joint associated with trauma or inflammation or haemorrhage that has occurred during the collecting process.

Dark yellow or pale amber samples are most commonly associated with chronic traumatic arthritis and are indicative of prolonged and low-grade haemorrhage, or of massive haemorrhage at some time prior to aspiration. Erythrocyte breakdown and the subsequent formation of unconjugated bilirubin impart a dark yellow or amber colour to the sample. Serosanguineous samples are indicative of continuous, minor haemorrhage of recent origin (van Pelt, 1974; McIlwraith and Trotter, 1996).

*Viscosity

Normal viscosity of synovial fluid is a result of the amount and polymerization of hyaluronic acid, which is a glycoprotein (Bonn, 1997). Viscosity is an indicator of the lubricating properties of the fluid in the joint, and decreased viscosity can be caused by several factors. Hyaluronic acid content can be reduced by decreased production as a result of synovial membrane damage, dilution by the influx of plasma or fluid, or degradation by white blood cells or bacteria. Intra-articular injection of drugs or joint lavage can also reduce fluid viscosity.

A drop of fluid between the thumb and finger should form a strand that is at least 2.5 cm long when the digits are pulled apart. A drop from a needle and syringe should produce a strand at least 2.5 cm before it breaks.

* Clot formation

Synovial fluid from normal joints does not clot in a tube or syringe but does tend to form a gel. It is important that gel formation not be confused with a clot. Agitation of the gel causes the sample to return to its fluid state. This reversible fluid-gel formation is called thixotropism (Fernadez et al., 1983).

Clotting rarely occurs in samples collected from horses affected with degenerative joint disease, osteochondritis dissecans, osteochondromatosis, or chronic traumatic arthritis. Samples collected from joints affected with acute or subacute traumatic arthritis, and acute to chronic idiopathic septic arthritis or infectious arthritis tend to clot rapidly (Cohen, 1967).

* Volume

Total volume of aspirated synovial fluid from any joint will generally depend directly to the size of the joint and its communication with another joint (for example, communication of the femoropatellar joint with the medial femorotibial joint; tibiotarsal joint with the proximal intertarsal joint; intercarpal joint with the carpometacarpal joint; van Pelt, 1962). Joints of young horses generally contain a proportionally greater volume of synovial fluid than do those of mature horses.

Synovial fluid volume is not appreciably increased in degenerative joint disease, osteochondritis dissecans, osteochondromatosis, or chronic traumatic arthritis (van Pelt et al, 1970 and 1971). It is markedly increased in hydrarthrosis of the stifle and tarsus, and less so in most forms of acute to subacute traumatic arthritis (van Pelt and Riley, 1969; van Pelt, 1971).

2.3.4 Chemical features

Most of the chemistry tests on synovial fluid produce results that support the cytologic and clinical assessment rather than providing new or discriminating information (MacWilliams and Friedrichs, 2003).

* Mucinous precipitate quality

The test for mucinous precipitate quality (MPQ) is a semiquantitative indicator of the amount of hyaluronic acid content based on acid precipitation. Synovial fluid collected in a plain tube or heparin tube is preferred, because the test is inhibited by the presence of EDTA (MacWilliams and Friedrichs, 2003). Both the degree of HA polymerization and its concentration in synovial fluid influence viscosity. Precipitate formation and turbigenic properties are determined by addition of 0.1 mL of 7N glacial acetic acid to 4mL of distilled water in a test tube and thoroughly mixing the solution. To the resultant 2.5% aqueous solution (pH 2.8 ant 25 C.), slowly add 1 mL of the synovial fluid sample, taking care that the sample does not come in contact with the wall of the test tube(van Pelt, 1962). The tube is gently swirled and allowed to stand at room temperature for 1 hour and graded as follows:

Normal: a tight, ropy clump in a clear solution;

Fair: soft mass in a slightly turbid and pale yellow or pale amber solution;

Poor: small, friable masses in a turbid and pale yellow or pale amber solution;

Very poor: a few flecks in a very turbid and pale yellow to dark yellow, or pale amber to dark amber, solution.

The MPQ is usually normal in degenerative joint disease, osteochondritis dissecans, osteochondromatosis, chronic traumatic arthritis, and tarsal hydrarthrosis; however, the size of the precipitate may be somewhat reduced in synovial fluid from joints affected with tarsal hydrarthrosis because of the low ratio of HA to plasma volume.

In idiopathic septic arthritis and infectious arthritis the MPQ varies from poor to very poor, with very poor MPQ most commonly encountered in the latter condition.

* Sugar content

In adult horses, under normal conditions, the sugar content in synovial fluid is closely parallels to that of the serum (van Pelt, 1962). With increasing inflammation, synovial fluid

sugar content falls below that of the simultaneous measured serum sugar content and may even approach a value of zero (van Pelt and Riley, 1969; van Pelt, 1971).

* Total protein content

Total protein content is best measured by a quantitative biochemical method. In joint inflammation, increased total protein content in the synovial fluid, especially the relative content of globulin to albumin is attributed to increased capillary permeability, which permits the higher molecular weight protein fractions, mainly globulins to enter the joint (Curtiss, 1964). Reduced quantity of hyaluronic acid or depolimerization, or both, of this essential component in the synovial fluid in joint disease, especially in inflammatory forms, permits passage and retention of proteins of increasingly higher molecular weight (Cohen, 1967; Schubert and Hamerman, 1968).

*Enzymes: Tests for determination of alkaline phosphatase (ALP), lactic dehydrogenase (LDH), and glutamic oxaloacetic transaminase (GOT) are best performed on synovial fluid after centrifugation (Sommer, 1954; Reitman and Frankel, 1957; Cabaud and Wröblewski, 1958). As a rule, there is a close correlation between its activities and the clinical severities of joint disease. The more severe the inflammatory reaction, the greater is the enzyme activity (Bartholomew, 1968).

2.3.5 Cytologic features

Fluid samples for cell counts and cytologic assessment can be stored for 24 hours in the refrigerator, but it is best to make slides immediately (Boon, 1997). If only a few drops of fluid are collected, most would agree that preparation of stained slides for microscopic evaluation provides the most useful information to the diagnostic process (Hardy and Wallace, 1974; Pedersen, 1978; Gibson et al., 1999)

Total nucleated cell counts are extremely valuable in separating inflammatory and noninflammatory arthropaties and in differentiating among the various causes of inflammatory arthritis (Ellison, 1988; Boon, 1997). Red blood cell counts provide little useful information (MacWilliams and Friedrichs, 2003).

2.3.6 Bacteriologic cultures

Aerobic and anaerobic cultures should be performed on synovial fluid wherein joint infection is suspected. Synovial fluid should be aspirated and inoculated into a blood culture bottle. Strict asepsis should be employed. The culture bottle should be incubated and plated after 1 and 7 days. Most isolates are apparent on the plates inoculated from the 24 hour broth. Culture bottles offer the advantages of convenience; enhancing organism proliferation with ideal media; diluting antimicrobial substances; containing inhibitors of antibiotics; lysing cells; and allowing a large volume of fluid to be inoculated (McIlwraith and Trotter, 1996).

One equine study however did not detect a significant difference in culture results with different methods of sample submission. Bottles should be cultured aerobically and anaerobically and susceptibility patterns obtained on all isolates (Madison et al., 1991).

Results from equine study show the most common isolates from all equine joint infections (foals and adults) were Enterobacteriaceae and *Escherichia coli*. Gram-positive organisms were isolated almost as frequently as gram-negative organisms, and more frequently in adult horses with infection (Schneider et al., 1992).

Table 1. Some properties of synovial fluid in joint disease in the horse. Osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA.

Diagnosis	Appearance	Viscosity	Total protein g/dL	Mucin	Leukocytes cells/µL
Normal	Pale yellow; clear	High	1.81 ± 0.26	good	167 ± 21
OCD	Colourless to yellow	High Low	2.1 ± 1.2	Good to fair	≤ 1000
NS	Colourless, yellow, Bloody	Low	1.7 ± 0.8	Fair to poor	≤ 1000
IA	Pale yellow to dark yellow; Turbid	Low	3.95 ± 0.79	Very poor	105 775 ± 25520
OA	Pale yellow; clear; opaque; some flocculent material	Low	1.84 ± 0.27	Good to fair	5000 -10000

Van Pelt, 1967; 1974; Korenek et al., 1992; Latimer et al., 2000

2.4 Joint disease biomarkers

Most equine joint lesions are induced by acute trauma, repetitive load or overload (Pool and Meagher, 1990). The inflammatory process results in an increase in levels of inflammatory mediators, and release of different macromolecules and their fragments into Synovial fluid and serum follow the anabolic and catabolic processes in the cartilage (Heinegard et al., 1985; Poole et al., 1986, 1994; Ratcliffe et al., 1988; Lohmander and Felson, 1997).

The terms biomarker, biochemical marker and molecular marker have all been used to describe either direct or indirect indicators of abnormal skeletal tissue turnover (Billinghurst, 2002). These markers are often molecules that are normal products and by-products of the metabolic processes occurring within the skeletal tissues. In disease, alterations occur in the balance between anabolic and catabolic processes and, consequently, concentrations of biomarkers can increase or decrease.

In joint disease, these molecules can be released into the synovial fluid when the source is articular cartilage, menisci, ligament or synovial membrane. If the underlying subchondral bone is involved, molecules from osseous tissue will usually be delivered into the bloodstream. The ability to identify and measure such markers molecules in synovial fluid, serum and urine offers researchers and clinicians the opportunity to use them as biomarkers of joint disease (McIlwraith, 2005).

According to the way they are detected, biomarkers can be subdivided into biochemical, which are demonstrated using biochemical techniques, and immunological markers, that appear to provide the most sensitive means to identify and quantify types and amounts of articular cartilage components (Fardale et al., 1986; Carrol, 1989; Frisbie et al., 1999).

According to the origin and specificity, they can be subdivided into direct and indirect.

2.4.1 Indirect Biomarkers

Indirect biomarkers are not derived principally from the tissues that make up the joint, but have the potential to influence the metabolism of these tissues or the integrity of the matrix. Examples include matrix metalloproteinases (MMPs), aggrecanases, tissue inhibitors of

MMPs, insulin-like growth factor 1, IL-1, IL-6, TNFα, hyaluronic acid (HA) and C-reactive protein (McIlwraith, 2005).

Collagenases 1, 2 and 3 (MMP-1, MMP-8 and MMP-13, respectively) have been implicated in collagen degradation. Stromelysin (MMP-3) and aggrecanases in the degradation of proteoglycans. There is good evidence in the horse that IL-1 drives the catabolic process, at least in the articular cartilage, by stimulating the release of MMPs, aggrecanases and PGE_2.

Studies have shown that the presence of IL-6 in synovial fluid was sensitive and specific of joint disease, whereas TNFα and IL-1β were no more useful than white cell counts in screening for joint disease. The role of TNFα in articular cartilage degradation is controversial (Joughlin et al., 2000; Bertone et al., 2001; van de Boom et al., 2004a)

MMPs play an important role in remodelling and degradation of both the PG and the collagen component of extracellular matrix of articular cartilage. MMP-9 monomer and dimer were significantly increased in synovial fluid from joints with severe cartilage alterations (Jouglin et a., 2000). An increase in total MMP activity was also correlated with the severity of cartilage change (Brommer et al., 2003; van de Boom et al., 2005). However, interpretation should have done carefully because other factors like repeated arthrocentesis could also lead to an increased MMP activity (van de Boom et al. 2004a, 2005).

Hyaluronate (hyaluronic acid) is somewhat intermediate between an indirect and direct marker. While it is found in the cartilage matrix, that source is minor compared with its synthesis by synoviocytes (Woessner, 1991). Elevated HA has been correlated with radiographic progression of joint disease (Paimella et al., 1991) and elevated plasma levels were demonstrated in patients with osteoarthritis, rheumatoid and experimental arthritis compared to controls (Bjork et al., 1989; Goldberg and Rubin, 1989; Goldberg et al., 1991).

Although these indirect biomarkers have been measured in equine joints (Clegg et al., 1997a,b; van de Boom et al., 2004a,b; Brama et al., 2004) and are useful in a research situation and experimental studies of osteoarthritis (Frisbie et al., 1997; Kawcak et al., 1997), it is considered that they are of minimal clinical value for the assessment of the amount of articular damage or defining the status of the joint, because of its unespecificity (Ray et al., 1996). For this purpose, direct biomarkers are more promising.

2.4.2 Direct Biomarkers

Direct biomarkers originate mostly from cartilaginous or bony structures or are enzymes that are active only in these structures. They provide specific information about alterations in cartilage matrix anabolism and catabolism (Thonar et al., 1999). They consist principally of breakdown products of type II collagen and PG fragments, which are liberated in increased concentrations into the synovial fluid and ultimately into the serum (Ray et al, 1996).

Direct biomarkers of anabolic processes of cartilage metabolism include carboxypropeptide of type II collagen (CPII) and chondroitin sulphate (CS).

Direct biomarkers of catabolic processes of cartilage metabolism include type II collagen fragments, glycosaminoglycans (GAGs), keratan sulphate (KS), chondroitin sulphate (CS) and cartilage oligomeric matrix protein (COMP).

Direct biomarkers of anabolic processes of bone metabolism include carboxy and amino terminal propeptides, osteocalcin and bone-specific alkaline phosphatase (BAP).

Direct biomarkers of catabolic processes of bone metabolism include type I collagen nonhelical telopeptide (ICTP), type I collagen C-telopeptides (CTX), bone sialoprotein (BSP) and deoxypyridinoline (DPYR).

2.5 Synovial fluid particle analysis

Studies revealed that radiographs, in general, underestimate the extent of cartilage damage, therefore, radiological features only are not enough to give clinicians a confident prediction of the status of articular cartilage. Its deterioration begins long before a radiological diagnosis can be made (Chan et al., 1991; Fife et al., 1991; Blackburn et al., 1994).

It has been known for decades that cartilaginous fragments occur in the synovial fluid (Horowitz, 1948). Pathologic changes and the accompanying mechanical destruction of articular surfaces can produce significant cartilage particles in the synovial fluid (Tew, 1980). Several researchers have suggested that the principles of wear particle analysis would provide a means of assessing the functional integrity of articular surfaces, as well as identifying lesions or deterioration of the articular cartilage (Mears et al., 1978; Evans et al., 1980). The

pathologic changes associated with arthroses and a number of diverse joint diseases of both humans and animals have been shown to produce significant debris within the joint capsule (van Pelt, 1974; Yehia and Duncan, 1975). The presence and morphology of such fragments have been suggested as a possible indicator of the extent of articular damage (Muirden, 1970; Tew, 1980).

Evans and coworkers, 1980, have demonstrated the feasibility of recovery and identification of cartilage fragments from osteoarthritic joints and have supported the potential diagnostic use of such analysis. Also in 1980, Tew has investigated the diagnostic implications of these particles in the synovial fluid of biomechanically impaired joints and found it an accurate indicator of lesions of the articular surfaces.

The identifying characteristics of cartilage fragments are not rapidly altered by the Synovial fluid. Although the ultimate fate of articular debris within the joint is not totally understood, such particles must eventually be reabsorbed. Cartilage particles greater than 20 microns long remain virtually unchanged in synovial fluid for a week (Tew, 1980).

Ferrographic analysis has shown interesting correlation between cartilage damage and cartilage particles in human joints (Evans et al., 1981a/b and 1982). Fractal methods have been applied to characterize the complexity of these wear particles (Kirk and Stachowiak, 1991; Hamblim and Stachowiak, 1993; Podsiadlo and Stakowiak, 1995). The shape of these fragments were also analysed and compared with osteoarthritic founds in human knee joints (Kuster et al., 1998).

2.5.1 Ferrography

Ferrography is a technique which was originally developed to analyse wear in machines (Sott et al., 1974). As the bearing surfaces of a machine wear, they shed particles into lubricating oil. These particles provide much information concerning the conditions of wear which produced them. For the ferrographic analysis, a sample of the lubricating oil is removed from the machine and pumped slowly along a thin glass microscope slide, the substrate, under the influence of a strong external magnetic field. Most of the wear particles are ferromagnetic. The magnet and substrate are so arranged that particles separate out under the influence of the magnetic field and deposit at various positions along the substrate. The position that a given particle will occupy depends largely upon its volume and magnetic susceptibility. When fixed

and dried, the substrate, with its magnetic graded wear particles, now termed ferrogram, is examined by light and electron microscopy. Ferrographic analysis of engine oil has become a highly sophisticated and accurate way of establishing the wear of a machine. Not only does it diagnose particular problems in machines, but it has a valuable predictive function (Evans et al., 1982).

Evans and coworkers (Evans et al., 1980; 1981a; Evans and Tew, 1981) were the first to apply the technique of ferrography to the analysis of wear particles present in synovial fluid. As ferrography employs magnetism to harvest particles and arrange them in an orderly fashion, it is first necessary to impart a positive magnetic susceptibility to the biological materials (Evans et al, 1980). In a preliminary study to evaluate this method, a series of synovial fluid aspirates and saline washings of joints was examined. Ferrograms were made using samples draw from patients presented with a range of different arthritides. The results were extremely promising. Wear particles, ranging in size from a few micrometers to several hundred micrometers, were clearly identifiable. As with machines, the size of the wear particles increased with the severity of mechanical erosion of the articular surface. Most of the particles were cartilaginous, but the few cases with osseous particles had radiological evidence of exposure of the subchondral bone (Evans et al., 1981a).

In 1982, ferrograms were made from saline washings retrieved from human knees after arthroscopy, the sensitivity of this method was confirmed (Evans et a., 1982). In this study, ferrography was found to be an extremely sensitive monitor of articular erosion, with a resolution far greater than that of arthroscopy. This was particularly apparent with knees suffering from a torn anterior cruciate ligament: arthroscopy detected no damage to the cartilaginous surfaces whereas ferrography detected a substantial level of micro damage. The spectrum of wear particles showed qualitative and quantitative alterations depending upon the condition of the knee (Casscells, 1980; Evans et a., 1982).

Rupture of the anterior cruciate ligament of canine knees produces a condition which closely resembles human osteoarthritis (Muir, 1977). Unrepaired damage of this kind in human knees may also lead to degenerative joint disease. As ferrograms detects high concentrations of cartilage wear particles in human knees with torn anterior cruciate ligaments before there is obvious damage to the cartilage, they may be involved with early events leading to osteoarthritic degeneration. One mechanism by which they could do so is by the cellular

release of enzymes, and perhaps other factors which mediate directly, or indirectly, in the degeneration of cartilage (Evans et al., 1981b). This hypothesis is supported by evidence from animal studies: injections of cartilaginous particles into the knee joints of dogs provoke a monarticular arthritis (Christian et al., 1965).

Also though the technique of ferrography, Kuster and coworkers, in 1998, have compared the shape of cartilage wear fragments and the extent of the cartilage damage in healthy an osteoarthritic human knee joints. The patients presented osteoarthritis grade 1 (fibrillation of less than half the cartilage thickness), grade 2 (fibrillation of more than half the cartilage thickness) and grade 3 (erosions down to bone). They examined a total of 565 particles extracted from synovial fluid samples by ferrography. Experiments demonstrated that there were significant differences between the numerical description calculated for wear particles from healthy and osteoarthritic knee joints, suggesting that the particle shape can be used as an indicator of the joint condition. In particular, the fractal dimension of the particle boundary was shown to correlate directly with the degree of osteoarthritis.

In this study (Kuster et al., 1998) several types of particles were identified. The appearance of the lamellar particles was flat with smooth surface morphology. These particles were thought to originate from the lamina splendens. Rod-shaped particles were elongated fragments which were most abundant in joints with the early stages of osteoarthritis (grade 1). Chunky particles were large and irregularly shaped with a cleavage or spongy type of surface. Their origin was thought to be from the deep cartilage zone. Osseous particles originated from the subchondral bone which implied that cartilage was completely worn off in some places. Their morphology resembled the trabecular structure of the bone.

2.5.2 Microporefiltration

Another method that makes possible this analysis of cartilage particles is the microporefiltration. Through this technique, cartilage fragments are recovered by filtration (millipore filters) of the synovial fluid after enzymatic treatment with hyaluronidase, to reduce viscosity and eliminate artefacts. The recovered debris are fixed directly on the filter with 10% buffered formalin and stained with hematoxylin and microscopic examination can be made (Tew, 1980; Tew and Hackett, 1981).

The technique of microporefiltration was used to test the presence of cartilage debris in synovial fluid from horses. In one study of several hundred race horses, a positive correlation between cartilage fragments suspended in synovial fluid and chronic joint problems was shown (Tew, 1980). In this study, more than 400 Standardbreds and Thoroughbreds with a variety of acute and chronic joint diseases where examined. The amount of cartilage debris recovered from the synovial fluids of these horses was qualitatively related to the degree of functional impairment.

In 1986 and 1993 Schossier and Erbacher, respectively, have also used this technique to test the presence of cartilage wear particles in equine joints. The first has collected synovial fluid from equine carpal and tarsal joints, that were placed in heparin-coated containers and frozen under a temperature of -22^{o}C until the examination. Erbacher has collected the synovial fluid samples from fetlock equine joints and placed in EDTA containers. Both have filtered the samples in milipore filters, fixed with formalin and stained on the filter with safranin 0. Schossier´s comparison of synovial and pathomorphologic findings with the number of particles per filter, size of each fragment showed partly positive tendencies but not significant differences between the various degrees of cartilage erosion. Erbacher found in this method a good means of assessing the degree of cartilage lesions in patients with osteochondritis dissecans after arthroscopic surgery. In his study, particle analysis one day after surgery showed much more fragments than the same test made 7 days after surgery in the same joints.

3 OBJECTIVES

* The main objective of this study was to compare the presence of clinical signs in various joint diseases and the presence, number and amount of cartilage wear fragments found in synovial fluid.

* A second aim of this study to analyse the relationship between several equine joint diseases, like osteochondritis dissecans, osteoarthritis, septic arthritis, podarthritis and navicular syndrome, and the presence, number and size of cartilaginous wear particles in the synovial fluid collected from different joints (coffin, fetlock, carpal, tarsal and stifle joints).

4 MATERIALS AND METHODS

4.1 Animals

Tested animals: synovial fluid samples were collected from 67 horses, among them mares, geldings and stallions, aging between 2 months and 23 years (average 10.79 ± 2.21), of several different breeds (Hanoverian, Traber, Mecklenburguer, Holsteiner, Friesian, Anglo-Arabian, Sachsener, Sachsen-anhaltiner, Brandenburguer and Lusitan). Most of which were used for sport, like dressage, horse jumping and race. General data like owner, name, and history of lameness of each animal was registered at the time of presentation in the clinic. The age of each horse was listed as recorded at the respective equine passport. The animals were chosen according to the casuistic of the Clinic for Horses, General Surgery and Radiology of the Faculty of Veterinary Medicine of the Freie Universität Berlin from April, 2006 to July, 2007 and diagnosis of articular problem. Horses that showed any other healthy problems besides joint disease where excluded from this study.

Control animals: synovial fluid samples were acquired from 18 sound horses of different ages (between 7 and 22 years old, average 10.92 ± 3.41), sexes (mares and geldings), and breeds (Hanoverian, Traber, Mecklenburguer, Brandenburguer, Sachsen-anhaltiner, and Friesian). To select the animals for control group we have taken the criteria according to Person, 1971 and Sander, 1990 as follows:

- The horse presented no signs of general disease;

- No factor causative of lameness could be attributed to the joint;

- The joint showed normal mobility;

- No swelling, tenderness or heat over the joint was noticed;

- No thickness or lack of elasticity on the joint capsule was felt on palpation;

- No signs of synovial effusion were apparent in the punctuated joint;

- Synovial fluid presented a normal appearance and physiological parameters after analysis.

The figure below shows the distribution of breeds among the horses that where part of this study.

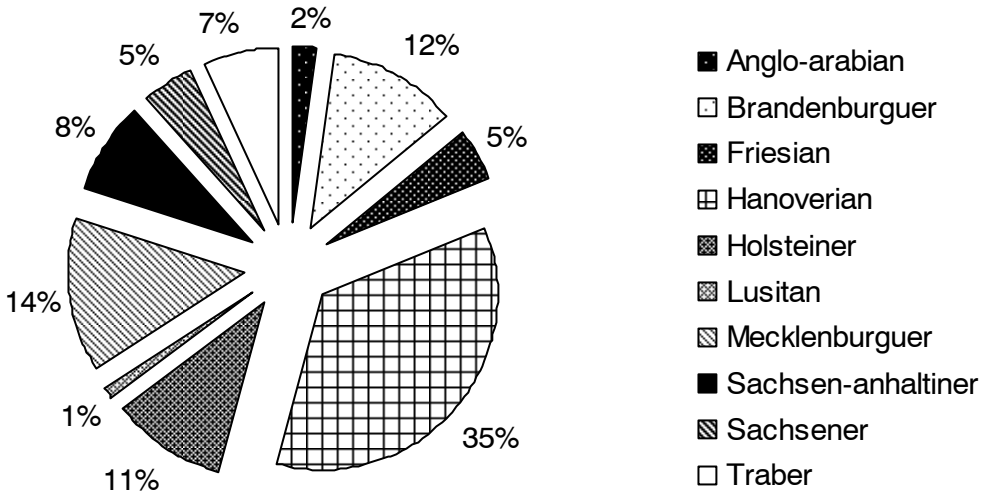

Figure 2. Distribution of breeds of the horses (18 sound animals and 67 patients with diagnosis of joint disease) in this study.

4.2 Samples

A total of 116 samples of synovial fluid were collected. As much synovial fluid as possible was withdrawn from different joints (coffin, fetlock, carpal, hock and stifle joint) by arthrocentesis, during the examination or during arthroscopy.

Aseptic preparation was always performed before each arthrocentesis with isopropyl alcohol and povidone-iodine. Clipping was not done, unless the horse coat was very long. When restraint was necessary, a nose twitch was used or mild sedation with detomidine hydrochloride (Domosedan, Pfizer GmbH), 20μg/Kg of body weight or romifidine hydrochloride (Sedivet, Boehringer Ingelheim, Vetmedica GmbH), 0.04 mg/Kg of body weight was done.

The coffin joint (distal interphalangeal joint) was punctuated at its dorsal surface, with the horse bearing weight, 1 to 1.5 cm proximal to the coronary band and 1.5 cm lateral to midline, using a 12-gauge needle. This punctuation was done mostly to measurement of the joint pressure and synovial fluid was collected after that, through the same needle. Most of the times, this procedure followed the diagnostic anesthesia and palmar digital block was made, which is very helpful to the arthrocentesis and sometimes avoids restraint methods.

To collect synovial fluid from the fetlock joint (metacarpo/metatarsophalangeal joints) it was used the palmar (plantar) pouch approach, with the limb bearing weight, through an 18-gauge needle, or during arthroscopy. The anatomic boundaries or landmarks for this approach are the suspensory ligament caudally, the apical border of the sesamoid bone distally, the third metacarpal (tarsal) bone dorsally, and the distal end of the small metacarpal (tarsal) bones (splint bones) proximally. The proximal border of the joint is approximately 2 cm distal to the end of the splint bone.

The approach for carpal joint arthrocentesis has been to flex the carpus and enter the antebrachiocarpal or middle carpal joints in the depressions between the extensor carpi radialis and common digital extensor tendons, using an 18-gauge needle. The carpometacarpal joint communicates with the middle carpal joint, making separate punctuation of that joint unnecessary.

Tarsocrural joint is the largest of the tarsal (hock) joints, and it communicates with the proximal intertarsal joint. A large palpable depression can be felt just distal to the medial malleolus of the tibia, and joint entry was made at this point. Synovial samples in this joint were mostly taken in arthroscopy, but also with the horse bearing weight, through an 18-gauge needle.

The medial femorotibial joint was injected by palpating between the medial collateral and medial patellar ligaments and directing a 20 gauge needle in the medial and slightly cranial direction. A direct medial or caudomedial direction results in contact with the medial meniscus.

After the arthrocentesis, synovial fluid began to flow through the needle. A 2mL sterile vacutainer containing calcium balanced heparin, 50 I.U./mL (Monovet) was inserted on the

needle hub and the fluid was carefully aspirated. Saline washes of five joints were also collected during arthroscopy and processed as well.

4.3 Synovial Fluid Analysis

A laboratory evaluation of all samples was made immediately after collection.

4.3.1 Physical examination:

Appearance: colour, degree of opacity, presence of flocculent material and viscosity.

Colour of synovial fluid was classified through visual examination in pale yellow, yellow straw, dark yellow and red (bloody). The opacity and presence of flocculent material were analyzed through visual examination as well. The samples were classified as transparent or opaque and for the presence of flocculent material: yes or no.

Viscosity was estimated by observing the length of the strand of synovial fluid between fingers before it drips. Samples were categorized into three groups:

	Length of strand
High	> 2.5 cm
Low	between 1 cm and 2.5 cm
Very low	< 1 cm

4.3.2 Chemical examination: protein content.

Total protein (g/dL) was determined using a handheld analog refractometer (HRM 18, A.Krüss Optronic GmbH). The refractometer works on the principle that light entering a prism has a unique characteristic. That characteristic is represented by a value on a scale in units known as ° Brix. When light enters a dry prism, the field of view remains blue. This is an indication that the light is not being interfered with as it passes through the prism.

Before each analysis, the calibration should be checked. Distilled water placed on the refractometer should result in a reading of zero. For this purpose, a few drops of distilled water were placed on the prism surface and the cover was closed. If the bubbles were formed, the cover was gently pressed to remove the bubbles and to help disperse the water over the entire surface. The refractometer was held up to natural light to obtain the readying.

Looking into the eyepiece, the examiner should see a distinct separation between a blue and white section, called the contrast line. If the contrast line is not directly at zero, the examiner should adjust by turning the screw on the top of the refractometer until it reads zero. The focus can be adjusted by twisting the eyepiece until the scale can be seen clearly. Once the refractometer was calibrated to zero with distilled water, the surface was dried with a clean cloth.

One drop of the synovial fluid sample was placed with a 10μL pipette on the prism surface and the cover was closed. The cover was gently pressed to help disperse synovial fluid over the entire surface. The refractometer was held up to natural light to obtain the readying.

The handheld analog refractometer used in this study is very convenient because it does not require an energy source. However, their measurements accuracy may change with the temperature and attention should be made regarding their use outdoors. In this study the analysis were always carried out in the laboratory, where the temperature was always around 20 °C.

4.3.3 Cytological properties: total leukocyte counts.

Total leukocytes cell counts were determined using an automated cell counter (T 840, Bedfordshire, United Kingdom) and a counting chamber (hemacytometer).

Automated cell counter:

In order to ensure a uniformity of the cellular content, each sample was gently shaken before measuring. The counter principle is based on the property of cells to behave as isolators. Cells are classified according to their number and size. In the counter, an electrical tension exists in an opening. Cells shortly interrupt this electrical tension as they are made to pass through this narrow opening. Electrodes placed at both sides of this opening register the difference in electrical tension produced by the passage of cells and send this information to the central computer where it is analyzed and interpreted. The size of the cells is calculated in femtoliters (fL), for example, only cells with size greater than 35 fL are considered leucocytes. Three different measuring periods are automatically carried out by the coulter for each sample. At least two of these three values must coincide. Otherwise, no valid value is reported. This can also happen when the total number of leucocytes in the samples is below the background, which is 200 cells/μL.

Counting chamber (hemacytometer)

Total leukocyte cell count was done in a counting chamber when the value could not be estimated in the automated cell counter, for example, when the total number of leucocytes was bellow 200 cells/μL. The counting chamber used in the present study was the improved Neubauer chamber, which has two counting nets in the middle support with one division. The counting net system, name and trademark of the manufacturer, chamber depth in millimeters and the area of the smallest square in mm^2 are printed on both unworked surfaces of the counting chamber. The chamber can be seen in figure 3.

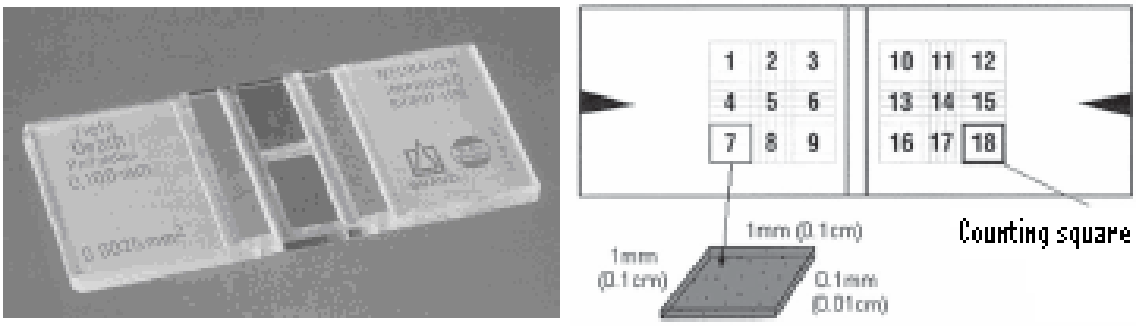

Figure 3. The Neubauer chamber at the left. At the right, its main divisions and the counting square with its dimensions.

In this chamber, there are four longitudinal grooves in the center third of a rectangular and thick base plate made of special optical glass. The grooves are parallel to the short sides of the base plate and the central third has the same size as the coverglass used. The central support and the two external supports are ground smooth and polished. The surface of the central support is deeper than that of the two external supports. The central support (chamber base) has two elevated platforms, each having etched on it the improved Neubauer ruling, which can be seen in figure 4.

Each platform on which the rulings are engraved is surrounded by a moat, and on each side of the platform there is an elevated glass support at the height that will allow for a distance of 0.1 mm between the bottom of the coverglass and the ruled area.

The improving Neubauer ruling of the platform consists of a system of squares in which there is a square measuring 3 by 3 mm that is divided into nine equal squares, each containing $1mm^2$. These divisions can be seen in figure 3.

The four corner squares are used for the total leukocyte count, which are marked in figure 4 with an A. These squares were used in this study to count white blood cells in synovial fluid as well. The center square millimeter is subdivided into 25 squares, and each of these is subdivided into 16 small squares that measure 0.05 by 0.05mm.

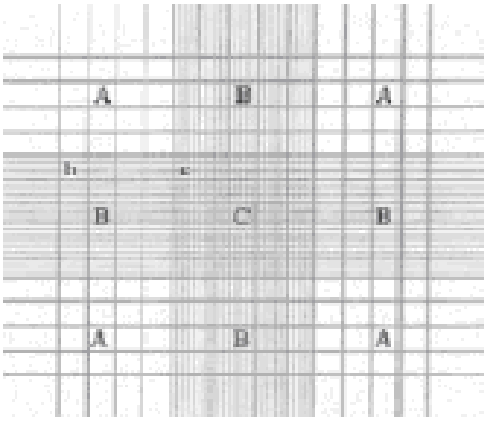

Figure 4. Ruled area on improved Neubauer hemacytometer. For total leukocyte count, the white blood cells are counted in the area A.

To prepare the chamber the mirror-like polished surface was carefully cleaned with lens paper. The coverglass was also cleaned. Coverglasses for counting chamber are specially made and are thicker than those for conventional microscopy, since they must be heavy enough to overcome the surface tension of a drop of liquid.

Before starting, the external support was moistened with distilled water and the coverglass was gently pushed onto the counting chamber from front. The formation of interface lines, called Newton rings, between the external support and the cover glass shows that the cover glass is correct positioned.

Synovial fluid was taken with a pipette and the first drop was put between the coverglass and the counting chamber. As a result of the capillary effect, the gap between the coverglass and the chamber base was filled up.

If any bubbles were visible or if the liquid has overflowed over the edges and into the grooves, the chamber was cleaned and filled again.

Counting Technique

Counting assumes precise knowledge of the limit lines of the counting chamber used. These are shown in the figure 5. To ensure that cells with are on or along the limit lines are not counted twice or are not missed during the count, certain rules have to be observed. Therefore, just the cells that touched the higher and left boundaries of the square were

counted. To make sure that the squares were also not duplicate counted, we followed the direction as can be seen in figure 5.

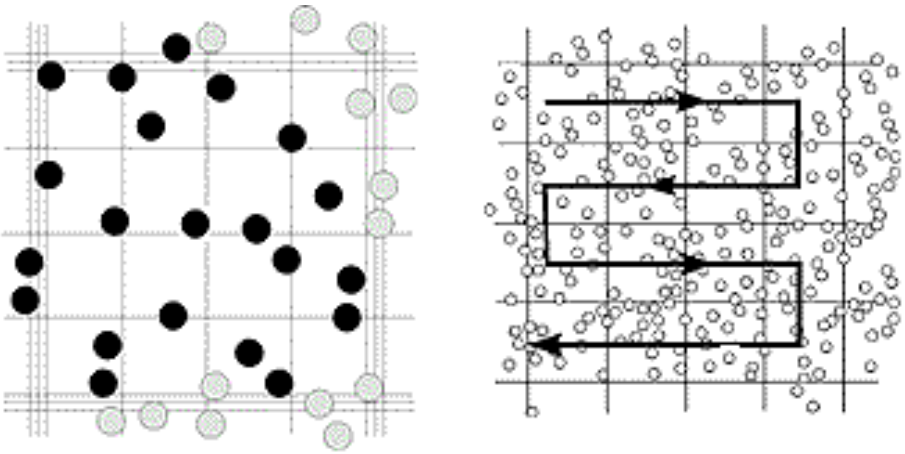

Figure 5. Counting technique to avoid duplicate counting at the left side. At the right side the direction of counting.

Calculation

A simple formula was used to calculate the number of leukocytes/ μL of synovial fluid.

$$\frac{\text{Number of cells counted}}{\text{Counted area (mm}^2\text{) x Chamber depth (mm)}} = \text{Cells / } \mu\text{L of synovial fluid}$$

As the counted area for leukocytes was 4 mm^2 and the chamber depth is 0.1 mm:

$$\frac{\text{Number of cells counted}}{4 \text{ mm}^2 \text{ x } 0.1 \text{ mm}} = \text{Cells / } \mu\text{L of synovial fluid}$$

4.4 Cartilage Particle Analysis

After physical, chemical and cytological analysis of the synovial fluid the samples were prepared for cartilage wear particle analysis.

Centrifugation

Centrifugation is a process used to separate or concentrate materials suspended in a liquid medium. The theoretical basis of this technique is the effect of gravity on particles in suspension. Two particles of different masses will settle in a tube at different rates in response to gravity.

With the objective of concentrating the cartilage wear particles suspended in the synovial fluid or washings, the samples were centrifuged (CPK-Centrifuge, Beckman) at 3000 rpm for 15 minutes. After this period, the supernatant was disposed off and with a 20μL pipette the sedimented material was collected and carefully dropped in the middle surface of the Neubauer chamber. The coverglass was slowly placed over the counting surface of the chamber, differently from the other analysis described above (total leukocytes account), were we have first putting the coverglass and than, the fluid between it and the chamber. Cartilage particles were then analyzed and quantified in the microscope.

Counting cartilage particles

For each sample, cartilage fragments were counted in both improved Neubauer ruling that were present in each chamber. These particles were counted in all the squares, following the counting technique described above to avoid duplicate counting. As each ruling has an area of 9mm^2, and the depth of the chamber is 0.1 mm, the total volume counted was 1.8 μL.

Estimating the length of cartilage particles

Using the improved Neubauer chamber it was also possible to estimate the size of the fragments as all the dimensions of the chamber are known (Figure 6). As it was described above, this chamber is divided in nine equal squares with an area of 1 mm^2. Based on the dimensions of the chamber, the greatest length of each fragment was estimated.

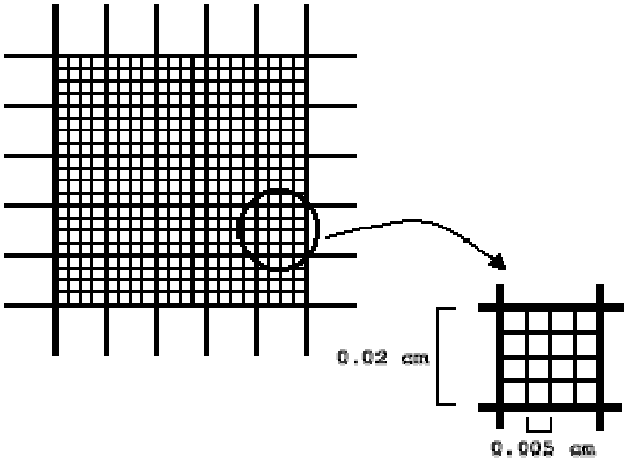

Figure 6. One of the nine equal squares of the Neubauer Chamber, with its divisions.

According to size, the fragments were classified into small (< 40μm), middle (≥ 40μm and ≤60μm) and big (> 60μm) particles.

Training cartilage visualization

Before starting the analysis it was necessary a great amount of practice and training. Without staining, it was in the beginning difficult to identify the cartilage fragments. We have then made a great number of cartilage slides to better characterize the appearance of the fragments. For that purpose it was used cartilage material collected from the arthroscopies, like small chips (covered with cartilage) or cartilage fragments that came out of the joint (Figures 7 and 8).

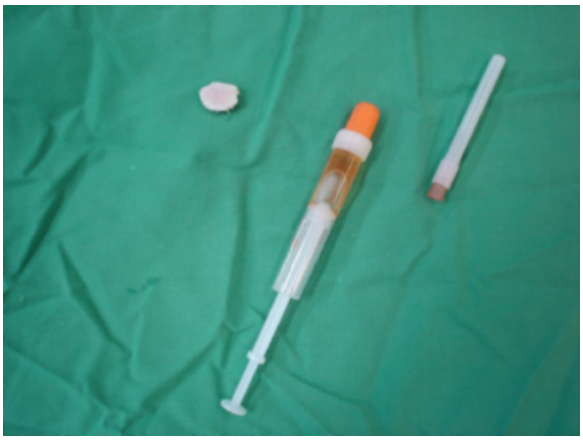

Figure 7. Synovial fluid collected through a 12-gauge needle during arthroscopy. At the left side, a chip extracted out of the joint.

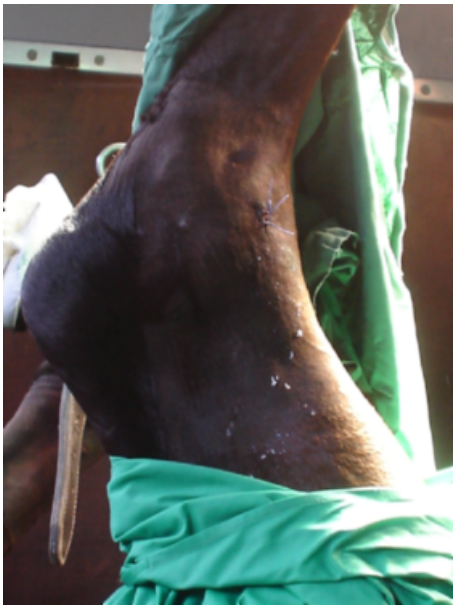

Figure 8. Tarsal joint during arthroscopy. Cartilage fragments that came out of the joint can be seen.

4.5 Groups

The groups were divided into “Control” (C): not lame horses, and “Test”(T): lame horses. These two main groups were subdivided into:

C1: no clinical signs, without radiography;

C2: no clinical signs, without radiographic alterations;

C3: no clinical signs, with radiographic alterations;

T1: lame horses, without radiographic alterations;

T2: lame horses, with radiographic alterations;

T3: lame horses, with radiographic and arthroscopic alterations.

A resume with the characteristics of the groups can be seen in table 2:

Table 2. Characteristics of the groups. Osteochondritis dissecans: OCD; navicular syndrome: NS; podarthritis: PO; infectious arthritis: IA; osteoarthritis: OA.

Groups: n	Lameness	Radiographic alterations	Arthroscopic alterations	Diagnostic: Number of samples
C1: 20	no	---	---	---
C2: 08	no	no	---	---
C3: 20	no	yes	---	OCD: 10; NS: 10
T1: 11	yes	no	---	IA: 8; PO: 3;
T2: 29	yes	yes	---	OA: 3; NS: 26
T3: 28	yes	yes	yes	OCD: 16; OA: 12

n: number of samples in each group

The difference between groups C1 and C2 was made because sometimes there was the opportunity of collecting synovial fluid from horses that, for any other purposes, were submitted to radiography. That was surely of great significance for the study, because this group was not just without clinical signs or history of lameness, but also without radiological founds. For financial reasons radiographies were not made in group C1.

The distribution between ages and sexes of the horses in each group can be seen in the following two tables.

Table 3. Age of the horses in each group.

Groups	n	Minimum	Maximum	Average age of the horses
C1	10	8 years	22 years	11.95 ± 2
C2	08	7 years	15 years	9.88 ± 1.39
C3	15	1 year	15 years	9.40 ± 2.12
T1	10	2 months	18 years	9.11 ± 2.55
T2	16	1 year	23 years	13.76 ± 2.23
T3	26	2 years	17 years	9.36 ± 1.61

Results expressed as means ± S.E.M.
n: number of horses

Table 4. Distribution of sexes among the groups.

Group	n	Sex		
		Stallions	Geldings	Mares
C1	10	-	8	2
C2	08	1	4	3
C3	15	2	9	4
T1	10	-	8	2
T2	16	-	11	5
T3	26	3	15	8
Total	85	06	55	24

n: number of horses

4.6 Diagnosis

Diagnoses were based on clinical examination, radiographic and arthroscopic features. The horses were diagnosed with osteochondritis dissecans: OCD; navicular syndrome: NS; infectious (septic) arthritis: IA; osteoarthritis: OA and podarthritis: PO. The number of horses with each diagnosis can be seen in table 7.

4.6.1 Clinical examination

First of all a complete clinical examination and a detailed anamnesis were carried out in each horse presented with a history of lameness. If no sings of general disease were present, lameness examination started with a careful observation of the limbs and its conformation. Size, shape, contour, heights, and widths of both sides were compared. It was also checked if any swelling was present.

The presence of lameness was assessed in a straight line on a hard surface, first at the walk and then at the trot. When needed the horses were also observed in a circle at both hands on a hard and soft surfaces.

The degree of lameness was classified in five categories:

1. Unclear: mild lameness is observed at the trot, but not consistently.

2. Mild: obvious lameness is observed at the trot, but still not observed at the walk.

3. Moderate: lameness is obvious at both walk and trot

4. Severe: the horse tends to stand with the dorsal angle of the hoof of the diseased limb and for a short period of time

5. Very severe: the horse does not bear weight on the limb. If trotted, the horse carries the limb.

The degree of lameness according to each diagnosis can be observed in tables 5 and 6.

Table 5. Number of horses and degree of lameness in the different diagnosis (osteochondritis dissecans: OCD; navicular syndrome: NS; podarthritis: PO; infectious arthritis: IA; osteoarthritis: OA).

Diagnosis	horses	Not lame	1	2	3	4	5
Sound	18	18	-	-	-	-	-
IA	08	-	-	-	02	06	-
NS	18	05	02	11	-	-	-
OA	15	-	-	-	07	08	-
OCD	24	10	-	06	07	01	-
PO	02	-	02	-	-	-	-
Total	85	33	04	17	16	15	-

Table 6. Number of samples and degree of lameness in the different diagnosis (osteochondritis dissecans: OCD; navicular syndrome: NS; podarthritis: PO; infectious arthritis: IA; osteoarthritis: OA).

Diagnosis	Samples	Not lame	1	2	3	4	5
Sound	28	28	-	-	-	-	-
IA	08	-	-	-	02	06	-
NS	36	10	04	22	-	-	-
OA	15	-	-	-	07	08	-
OCD	26	10	-	08	07	01	-
PO	03	-	03	-	-	-	-
Total	116	48	07	30	16	15	-

A general flexion test or a flexion of the examined joint was also carried out in order to localize the source of pain. Perineural blocks with lydocain were performed using a 23-gauge needle. Before anesthesia, the local was aseptic prepared with isopropyl alcohol. The response was assessed 10 minutes later, at the trot.

4.6.2 Radiographic examination

A complete radiographic examination of the affected joint was performed with an Philips Super 100 CP x-ray machine. Screen x-ray XOD TM (Fototechnische Werke GmbH) were used. Rare-earth intensifying screens of the type Trimax T2 – 100 (Company 3M GmbH) were used for the distal interphalangeal, metacarpo/metatarsophalangeal, and tarsocrural joints. Intensifying screens type Trimax T6 – 300 were preferred for the carpal and femoropatellar joints.

The radiographic views performed for each joint were:

Distal interphalangeal joint (coffin joint) :

Dorsopalmar/plantar;
Lateromedial;
Flexed dorsolateral-palmaro/plantaromedial oblique;
Flexed dorsomedial-palmaro/plantarolateral oblique;
Oxpring view;

Metacarpo/metatarsophalangeal joint (fetlock joint):

Dorsopalmar/plantar;
Lateromedial;
Flexed lateromedial;
Dorsolateral-palmaro/plantaromedial oblique;
Dorsomedial-palmaro/plantarolateral oblique;

Carpal joint:

Dorsopalmar;
Lateromedial;
Flexed lateromedial;
Dorsolateral-palmaro/plantaromedial oblique;
Dorsomedial-palmaro/plantarolateral oblique;

Tarsocrural joint (hock joint):

Dorsoplantar;
Lateromedial;
Dorsolateral-palmaro/plantaromedial oblique;
Dorsomedial-palmaro/plantarolateral oblique;

Medial femorotibial joint (stifle joint):

Lateromedial;
Caudocranial.

4.6.3 Arthroscopic examination

In the present study, arthroscopy was carried out in 26 horses, 14 with osteochondritis dissecans and 12 with osteoarthritis. For this procedure general anesthesia was required. The horse was sedated with 0.08 mg/kg of body weight of romifidin (Sedivet) and 0.1 mg/kg of body weight of L-Polamivet. After 10 minutes, 0.04 mg/Kg of body weight and 2.2 mg/kg of body weight were administered in the same syringe. Anesthesia was maintained with isofluran. The patient was prepared as for any other aseptic orthopedic procedure and put in dorsal recumbence. Before inserting the arthroscope, synovial fluid was collected through a 12-gauge needle. The joints punctuated were metacarpo/metatarsophalangeal, tarsocrural and femoropatellar. During five arthroscopic surgeries, all from horses with osteoarthritis, saline washings were collected from the joint as well.

Osteoarthritis (OA)

The diagnosis of osteoarthritis was based on radiographic and/or arthroscopic findings. The radiographic findings were joint space narrowing, subchondral sclerosis, subchondral lyses or fragmentation, presence of peri-articular osteophytosis. Arthroscopic findings were defects in articular cartilage, like diffuse fibrillation (parallel scoring line), erosions and ulceration at the cartilage surface.

Navicular syndrome (NS) and Podarthritis (PO)

Diagnosis of navicular syndrome was based on clinical signs, perineural anesthesia and radiographic findings. Intraarticular anesthesia was not performed, because of the increased risk and inespecificity, as an anesthetic diffusion to the adjacent areas occurs. An aid in the diagnosis of podarthritis and navicular syndrome was the direct manometry in the coffin joint, according to Hertsch and Höppner, 1999. When the pressure was high (higher than 40 mmHg), but no radiographic findings were present, the patient became a diagnosis of podarthritis only. The radiographic findings were the presence of several lucent zones of variable shapes along the distal border of the bone; poor definition between the palmar cortex and the medulla as a result of subcortical sclerosis; crescent-shaped lucent zone in the central eminence of the flexor cortex of the bone; entheseophyte formation on the proximal border of the navicular bone; cyst-like lesion within the medulla; new bone on the flexor cortex of the navicular bone.

The presence of these lucent zones along the distal border of the bone can be normal if they are less than six and if they are narrow and have a conical shape.

Osteochondritis dissecans (OCD)

Diagnosis of osteochondritis dissecans was based on the characteristic radiographic findings (the presence of a chip fracture). Lameness in patients with OCD ranged from none to severe. Effusion was rarely observed in these animals.

Infectious (septic) arthritis (IA)

Diagnosis of infectious (septic) arthritis was based on clinical signs, history and synovial fluid analysis. The joints presented classic cardinal signs of inflammation centered around the joint involved. These include heat, swelling (edema or joint effusion), pain on palpation and flexion, erythema of surrounding skin, and lameness grade 3 or 4. The history of a recent joint injection, recent surgery, or a recent wound was oft present. Synovial fluid analysis presented a total protein greater than 3.0 g/dL and the total leukocyte count exceeded 30 000 cells/μL.

Table 7. Number of horses with different joint diseases diagnoses (osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA; podarthritis:PO).

Group	Sound	PO	OCD	NS	IA	OA	n
C1	10	-	-	-	-	-	10
C2	08	-	-	-	-	-	08
C3	-	-	10	05	-	-	15
T1	-	02	-	-	08	-	10
T2	-	-	-	13	-	03	16
T3	-	-	14	-	-	12	26
Total	18	02	24	18	08	15	85

n: number of horses

4.7 Statistical analysis

For the statistical analyses were used one-way ANOVA (analysis of variance) and Student's t test, with the level of significance set at $p < 0.05$. The null hypothesis for ANOVA is that the mean (average value of the dependent variable) is the same for all groups. With this statistical test, if the null hypothesis was not rejected, none of the groups were different. However, if the null hypothesis was rejected, that means that at least two groups are different from each other. In order to determine which groups are different from which, Student's t test was made. When the sample sizes were very small, Fisher's exact test was done.

Each group consisted of different types of joints. On most of the occasions, one joint per horse was punctuated. However sometimes synovial fluid was obtained from two joints of the same animal. For example, in the horses with diagnosis of navicular disease, in the majority of cases, both right and left coffin joints of the forelimbs were punctuated. Each individual sample was considered as an independent statistical unit, whether or not they were obtained from the same or from different horses.

The data were presented as mean value with standard errors. Statistical analyses were performed using GraphPad Prisma V.4 Statistical Software (GraphPad Software, 2003).

5 RESULTS

5.1 Clinical sings and cartilage particles

The main idea of this study was to compare the presence of clinical signs (pain, lameness) in different joint diseases and the presence, number and amount of cartilage wear fragments. Therefore the first graphic presents this relationship, showing lame and no lame horses in comparison with number and size of cartilage fragments encountered in the 6 different groups that were analysed (figure 9). A detailed description of each group can be seen in table 2.

The cartilage wear particles found in this research had a length between 20 and 200 μm. They were subdivided according to size into small (< 40μm), middle (≥ 40μm and ≤ 60μm) and big (> 60μm) particles, as can be observed in the following graphics.

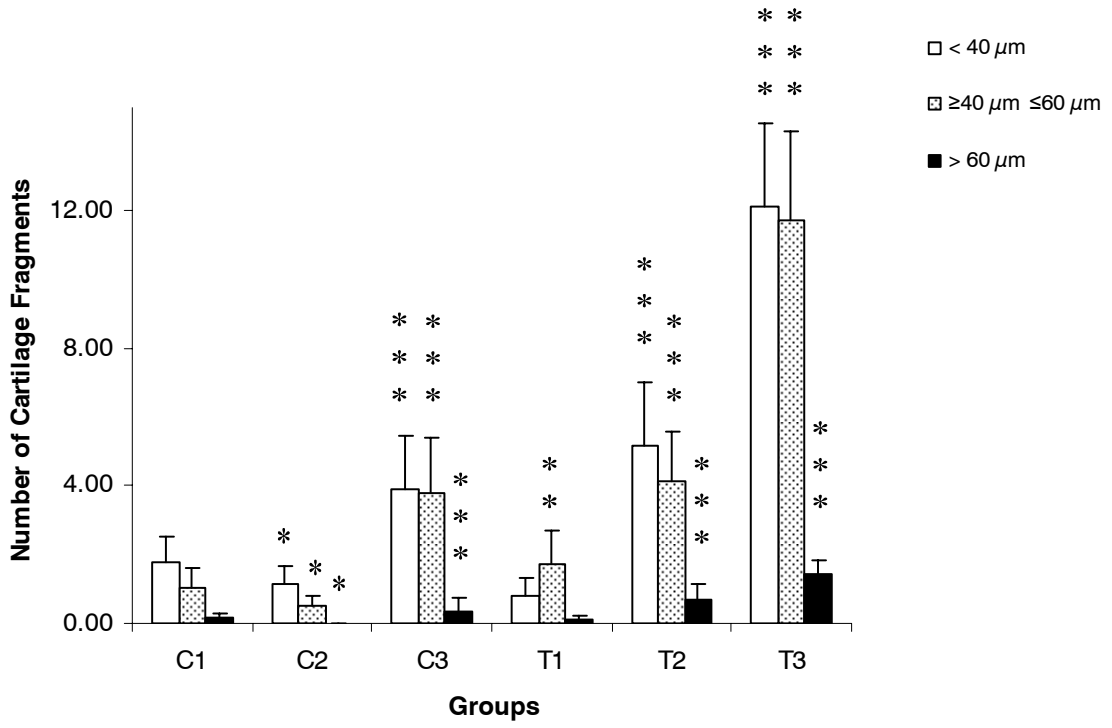

Figure 9. Number and size of cartilage fragments from all samples (n:116) versus groups.
*: P<0.05 compared with the same length in control group C1;
**: P<0.05 compared with the same length in control group C2;
***: P<0.05 compared with the same length in both C1 and C2.

Some cartilage particles were also found in control groups C1 and C2, but in significant smaller quantity when compared to C3 and to "test" groups T2 and T3. In these last 3 groups, the average number of particles, no matter if of small, middle or big size, was statistically greater than in C1 and C2. However, in test group T1 this was not observed and it has shown statistical increase just when middle size fragments were compared to control group C2. Control group C2 showed lower average number of cartilage fragments than C1. No cartilage fragments greater than 60μm was found in C2, whereas in control group C1 some big fragments could be seen. Data and standard error of measurement (S.E.M.) can also be analysed in table 8.

Table 8. Average number and size of cartilage fragments from all samples (n:116) versus groups.

Group	n	$< 40\mu$m	$\geq 40\mu$m and $\leq 60\mu$m	$> 60\mu$m
C-1	20	1.80 ± 0.76	1.05 ± 0.58	0.15 ± 0.15
C-2	08	1.13 ± 0.55 *	0.50 ± 0.31 *	0 *
C-3	20	3.90 ± 1.56 ***	3.80 ± 1.61 ***	0.35 ± 0.38 ***
T-1	11	0.82 ± 0.48	1.73 ± 0.97 **	0.09 ± 0.12
T-2	29	5.17 ± 1.81 ***	4.14 ± 1.44 ***	0.69 ± 0.46 ***
T-3	28	12.11 ± 2.41 ***	11.75 ± 2.56 ***	1.43 ± 0.44 ***

Results are expressed as means ± S.E.M.
n: number of samples
*: P<0.05 compared with the same length in control group C1;
**: P<0.05 compared with the same length in control group C2;
***: P<0.05 compared with the same length in both C1 and C2.

In table 9, the relation between the degree of lameness and the average number and size of cartilage fragments found can be observed. Horses which were not lame showed a small average number of small, middle and big cartilage fragments. In this group there were not only sound horses, but always patients with diagnosis of osteochondritis dissecans and navicular syndrome. Horses with grade 1 lameness did not showed a great average of small and middle particles as well. However, the average number of big fragments was already statistically greater than in no lame horses. In this group there were patients with navicular syndrome and podarthritis. Animals with lameness grade 2, 3 or 4 presented an average number of small, middle and big cartilage particles statistically greater than no lame horses. Patients with grade 3 of lameness showed the greatest number of small, middle and big fragments.

Table 9. Average number and size of cartilage fragments according to degree of lameness. (osteochondritis dissecans: OCD; navicular syndrome: NS; podarthritis: PO; infectious arthritis: IA; osteoarthritis: OA).

Degree of lameness	n	Diagnosis	$< 40\mu$m	$\geq 40\mu$m and $\leq 60\mu$m	$> 60\mu$m
No lame	48	Sound; OCD; NS	2.56 ± 1.22	2.10 ± 1.25	0.21 ± 0.27
1	07	NS; PO	2.43 ± 1.18	3.57 ± 1.97	0.86 ± 0.60 *
2	30	OCD; NS	7.13 ± 2.72 *	5.83 ± 2.37 *	0.90 ± 0.45 *
3	16	IA; OCD; OA	10.38 ± 1.87 *	8.88 ± 1.87 *	1.13 ± 0.49 *
4	15	IA; OCD; OA	6.73 ± 3.03 *	8.13 ± 3.52 *	0.67 ± 0.40 *
5	0	-	-	-	-

Results are expressed as means ± S.E.M.
n: number of samples
* $P<0.05$ compared with the same length in the group of not lame, sound horses.

5.2 Shape of cartilage particles

Cartilage fragments showed different shapes, some of which can be seen in figure 10. According to their shape cartilage particles were classified in lamellar, elongated and chunky fragments (Figure 11).

Lamellar: their appearance was flat with a smooth surface morphology. The majority of particles (61%) presented this kind of shape. They were found in all the groups in this study, including controls.

Elongated: these particles have been found in all groups, except the control group C2. They were so classified when the greater length was approximately double or bigger than the perpendicular length.

Chunky: there were large and irregularly shaped fragments with a spongy type of surface. This kind of cartilage wear particles have just appeared in samples from horses diagnosed with osteoarthritis and osteochondritis dissecans that showed pain and radiographic alterations. Most of them were seen in group T3 and just in one sample in group T2 (Figure 12).

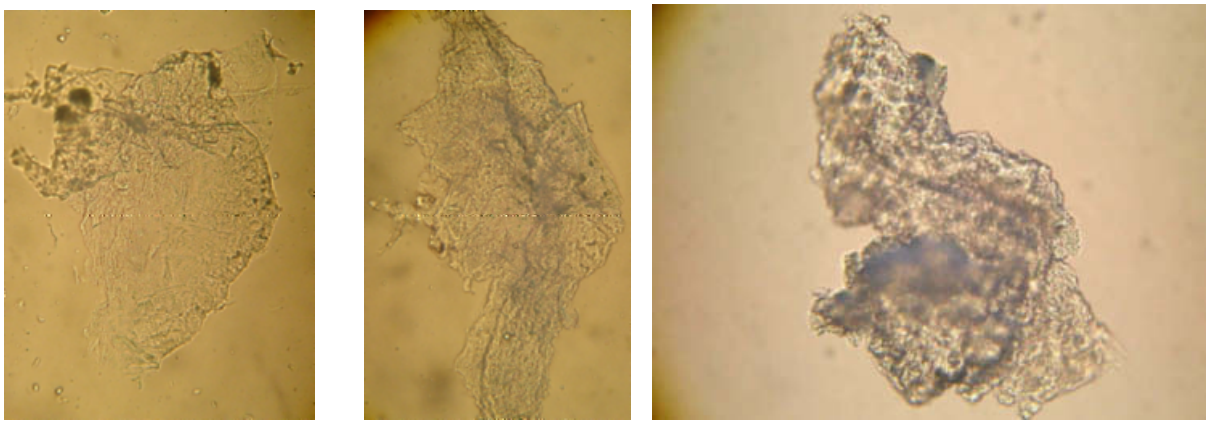

Figure 10. Different cartilage fragments isolated from synovial fluid. From left to right: lamellar, elongated and chunky shaped wear fragments (magnification x 200).

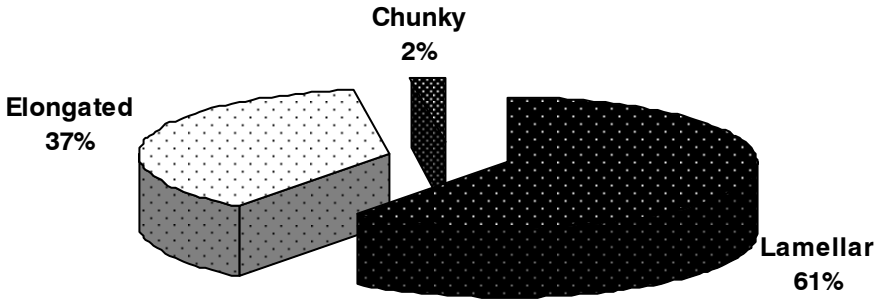

Figure 11. Distribution of the shapes of the cartilage wear fragments found in all the samples (116) of this study.

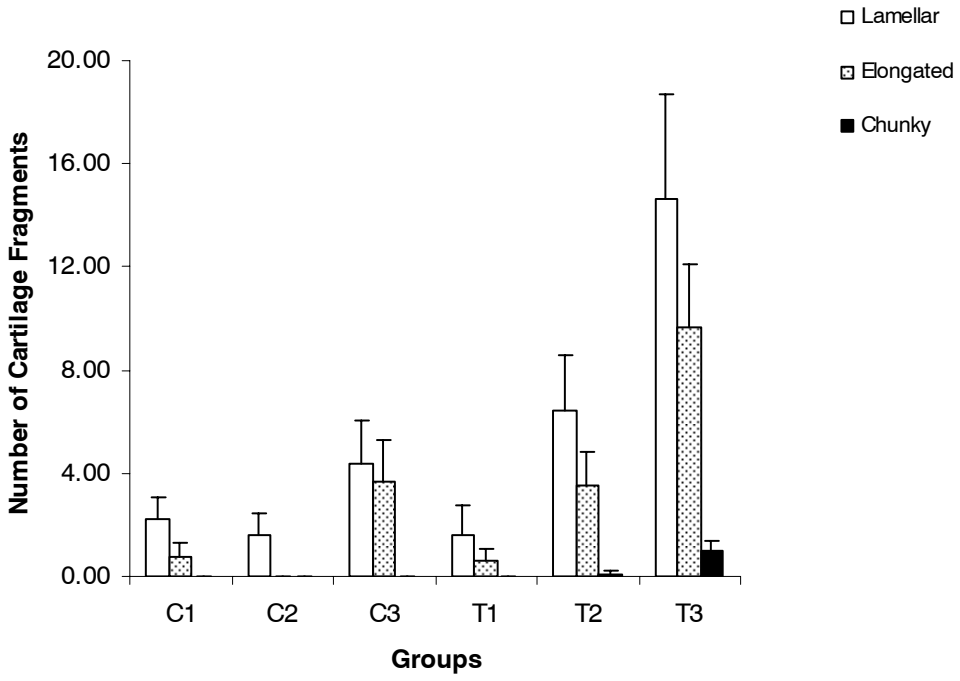

Figure 12. Distribution of the shapes of the cartilage wear fragments found in each group.

5.3 Different joint disease versus cartilage particles

The samples were collected from 116 joints: 28 from sound joints, 36 from animals with navicular syndrome (NS), 26 from patients with osteochondritis dissecans (OCD), 15 from osteoarthritic joints (OA), 8 from horses with infectious arthritis (IA), and 3 from horses with podarthritis (PO).

In table 10 it can be observed the relationship between these different joint diseases encountered in this study, average number and size of cartilage fragments found. In this table we can observe that the average number of cartilage fragments was greater in horses with osteoarthritis, followed by patients with osteochondritis dissecans, when related to small and middle fragments. However, horses with OCD presented the greatest average number of big cartilage wear particles. Horses with septic arthritis or navicular syndrome showed a smaller average number of particles.

Table 10. Relationship between presence, number and size of cartilage particles found and different diagnosis of joint disease (osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA; Podarthritis: PO). It presents also control groups C-1 and C-2.

n	Diagnostic	Groups	$< 40\mu$m	$\geq 40\mu$m and $\leq 60\mu$m	$> 60\mu$m
20	normal	C-1	1.80 ± 0.76	1.05 ± 0.58	0.15 ± 0.15
08	normal	C-2	1.13 ± 0.55	0.50 ± 0.31	0
08	IA	T-1	4.46 ± 1.67	3.77 ± 1.43	0.77 ± 0.48
26	OCD	C-3; T-3	9.96 ± 2.64	9.5 ± 2.37	1.15 ± 0.54
15	OA	T-2; T-3	10.73 ± 2.18	10.27 ± 2.82	0.93 ± 0.39
36	NS	C-3; T-2	3.87 ± 1.67	3.28 ± 1.49	0.62 ± 0.41
03	PO	T-1	1.38 ± 0.94	1.33 ± 0.62	0.33 ± 0.25

Results are expressed as means ± S.E.M.
n: number of samples

When comparing just patients with osteochondritis dissecans (OCD) and control group C-2, there was an increased number of cartilage fragments even in those horses that did not show clinical sighs, represented in group C3 (Figure 13, table 11). This increased number was significant in this group in small and middle cartilage particles. In group T-3, where patients showed joint pain, this significance was seen also in big particles.

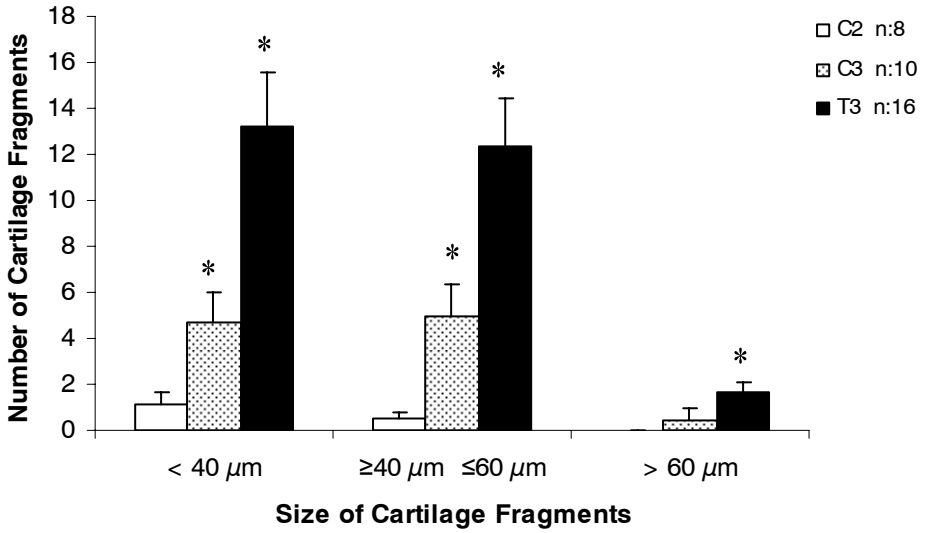

Figure 13. Number (per 20μL of synovial fluid) and size of cartilage fragments from the animals with osteochondritis dissecans. *P<0.05 compared with the same length in control group C2.

Table 11. Number and size of cartilage fragments from the animals with osteochondritis dissecans.

Group	n:	< 40μm	≥ 40μm and ≤ 60μm	> 60μm
C-2	08	1.13 ± 0.55	0.50 ± 0.31	0
C-3	10	4.70 ± 1.32 *	5.00 ± 1.35 *	0.40 ± 0.52
T-3	16	13.25 ± 2.35 *	12.31 ± 2.15 *	1.63 ± 0.47 *

Results are expressed as means ± S.E.M.
n: number of samples
*P<0.05 compared with the same length in control group C2.

Osteoarthritic joints also presented a great amount of cartilage wear particles, as can be seen in figure 14 and table 12. The number of small and middle fragments was significant increased in T2 and T3, and when related to big particles this increase was significant only in T3.

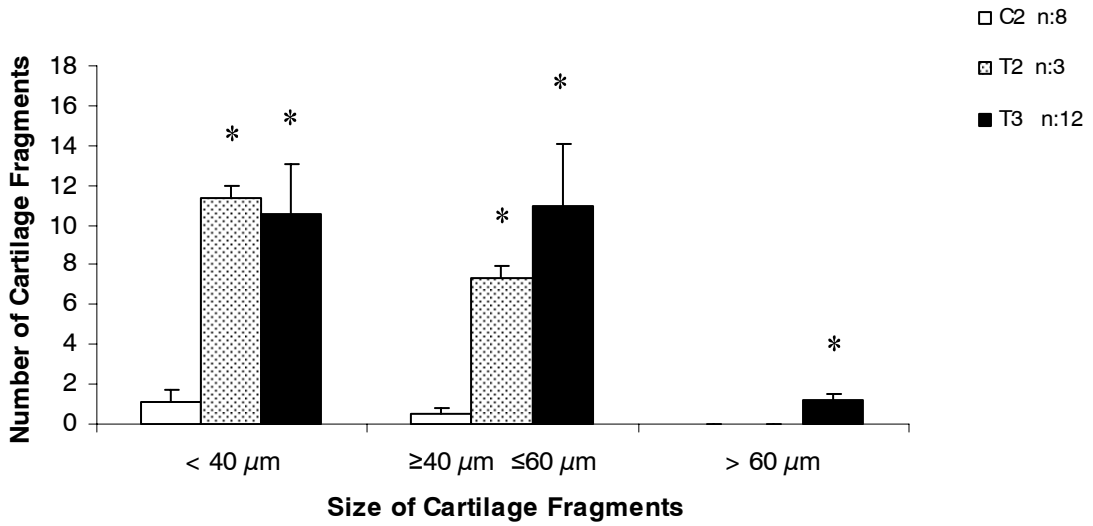

Figure 14. Number and size of cartilage fragments from the animals with osteoarthritis. *P<0.05 compared with the same length in control group C2.

Table 12. Number and size of cartilage fragments from the animals with osteoarthritis.

Group	n:	< 40μm	≥ 40μm and ≤ 60μm	> 60μm
C-2	08	1.13 ± 0.55	0.50 ± 0.31	0
T-2	03	11.33 ± 0.62 *	7.33 ± 0.62 *	0
T-3	12	10.58 ± 2.44 *	11.00 ± 3.09 *	1.17 ± 0.38 *

Results are expressed as means ± S.E.M.
n: number of samples
*P<0.05 compared with the same length in control group C2.

In synovial fluid from animals with navicular disease, there was a significant increased number of small, middles and big cartilage wears in groups C3 and T2 (figure 15, table 13). These group represent respectively patient without and with joint pain. However, the number of particles found, no matter which size, was not so high as in animals with diagnosis of osteochondritis dissecans or osteoarthritis.

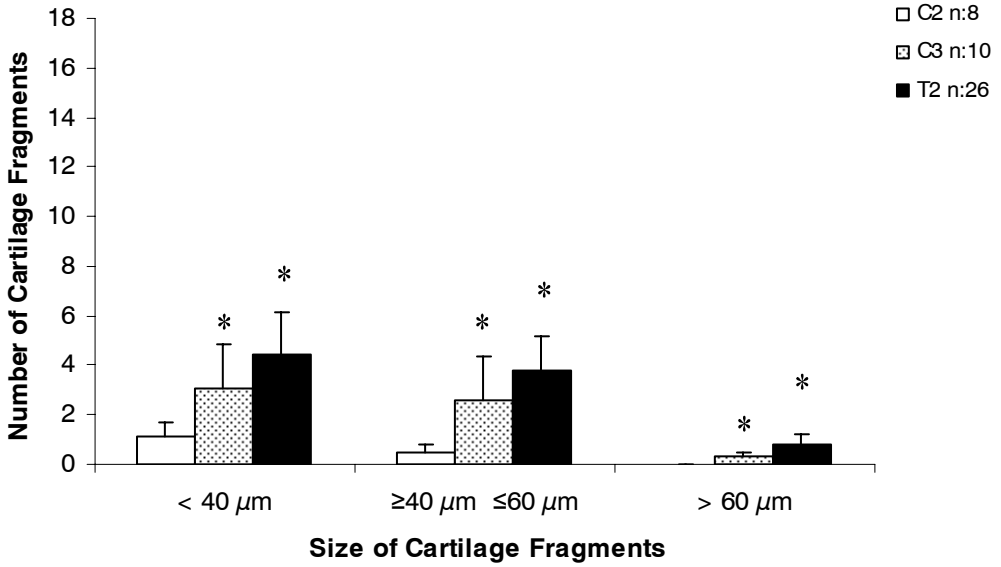

Figure 15. Number and size of cartilage fragments from the animals with navicular syndrome. *P<0.05 compared with the same length in control group C2.

Table 13. Number and size of cartilage fragments from the animals with navicular syndrome.

Group	n	< 40μm	≥ 40μm and ≤ 60μm	> 60μm
C-2	08	1.13 ± 0.55	0.50 ± 0.31	0
C-3	10	3.10 ± 1.78 *	2.60 ± 1.78 *	0.30 ± 0.20 *
T-3	16	4.46 ± 1.67 *	3.77 ± 1.43 *	0.77 ± 0.48 *

Results are expressed as means ± S.E.M.
n: number of samples
*P<0.05 compared with the same length in control group C2.

5.4 Age of the horses versus cartilage particles

In table 14 we can see the relationship between age and the different diagnosis of joint disease found in this study. Most of the animals were between 2 and 10 years old (41 animals). 37 horses were between 11 and 20 years old. There were also 5 patients less than 1 year and 5 older patients with more than 21 years old. As can be seen in table 15, the greatest average number of small, middle and big cartilage particles was presented in patients between 2 and 10 years old. There is exactly where we find most of the horses with OCD and OA.

Table 14. Relationship between the age of the horses, and the various diagnoses of joint disease (osteochondritis dissecans: OCD; navicular syndrome: NS; Podarthritis: PO; infectious arthritis: IA; osteoarthritis: OA).

Age of the horse	n	OCD	NS+PO	IA	OA
1 year old or younger	05	01	01	03	0
Between 2 and 10 years old	41	16	12	03	10
Between 11 and 20 years old	37	09	21	02	05
Older than 21 years	05	0	05	0	0
Total	88	26	39	08	15

n: number of horses

Table 15. Age of the patients and its relationship with the amount and size (small: <40μm, middle: ≥40μm and ≤60μm and big: >60μm) of cartilage fragments found in synovial fluid.

Joint	n	< 40μm	≥40μm and ≤60μm	>60μm
1 year old or younger	05	2.4 ± 1.4	3.0 ± 2.3	0.2 ± 0.2
Between 2 and 10 years old	41	9.0 ± 2.8	8.6 2.9	1.0 ± 0.5
Between 11 and 20 years old	37	4.7 ± 1.9	4.0 ± 1.6	0.6 ± 0.4
Older than 21 years	05	3.6 ± 0.8	3.8 ± 0.8	0.4 ± 0.2
Total	88			

Results are expressed as means ± S.E.M
n: number of horses

5.5 Different joints versus cartilage particles

The samples were collected from different joints, including coffin, fetlock, carpal, tarsal and stifle joints. The relationship between the number of samples in each joint and the different diagnosis of the horses can be seen in table 16.

Table 16. Several joints and amount of samples from horses with different diagnoses (osteochondritis dissecans: OCD; navicular syndrome: NS; podarthritis:PO; infectious arthritis: IA; osteoarthritis: OA).

Joint	Number of samples	OCD	NS + PO	IA	OA
Coffin	39	0	39	0	0
Fetlock	17	13	0	02	02
Carpal	06	0	0	03	03
Tarsal	20	10	0	03	07
Stifle	06	03	0	0	03
Total	88	26	39	8	15

The following table shows a relationship between the joint and number and size of cartilage particle. The average number of cartilage wear particles was highest in stifle joints in small, middle and big particles, whereas the coffin joints showed the smallest average number. Big fragments, greater than 60μm, appeared in all joints.

Table 17. Several joints and their relationship with the amount and size (small: <40μm, middle: ≥40μm and ≤60μm and big: >60μm) of cartilage fragments found in synovial fluid.

Joint	Number of samples	< 40μm	≥40μm and ≤60μm	>60μm
Coffin	39	3.9 ± 1.6	3.5 ± 1.5	0.6 ± 0.4
Fetlock	17	5.5 ± 1.9	9.1 ± 3.5	0.6 ± 0.5
Carpal	06	8.0 ± 2.4	7.1 ± 1.3	0.7 ± 0.4
Tarsal	20	9.1 ± 2.7	7.5 ± 2.4	1.1 ± 0.5
Stifle	06	14.8 ± 3.3	13.0 ± 2.3	1.2 ± 0.5

Results are expressed as means ± S.E.M

5.6 Synovial fluid analysis

5.6.1 Colour

From the normal patients, 3 presented a yellow straw synovial fluid. The other samples from sound joints were pale yellow (89.3%), almost colorless. Some samples (15) were bloody, ranging from pale to dark red. From these samples, 8 came from horses with navicular disease, 4 from patients with infeccious arthritis, 2 from horses with osteochondritis dissecans (OCD) and only 1 from an osteoarthritic joint. Most of the samples from patients with osteoarthritis (38.9%) and OCD (53.3%) were pale yellow. These results can be observed in table 18.

Table 18. Relationship between the colour of the synovial fluid sample and the different diagnoses of joint disease (normal; osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA and Podarthritis: PO).

n	Diagnostic	Pale yellow	Yellow straw	Dark yellow	Red (bloody)
28	normal	25 (89.3%)	03 (10.7%)	-	-
8	IA	01 (12.5%)	-	03 (37.5%)	04 (50%)
26	OCD	15 (57.7%)	07 (26.9%)	02 (7.7%)	02 (7.7%)
15	OA	8 (53.3%)	06 (40%)	-	01 (6.7%)
36	NS	14 (38.9%)	12 (33.3%)	02 (5.6%)	08 (22.2%)
3	PO	03 (100%)	-	-	-
116		66	28	7	15

n: number of samples

The following table presents the relationship between the colour and the number of small, middle and big cartilage fragments found in the synovial fluid. Samples yellow straw showed an average number of small, middle and big cartilage fragments significantly greater than other colours. Bloody samples also presented significant increased average number of big particles, when compared to pale and dark yellow samples.

Table 19. Relationship between the colour of the synovial fluid sample and the amount and size of cartilage particles found.

Colour	n	$< 40\mu$m	$\geq 40\mu$m and $\leq 60\mu$m	$> 60\mu$m
Pale yellow	66	4.82 ± 2.28	4.30 ± 2.53	0.35 ± 0.31
Yellow straw	28	7.61 ± 2.71 *	7.18 ± 2.19 *	1.21 ± 0.55 *
Dark yellow	07	4.29 ± 2,05	4.29 ± 1.95	0.29 ± 0.20
Red/Bloody	15	5.00 ± 2.14	3.93 ± 1.87	0.67 ± 0.40 *

n: number of samples
* $P < 0.05$

5.6.2 Opacity

All the samples from control groups (normal patients) and from horses with podarthritis, navicular disease and osteochondritis dissecans presented a transparent synovial fluid. 75 % of the synovial fluid from horses with infectious arthritis showed great opacity. Most of the samples from horses with osteoarthritis (93.3%) were transparent, just 1 was opaque.

Table 20. Relationship between the opacity of the synovial fluid sample and the different diagnoses of joint disease (normal; osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA and Podarthritis: PO).

n	Diagnostic	Transparent (Clear)	Opaque
28	normal	28 (100%)	-
08	IA	02 (25%)	06 (75%)
26	OCD	26 (100%)	-
15	OA	14 (93.3%)	01 (6.7%)
36	NS	36 (100%)	-
3	PO	03 (100%)	-
116		109	07

n: number of samples

The average number of small, middle and big cartilage wear particles was greater in transparent samples. Concerning middle and big fragments, this number was indeed significant decreased in opaque synovial fluid, as can be seen in the following graphic:

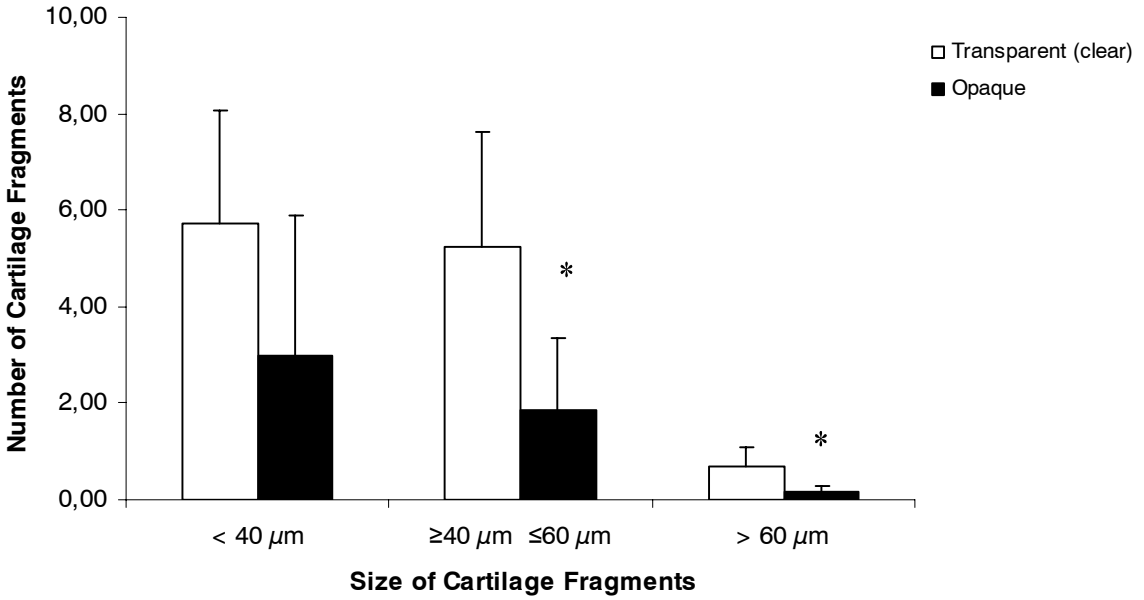

Figure 16. Average number and size of cartilage fragments found in transparent or opaque samples. * $P < 0.05$

5.6.3 Presence of flocculent material

All the samples from control groups (normal patients) and from horses with podarthritis and navicular disease were free of flocculent material. The presence of flocculent material was observed in 5 samples: 2 from horses with septic arthritis, 1 from a patient with OCD and 2 from osteoarthritic joints. In these samples, as can be seen in figure 21, horses with osteochondritis dissecans and osteoarthritis presented a great number of cartilage wear particles, whereas patients with infectious arthritis showed just a few fragments.

Table 21. Relationship between the 5 samples with the presence of flocculent material and the number of cartilage particles found (osteochondritis dissecans: OCD; infectious arthritis: IA; osteoarthritis: OA).

Sample	Diagnostic	$< 40\mu$m	$\geq 40\mu$m and $\leq 60\mu$m	$> 60\mu$m
1	IA	01	0	0
2	IA	01	01	0
3	OCD	22	10	02
4	OA	12	09	02
5	OA	19	15	03

5.6.4 Viscosity

All synovial fluid samples from control groups (normal patients) presented high viscosity. Most of the samples from horses with septic arthritis, osteoarthritis and navicular disease presented low or very low viscosity. The viscosity was high in 53.8% of the synovial fluid from horses with osteochondritis dissecans. All the samples from patients with diagnosis of podarthritis (3) showed low viscosity. The majority of samples from patients with osteoarthritis presented low (40%) or very low (26.7%) viscosity.

In table 22 this data can be observed. Figure 17 presents the relationship between samples with high, low or very low viscosity and number and size of cartilage wear particles. Statistical analysis showed no significance among the groups.

Samples with high viscosity were those in which the separation of fingers produced a string 2.5 cm long or more before braking. Samples with low viscosity were those in which the separation of fingers produced a string between 1 and 2.5 cm long before braking. When this string was less than 1 cm long, the samples were classified as very low viscosity.

Table 22. Relationship between the viscosity of the synovial fluid sample and the different diagnoses of joint disease (normal; osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA and Podarthritis: PO).

n	Diagnostic	Very low	low	High
28	normal	0	0	28 (100%)
08	IA	02 (25%)	05 (62.5%)	01 (12.5%)
26	OCD	02 (7.7%)	10 (38.5%)	14 (53.8%)
15	OA	04 (26.7%)	06 (40%)	05 (33.3%)
36	NS	19 (52.8%)	09 (25%)	08 (22.2%)
03	PO	0	03 (100%)	0
116		27	33	56

n: number of samples

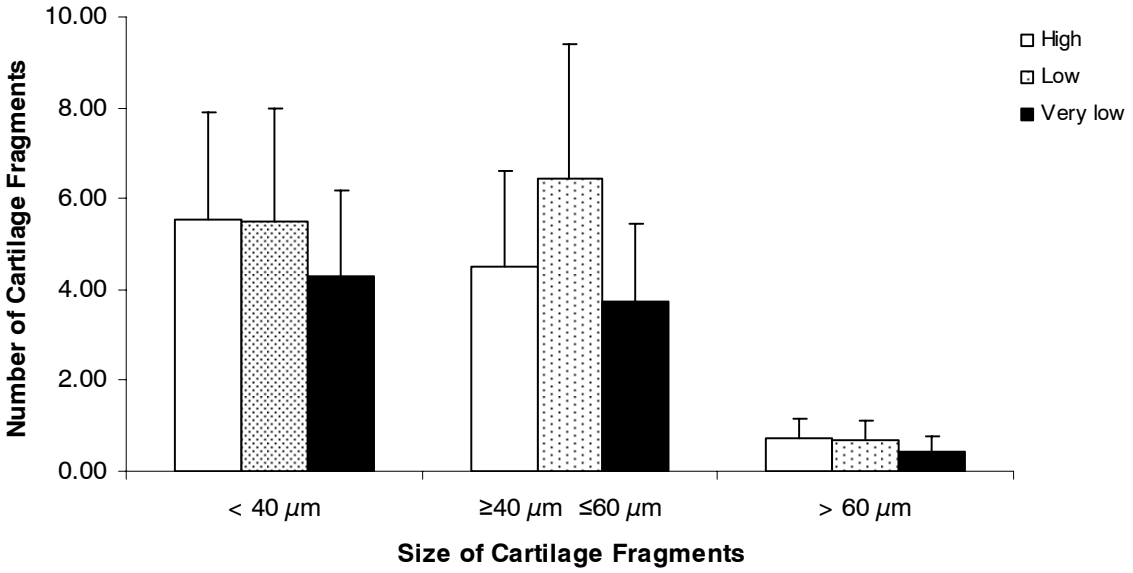

Figure 17. Number and size of cartilage fragments found in samples of very low, low and high viscosity.

5.6.5 Total protein content

Total protein content was increased in patients with infectious arthritis. As can be observed in table 23, in these patients the number of cartilage wear particles was very low. Patients with osteochondritis dissecans and osteoarthritis presented increased number of cartilage fragments, but no significant increased total protein content. There is not seen to be a relation between protein content and presence of cartilage wear particles.

Table 23. Relationship between presence, number and size of cartilage particles found in different diagnoses of joint disease (osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA) and total protein account. It presents also control groups C-1 and C-2.

Diagnostic Groups	Total protein content g/dl	$< 40\mu$m	$\geq 40\mu$m and $\leq 60\mu$m	$> 60\mu$m
Normal				
C-1	1.87 ± 0.30	1.80 ± 0.76	1.05 ± 0.58	0.15 ± 0.15
C-2	1.97 ± 0.26	1.13 ± 0.55	0.50 ± 0.31	0
OCD				
C-3	2.02 ± 0.28	4.70 ± 1.32	5.00 ± 1.35	0.40 ± 0.52
T-3	2.42 ± 0.44	13.25 ± 2.35	12.31 ± 2.15	1.63 ± 0.47
OA				
T-2	2.51 ± 0.36	11.33 ± 0.62	7.33 ± 0.62	0
T-3	2.87 ± 0.65	10.58 ± 2.44	11.00 ± 3.09	1.17 ± 0.38
NS				
C-3	1.79 ± 0.35	3.10 ± 1.78	2.60 ± 1.78	0.30 ± 0.20
T-3	1.84 ± 0.41	4.46 ± 1.67	3.77 ± 1.43	0.77 ± 0.48
IA				
T-1	4.11 ± 0.49	0.50 ± 0.22	1.38 ± 0.97	0

Results are expressed as means ± S.E.M.
n: number of samples

5.6.6 Total white blood cell count

In table 24 the relation between total white blood cell count and number and size of cartilage particles can be observed. An increased number of total leukocytes was presented in synovial fluid of patients with infectious arthritis, by whom a very low number of cartilage fragments was found. In contrast, patients with osteochondritis dissecans and osteoarthritis showed an increased number of fragments, but no significant increased number on total leukocyte count was observed.

Table 24. Relationship between presence, number and size of cartilage particles found in different diagnoses of joint disease (osteochondritis dissecans: OCD; navicular syndrome: NS; infectious arthritis: IA; osteoarthritis: OA) and total white blood cell account. It presents also control groups C-1 and C-2.

Diagnostic Groups	White blood cell (cells/μL)	$< 40\mu$m	$\geq 40\mu$m and $\leq 60\mu$m	$> 60\mu$m
Normal				
C-1	155 ± 26	1.80 ± 0.76	1.05 ± 0.58	0.15 ± 0.15
C-2	172 ± 71	1.13 ± 0.55	0.50 ± 0.31	0
OCD				
C-3	170 ± 39	4.70 ± 1.32	5.00 ± 1.35	0.40 ± 0.52
T-3	281 ± 87	13.25 ± 2.35	12.31 ± 2.15	1.63 ± 0.47
OA				
T-2	192 ± 72	11.33 ± 0.62	7.33 ± 0.62	0
T-3	265 ± 95	10.58 ± 2.44	11.00 ± 3.09	1.17 ± 0.38
NS				
C-3	159 ± 33	3.10 ± 1.78	2.60 ± 1.78	0.30 ± 0.20
T-3	189 ± 42	4.46 ± 1.67	3.77 ± 1.43	0.77 ± 0.48
IA				
T-1	75000 ± 29000	0.50 ± 0.22	1.38 ± 0.97	0

Results are expressed as means ± S.E.M.
n: number of samples

5.6.7 Saline washings of the joints

It was collected washes from just 5 samples. All of them during arthroscopy and from horses with osteoarthritis. The Next table showed the relation between the cartilage wear particles found in them and in the synovial fluid collected from the same joint before the washes were make.

Table 25. Number of cartilage particles found in washes (WA) and synovial fluid (SF) in 5 osteoarthritic joints.

Sample	$< 40\mu$m		$\geq 40\mu$m and $\leq 60\mu$m		$> 60\mu$m	
	WA	SF	WA	SF	WA	SF
1	30	19	22	15	4	3
2	12	9	8	5	3	0
3	11	10	6	8	0	0
4	7	8	6	9	0	1
5	18	12	12	9	1	2

6 DISSCUSSION

6.1 General considerations

It is well known that every affliction to a joint that causes arthritis and consequently, changes in the intraarticuar biochemistry, release of several different inflammatory molecules and products of modified anabolism and catabolism, have the potential to lead to osteoarthritis (OA; degenerative joint disease). Management of OA is one of the greatest challenges both in veterinary and human medicine, with poor prognosis and frustrating evolution in the majority of the cases. For this reason it is of paramount importance to develop a method that enables early diagnosis of degenerative joint disease.

Studies demonstrated that approximately 41% of the human population will develop arthritic disorders by the age of 65 years (Wood, 1977). Other studies showed that 60 to 70% of people in their 60s and 70s have evidence of osteoarthritis in their knee (Felson, 1988; Copper, 1994). Regarding equine patients, this degenerative joint disease is also of great relevance. A research from Todhunter and Lust, in 1992, showed that osteoarthritis was responsible for 54% of lame horses.

Small lesions, micro damages in the articular cartilage, appear long before a radiographic diagnosis could be made (Chan et al., 1991; Fife et al., 1991; Blackburn et al., 1994). Until the present date, the best method to make this diagnosis is still arthroscopy. Several studies have suggested that the presence of cartilage wear debris in the synovial fluid would provide a good prediction of articular damage (Tew, 1980; Tew and Hackett, 1981; Evans et al., 1980, 1981a/b). The objective of this study was to check the relationship between these cartilage particles (presence, number and size of cartilage fragments in the synovial fluid) and osteoarthritis. As other joint diseases, as osteochondritis dissecans and all cases of arthritis (traumatic, inflammatory or not), can also lead to secondary osteoarthritis, it was decided to check the presence of cartilage fragments in these joints as well. It was also matter of this study to exam these cartilage debris in joints with podarthritis, with or without involvement of the navicular bone (podarthritis + navicular disease).

So we came to this main objective of this study that was to compare the presence of clinical signs in different joint diseases and the presence, number and amount of cartilage fragments found in these joints. It was from the beginning very important that the method used in this research be simple and not expensive. So, costly or time-consuming analytic methods have been avoided to make the overall analysis practical for routine use.

6.2 Cartilage particle analysis

This data shows a significant increase in the number of cartilage fragments in osteoarthritic joints, as has been previously described in the literature (Evans et al, 1981 and 1982; Kuster et al, 1998). The same was noticed in the samples from horses with osteochondritis dissecans (OCD), which is in contrast with the study of Tew in 1980, where he rarely found large amounts of particles in these joints. Patients diagnosed with navicular syndrome in group C3 were horses in the chronic stage of this disease. These animals were pain-free, although the intraarticular pressure was still high and they were, therefore, still being treated. Acute cases of joint disease can be seen in figure 9, group T1, where patients with infectious arthritis were included. There was no significantly increased number of fragments in these samples, in contrast with the study from Tew, 1980, where 65% of acute cases showed a great quantity of cartilage wear particles. However, in both studies there was no correlation between the characteristics of effusion or protein concentration and the number of cartilage fragments.

In the present study cartilage wear particles with a length between 20 μm and 200 μm were detected. A comparison with the findings from Kuster and coworkers, who searched for cartilage fragments in human knee joint, regarding the size of fragments is appropriate. Using ferrographic analysis, they have found a range from 7.8 μm and 268.3 μm. The range is quite similar to what we found, but probably with ferrography it is possible to detect smaller particles than with the technique applied here. This could also be due to the quantity of sample analysed. For instance, ferrograms are made in the whole synovial fluid or washing collected, whereas in our study we analysed in microscope just 1.8μL of the sediment of each sample after centrifugation. Although different methodology, both results suggest a very good correlation between cartilage damage in osteoarthritis and wear fragments analysis. In their research, it is interesting to notice that the average length of particles does not increase with the severity of the disease (grade 1: 66.6 μm; grade 2: 51.7 μm; grade 3: 50.7 μm). We have

found a significant increase in the number of fragments when comparing osteoarthritic and healthy joints, no matter if small, middle or big particles.

Like other authors, we have also found cartilage wear particles in healthy, or at least apparently healthy joints (Evans et al., 1981a; Evans et al, 1982; Kuster et al, 1998). Our control group C1, where no radiographies were taken, showed a greater number of particles than group C2, without radiographic alterations. For this reason we decided to use C2 as control in the last three graphics (figure 7,8 and 9). In control group C1 there were even particles greater than 60μm, although few, which was not seen in C2. Kuster and colleagues in 1998 have also found particles ranging from 13.6 to 97.4 μm (average 37.6 μm) in healthy joints, which is very similar to the results of the present study, whereas in the study from Tew and Hackett, in 1981, cartilage fragments were not found in 42 from the 47 apparently normal joints analysed. Evans and coworkers, in 1982, have found particles in healthy knee, but all smaller than 40 μm.

An important point to consider is whether it is normal to find these fragments in sound joints, specially in those submitted to great load, or if that already represents micro damages, so small that could not be evidenced in arthroscopy, as proposed by Evans in 1982. In 1980, Tew suggested that biomechanically sound joints would not produce any significant number of cartilage wear particles when placed under normal stress, whereas impaired cartilage even under normal stress could be expected to produce wear debris (Tew and Hackett, 1981). According to these authors, the absence of cartilage particles in joint fluid could be even equally important in accurate diagnosis of the lameness cause.

6.3 Pain and cartilage particles

In the present study, there was a correlation between pain and amount of fragments, as can be seen in figures 9, 13 and 15. In figure 9, a general graphic with all the groups and different joint diseases this correlation is already clear, but it becomes more evident in the graphics of navicular syndrome (NS) and osteochondritis dissecans (OCD). It has been described in the literature that in patients with OCD, significant damage only occurs when the chip lies between articular surfaces (Tew, 1980). That is also when they would probably cause pain. However, our results indicate that, even when there are no clinical signs, there can already be cartilage micro damage. Unfortunately, there were no samples from horses with osteoarthritis

in group C3, where the horses showed radiographic finding, but no pain. It should be very interesting to compare these findings in asymptomatic osteoarthritic joints as well. However, all the patients with osteoarthritis (OA) in this study presented lameness grade 3 or 4.

It could be expected a positive relation between the grade of lameness and the number of cartilage wear particles. This positive relation was observed in grade 1, 2 and 3 in all sizes of fragments, as can be seen in table 9. However there was a subtle decrease in grade 4. This was probable due to the great number of samples from horses with infectious arthritis in this group. This patients presented severe pain, but no large amounts of cartilage fragments. They could have pushed down the average number, although the horses with osteochondritis dissecans and osteoarthritis in this group have shown increased number of cartilage particles. However, several studies have proved a weak correlation between the magnitude of pain and the severity of cartilage damage observed (Ross and Dyson, 2003).

Incipient cartilage lesions do not cause pain, which is a potential reason for delayed detection of articular cartilage damage (Wojtys et al, 1990). The hallmark of OA is articular cartilage degeneration, a process occurring in a tissue devoid of sensory innervation. As a result, lameness is typically attributed to involvement of peri-articular soft tissues and bony, the former being relatively richly innervated. In capsular and ligamentous tissues, unmyelinated sensory nerve fibers conduct painful sensations from widely distributed free nerve endings (Murrel et al., 1995; Sasaki et al., 1998). With joint inflammation, these receptors exhibit increased sensibility. Specifically the threshold for these receptors is reduced by inflammatory mediators such as prostaglandins, and increased receptor activity accompanies physiological joints excursions (Hepplemann et al., 1986).

Although the severity of soft tissue changes are related, horses with significant peri-articular fibrosis occasionally demonstrate less than the expected degree of lameness. Studies of joint capsule innervation in arthritic specimens have revealed that with time degeneration of neurons is common, which provides a potencial reason for the less than expected magnitude of pain in some patients having clearly demonstrable changes in peri-articular soft tissues (Nilsson, 1973; Nilsson and Olsson, 1973). That could also be a reason for the pain free horses with radiographic findings for navicular disease in group 3. Radiographic findings can last for a lifetime in these horses, even if they become sound, are no lame and return to work.

However, the patients of the present study still presented high coffin joint pressure and the number of small, middle and big cartilage wear fragments were significant increased.

Bone and periosteum also contribute to osteoarthritis pain. The periosteum is well innervated, and the periosteal disruption that accompanies the development of peri-articular osteophytes is a source of joint pain (Kellgren, 1983). The subchondral plate and epiphyseal trabecular bone make variable contributions to clinical signs. For example, many, but not all, horses with subchondral cystic lesions demonstrate lameness (Verschooten and DeMoor, 1982).

6.4 Age and cartilage particles

The age range in this study was quite large. As cartilage damages are generally related to age, it could be expected that old patients would present the greatest amount of cartilage fragments in their affected joints. However, the group between 2 and 10 years old showed the greater number of cartilage fragments, whereas animals older than 20 years showed not so many particles. It can be observed that here diagnosis played a more important role than age. Osteoarthritic joints presented the greatest number of particles and they were exactly more prevalent in this study in patients between 2 and 10 years old. In the group of older patients, most of them were diagnosed with navicular disease. In this disease, the average number of wear particles was pretty smaller than in osteoarthritic joints.

6.5 Human and equine osteoarthritis

The pathogenesis of osteoarthritis is still not fully understood but believed to be multifactorial including genetic, environmental, metabolic, and biomechanical factors (Buckwalter and Mankin, 1998). Human OA is a mainly age-related disease. One of the remarkable changes of cartilage in the human aging population is a diffuse and profound loss of chondrocytic cellularity (up to 50% decrease) with an increase in the number of empty lacunae, matrix calcification, and fibrillation in the superficial layer (Stockwell, 1971; Buckwalter and Mankin 1998).

It is interesting to notice that whereas the prevalence of degenerative joint disease in humans is very correlated with aging, in this study, that was not observed, suggesting that in horses the process of onset of this disease may be different of what occurs in humans. In horses, trauma has been suggested to be a common etiological factor in the occurrence of OA. This

may be a single event trauma or more insidious damage caused by multiple repetitive traumatic insults, which implies that the traumatic damage is due to the normal, day-to-day activities, which may include athletic training and competition (Kidd et al. 2001). In Horses there is an increased incidence of OA in young horses used for athletic activity (Hoffman et al. 1984).

In the patients of the present study, osteoarthritis caused by trauma or secondary to other joint disease, was more frequently than primary degeneration caused by age. In the study from Tew and Hackett, in 1981, where they compared the presence of cartilage debris and visual inspection of the joint, of the 8 horses between 6 and 14 years of age, only 1 was free of articular damage, which supports this suggestion that in horses, these damages appears much earlier than in human joints.

In other studies it was reported that samples from patients of similar age, with an identical diagnosis, yielded not always similar populations of wear particles (Evans et al., 1981a; Evans et al., 1982). In this study that was also observed. There was always a variation in the number and size of fragments between animals of the same age, in all the joint diseases analysed. One explanation is that many of the standard diagnostic “labels” cover heterogeneous collections of abnormalities. Other explanation would be that the sensitivity of the technique of cartilage wear particles analysis enables arthritis to be examined at a more subtle level than with other diagnostic tools and, consequently, previously unrealised variations in the joint disease may be uncovered.

6.6 Synovial Fluid Analysis

Routine synovial fluid analysis was made. Most of the samples where pale yellow, just in the animals with infectious arthritis, the majority where bloody or dark yellow. According to van Pelt, in 1974, if the sample contains blood, it is important to determine whether haemorrhage occurred prior to aspiration as a result of injury or resulted from rupture of subsynovial capillaries associated with needle puncture of the joint. In acute traumatic arthritis, the sample is usually markedly hemorrhagic, whereas samples streaked with fresh blood are indicative of haemorrhage incurred as a result of aspiration. That could have occurred in this study in some samples from horses with navicular disease, because of the strongly irrigated area from where the samples are collected.

The average number of cartilage wear particles was significant increased in yellow straw samples. Pale yellow and dark yellow samples showed a statistically equal average number of fragments. In bloody samples, just the number of big particles was statistically increased. However, colour does not seem to have any direct correlation with the amount of particles.

High opacity was mostly seen in the present study in the samples from horses with infectious arthritis, with is probably due to presence of great number of nucleated cells (leukocytes) in synovial fluid.

Flocculent material was found in 5 samples. It was suggested in the literature, that in degenerative joint disease, osteochondromatosis, or osteochondritis dissecans, cartilage shreds give the joint sample a markedly flocculent appearance (van Pelt, 1974). The number of samples in this study was to slow to make statistical analysis that could confirm this suggestion. However, in table 21, the number of fragments in this 5 samples can be observed. In the synovial fluid from horses with OA (2) and OCD (1), the number of small, middle and big particles is high, suggesting that this could really be the reason of this flocculent material. Whereas in patients with infectious arthritis (2), the number of cartilage fragments is very low, which suggests that here the presence of flocculent material is probably related to other factors. Further studies should be done to add other observations regarding this thema.

There is not seem to be a direct correlation between viscosity, total protein or total white cell count with the presence, number and size of cartilage wear particles. Statistical analysis of the viscosity data showed no significance among the samples. Total protein was increased in samples from horses with infectious arthritis, which presented a decreased number of cartilage fragments. Total white cell count was also increased in these patients. For example, patients with OCD showed increased number of cartilage fragments and low total white cell count.

6.7 Saline Washings

We have just been able to collected 5 saline washings (more than 100mL). All of them were collected during arthroscopy and from osteoarthritic joints. Unfortunately, the number of samples was small and, therefore, statistical analyses were not made. However, the data was put on a graphic to compare the finding in synovial fluid and saline washings in the same joint (table 26). It could be expected that more particles would be found in washings than in

synovial fluid, but the small number of samples does not allow this conclusion. Evans and colleagues, in 1982, have analysed just saline washings (100mL injected). Kuster and coworkers, in 1998, have extracted synovial fluid from joints with effusion and saline washings (10mL injected) from the other joint tested, but comparison between the presence of cartilage fragments in synovial fluid and saline washings at the same joint were not made.

6.8 Shape of fragments and cartilage zone

The articular cartilage is divided in four anatomic zones. The thin tangential or superficial layer, in contact with synovial fluid, is only a few microns thick and contain small and flat chondrocytes. Immediately below is the transitional zone, characterized by larger and rounded cell profiles. In radiate zone, the cells are larger and arranged with their long axis perpendicular to the surface. The deepest layer of cartilage is continuous with the calcified tissue (McIlwraith and Trotter, 1996).

Several authors have tried to correlate the quality of cartilage wear particles with the respective zone. The unique chondrocyte morphology of articular cartilage at various depths from the articular surface to the subchondral bone has been suggested as a basis for determining the anatomic zone from which the cartilage particles arise. Based on the characteristics of the cells, Tew in 1980 found, in acute cases, small fragments, that appeared microscopically as noncellular flakes and he concluded they should have come from superficial and transitional layers. In cases of chronic lameness, the fragments were thought to originate from deeper layers. Tew also found particles originating from deep layers and subchondral bone in patients with OCD. As we did not stain the fragments, it was impossible to identify the cells and, therefore, the zone of origin.

In the study from Tew and Hackett, in 1981, they also tried to recognize the cartilage zone. The most common articular lesions encountered in this work were cartilage erosion of distal metacarpus and ulceration and punctuate erosion of craniomedial carpal bone. The cartilage fragments in these joints appeared as thin particles, with clearly identifiable chondrocytes. In this study, they also observed that many of the cartilage fragments isolated from the synovial fluid resemble those produced by artificial means. Other particles recovered appeared to have separated from the articular cartilage as tangential rather than transverse fragments. In these

cases, the chondrocyte morphology was similar to that observed in tangential sections of articular cartilage.

Other researchers have proved the relationship between the shape of the wear particles and the extent of articular cartilage damage. They have then correlated these findings with the most probable zone. Lamellar, leaf wear particles found in healthy joints should have come from the lamina splendens. Damage in tangential zone would probably originate elongated, rod-shaped particles and deep cartilage zone damage would produce chunky fragments (Kuster et al, 1998).

In the present study the cartilage wear fragments were classified according to their shapes in lamellar, elongated and chunky. Most of the particles presented a lamellar shape. Chunky particles were just seen in synovial fluid from horses that showed pain and radiographic alterations, from horses with diagnosis of osteoarthritis or osteochondritis dissecans. It is probable that these fragments were originated in deep cartilage layers. Most of them came from patients with severe arthroscopic findings, like cartilage erosion.

However, sometimes it was difficult to classify the shape. This is a subjective analysis and for some particles the visual differentiation might be difficult, due to the fact that wear particles can exhibit similar morphology. This fact was also noticed by Kuster and coworkers, in 1998.

The biochemical disturbances of joint tissues as a direct or indirect consequence of cellular reactions to wear particles should foster the generation of yet more particles. Owing to the altered ambient conditions under which these fragments are produced, their morphology and chemistries might well be changed. Through ferrography, morphologic changes were detected (Evans et al., 1980). Chemical characterisation of the wear particles should prove a fruitful study, but one requiring extremely sensitive techniques. Although a single synovial fluid aspirate often contains sufficient wear debris for its aggregate chemical properties to be established (Cheung et al., 1980), characterisation of individual particles will probably require monospecific antibodies to the different types of collagen and to other antigenic components of cartilage such as the “link” and “core” proteins of proteoglycans. In this way damage to the articular or meniscal cartilage, for instance, could be readily distinguished.

6.9 Considerations on the methodology

With the methodology performed in the present study it is not possible to quantify the number of cartilage wear particles per μL of synovial fluid. We have presented just a means of comparison among the number of fragments in several joints. That must be taken into consideration because this number could not be compared, for example, with the number found through a different method.

Before performing arthrocentesis, there is an agreement that aseptic preparation should always be carried out. However, considerable debate and variation exists among clinicians regarding the need to clip the hair over the site. Some clinicians always clip the hair, whereas others never do. Still others shave the hair in a small area directly over the injection. The results of the study from Hague and colleagues in 1997 indicated no significant difference in the number of post-scrub colony-forming units (bacterial flora) between clipped and unclipped skin over the distal interphalangeal and carpal joints. According to the experience of Prof. Dr. Hertsch, there are no differences between clipped or unclipped joints as well, but shaving should always be avoided.

It is important to have a standardized lameness scoring system that allows the clinician to quantify lameness within and between horses. Unfortunately, there is not one system that is used worldwide, as it should be. The system adopted by the American Association of Equine Practitioners is as follows (AAEP, 1999):

Grade 1 lameness is difficult to observe and not consistently apparent regardless of circumstances (such as weight carrying, circling, inclines, hard surfaces).

Grade 2 lameness is difficult to observe at a walk or trotting a straight line but is consistently apparent under certain circumstances (such as weight carrying, circling, inclines, hard surfaces).

Grade 3 lameness is consistently observable at a trot under all circumstances.

Grade 4 lameness is obvious, with marked nodding, hitching, or shortened stride.

Grade 5 lameness is characterized by minimal weight bearing in motion or at rest and the inability to move.

In the present study, a scale from 0 to 5 was used as well, which shows some similarities with the system adopted by AAEP. Sue Dyson, 2003, presented a lameness scoring just based on the observation of the horse at a trot in hand, in a straight line, on firm or hard surface.

Grade 1 mild lameness is observed while the horse is trotted in a straight line. When the lame forelimb strikes, a subtle head nod is observed; when the lame hindlimb strikes, a subtle pelvic hike occurs. The head nod and pelvic hike may be inconsistent at times.

Grade 2 obvious lameness is observed. The head nod and pelvic hike are seen consistently, and excursion is several centimeters.

Grade 3 pronounced head nod and pelvic hike of several centimeters is noted. If the horse has unilateral singular hindlimb lameness, a head and neck nod is seen when the diagonal forelimb strikes the ground (mimicking ipsilateral forelimb lameness).

Grade 4 severe lameness with extreme head nod and pelvic hike is present. The horse can still be trotted, however.

Grade 5 the horse does not bear weight on the limb. If trotted, the horse carries the limb.

For Dyson the AAEP system is a little confusing because it grades lameness at both the walk and trot. It does not account for a horse that has a shortened stride at walk that trots sound.

6.10 Potential application of this method

Some potential applications of synovial fluid analysis suggested with the use of this method are:

* Assessing cases of acute trauma that may involve damage of the articular cartilage and identifying chronic conditions in which articular degeneration may contribute to lameness;

* Determining the severity and depth of articular degeneration, and predicting degree of recovery.

* Identifying fractures that extend into synovial cavity. Blood, fat and mineralized cartilage in the synovial fluid indicate acute fractures. In chronic cases, the blood and fat may have been reabsorbed, but cartilage particles remain in the joint fluid for some time.

* Evidence of extensive deep articular degeneration may indicate severe biomechanical instability of a joint.

* Monitoring the efficacy of therapeutic measures designed to arrest articular degeneration and determining when it is safe to resume training, if ever.

6.11 Last considerations

The method used here is simple and good, but the veterinarian or technician needs some practical visual training to identify the cartilage fragments and not to confound them with artefacts, like pieces from synovial membrane. Amorphous debris may originate from the synovial membrane. The basis of histologic identification of cartilage wear debris can be demonstrated using artificially generated particles of articular cartilage. In this study were used a lot of pieces of cartilage and synovial membrane to make slides for this purpose. Training with serum can also bring benefits in avoiding artefact confounders. According to Tew and Hackett, in 1981, particles of 10μm or bigger are easily distinguished from amorphous protein and fibrin clots by their appearance under polarized light. However, in this study, just particles bigger than 20 μm were identified. Little is known about the fate of these fragments arising from articular cartilage presumably they are eventually degraded or absorbed. However, there is evidence to suggest that, once produced, they would remain in the synovial fluid at least for some weeks (Tew and Hackett, 1981).

7 CONCLUSION

Synovial fluid cartilage wear particle analysis may be a significant diagnostic aid for equine practitioners. The simplicity of the technique makes its routine use practical.

There is a significant relation between the number of cartilage fragments and the degree of cartilage damage.

The greatest number of cartilage debris was found in patients with osteoarthritis and osteochondritis dissecans, but in navicular disease, this number was also significantly increased.

No direct relation between the number of cartilage fragments and colour, opacity, viscosity, total protein content and total white blood cell count was observed in this study.

The presence of cartilage wear particles in other joint disease, as osteochondritis dissecans and podarthritis, and in navicular disease could help the evaluation and prognoses. As it is known that any of these affection could lead to degenerative joint disease, this foresee would be of crucial importance even to evaluate the efficacy of the chosen treatment.

This simple minimal invasive method could allow an early diagnosis of osteoarthritis, possibly even before the onset of clinical signs. Further studies should be performed, because this technique has potential to provide a great assessment of degenerative joint disease progress, long-term prognosis and evaluation of therapeutic regimes at any point in the processes, without repeated surgical intervention.

8 SUMMARY

Analysis of cartilage wear particles in equine joints and their relationship to several joint diseases.

Objective: the main objective of this study was to compare the presence of clinical signs in various joint diseases and the presence, number and amount of cartilage wear fragments found in synovial fluid. It was also matter of this study to analyse the relationship between several equine joint diseases, like osteochondritis dissecans, osteoarthritis, septic arthritis, podarthritis and navicular syndrome, and the presence, number and size of cartilaginous wear particles in the synovial fluid collected from different joints (coffin, fetlock, carpal, tarsal and stifle joints).

Materials and Method: 116 samples of synovial fluid from 85 horses were analysed for the presence, number and size of cartilage wear particles. Other synovial fluid analyses, like colour, opacity, viscosity, total protein content and total white blood cell count were also made and compared to the presence of cartilage wear particles. The material was centrifuged and prepared to microscopic examination. The animals presented different joint affections and were divided into 6 groups, accordingly to the presence of clinical signs, radiographic and arthroscopic alterations.

Results: There was a significant correlation between cartilage debris and lesions of the articular surfaces in horses with osteoarthritis (middle particles: 11.00 ± 3.09; control: 0.50 ± 0.31) and osteochondritis dissecans (middle particles: 12.31 ± 2.15; control: 0.50 ± 0.31). Horses with osteochondritis dissecans that showed lameness presented a number of small, middle and big fragments greater than no lame horses. However, the number of cartilage particles in these no lame patients was already significantly increased when compared to control group (small particles found in lame horses: 13.25 ± 2.35; no lame horses: 4.70 ± 1.32; control: 1.13 ± 0.55), which means that even when there is no pain, there can be already cartilage damage. Patients that showed lameness presented in general a greater number of cartilage fragments than horses without pain (small particles in group T3: 12.11 ± 2.41;

control: 1.13 ± 0.55). This just did not occurred in acute cases, for example, in horses with septic arthritis (middle particles in group T1: 1.73 ± 0.97; control: 0.50 ± 0.58), that showed no significant number of cartilage fragments, although severe lameness. Horses with navicular syndrome (small particles: 4.46 ± 1.67; control: 1.13 ± 0.55) presented few cartilage fragments when compared to patients with osteoarthritis and osteochondritis dissecans. However, this number was significantly increased when compared to control group. No direct relation between the number of cartilage fragments and colour, opacity, viscosity, total protein content and total white blood cell count was observed in this study.

Conclusion: This is a simple minimal invasive method that could permit a better assessment of degenerative joint disease progress, long-term prognosis and evaluation of therapeutic regimes. The presence of cartilage wear particles in other joint disease, as osteochondritis dissecans and podarthritis, and in navicular disease could help the evaluation and prognoses. As it is known that any of these affection could lead to degenerative joint disease, this foresee would be of crucial importance even to evaluate the efficacy of the chosen treatment.

Keywords: synovial fluid, cartilage particles, joint disease, articular cartilage, arthritis, osteochondritis dissecans.

9 ZUSAMMENFASSUNG

Knorpelabriebprodukte in der Synovia beim Pferd und ihre Beziehung zu verschiedenen Gelenkerkrankungen.

Fragestellung: Lahmheit ist eine der Hauptursachen für Schmerzen und Unwohlsein bei Pferden und führt zu finanziellen Verlusten bei den Besitzern. Die Frühdiagnose von Gelenkerkrankungen, insbesondere degenerative Gelenkerkrankungen, stellt eine große Herausforderung für Tierärzte auf der ganzen Welt dar. Eine Untersuchung der Synovia auf Knorpelfragmente könnte einen Gelenkknorpelschaden frühzeitig nachweisen. Das Ziel dieser Studie war nicht nur einen Bezug zwischen Zahl und Größe der Knorpelabriebprodukte zu verschiedenen Gelenkerkrankungen nachzuweisen, sondern eine sehr einfache Methode auch zu entwickeln, die als Diagnosewerkzeug schließlich verwendet werden könnte.

Material und Methode: Insgesamt wurden 116 Gelenke (Huf-, Fessel-, Vorderfußwurzel-, Sprung- und Kniegelenk) von 85 Pferden, die zwischen 2 Monaten und 23 Jahre alt waren, punktiert und auf die Anzahl und Größe von Knorpelabriebprodukte untersucht. Die Proben wurden 15 Minuten zentrifugiert und anschließend mikroskopisch ausgewertet. Andere Synovia Untersuchungen, so wie Farbe, Transparenz, Konsistenz, Protein und Gesamtleukozytenzahl wurden auch dargestellt. Die Pferde kamen in die Klinik zur Lahmheitsuntersuchung. Bei diesen Patienten wurde die Diagnose einer Gelenkerkrankung gestellt. Die Pferde wurden anhand klinische, röntgenologische und arthroskopische Befunde in 6 Gruppen geteilt.

Ergebnisse: Bei Pferden mit Arthrose konnte ein signifikanter Zusammenhang zwischen Knorpelrückstand in der Synovia und der Beschädigung der Gelenkoberfläche festgestellt werden (mittlere Knorpelpartikel: 11,00 ± 3,09; Kontrolle: 0,50 ± 0,31). Dies deckt sich mit der Untersuchung von Evans et al, 1981 und 1982; Kuster et al, 1998. Bei Pferden mit Osteochondritis dissecans gab es eine signifikant erhöhte Anzahl von kleineren, mittleren (mittlere Knorpelpartikel: 12,31 ± 2,15; Kontrolle: 0,50 ± 0,31) und großen Knorpelfragmenten. Bei diesen Patienten konnte auch eine Beziehung zwischen Schmerzen und der Menge der Knorpelfragmente festgestellt werden, aber die Anzahl der Knorpelfragmente, bei diesen lahmen Pferden, war trotzdem signifikant erhöht (Kleinere

Knorpelpartikel bei lahmen Pferden: 13,25 ± 2,35; bei nicht lahmen Pferden: 4,70 ± 1,32; Kontrolle: 1,13 ± 0,55). Allgemein wurden mehr Knorpelfragmente in Synovia von lahmen Pferden gefunden (kleinere Knorpelpartikel in Gruppe T3: 12,11 ± 2,41; Kontrolle: 1,13 ± 0,55). Allerdings, bei akuten Fällen, wie zum Beispiel in Synovia von Pferden mit septischer Arthritis, konnte ein derartiger Zusammenhang nicht festgestellt werden (mittlere Knorpelpartikel in Gruppe T1: 1,73 ± 0,97; Kontrolle: 0,50 ± 0,58). Diese Patienten zeigten sehr wenig Knorpelfragmente in ihrer Synovia, dafür aber hochgradige Lahmheit. Im Vergleich zu Pferden mit Arthrose und Osteochondritis dissecans wurden in der Synovia von Pferden mit Hufrehe nur wenige Knorpelfragmente gefunden. Allerdings ist auch hier die Menge der gefundenen Knorpelfragmente significant erhöht im Vergleich zur Kontrollgruppe (kleinere Knorpelpartikel: 4,46 ± 1,67; Kontrolle: 1,13 ± 0,55). In diese Studie wurde kein Zusammenhang zwischen Farbe, Transparenz, Konsistenz, Protein oder der Gesamtleukozytenzahl und der Menge von Knorpelfragmenten festgestellt.

Schlussfolgerung: Der Nachweis von Knorpelabriebprodukten in der Synovia stellt eine einfache, minimalinvasive Methode zur besseren Einschätzung von degenerativen Gelenkkrankheiten und einen Fortschritt, bezüglich der Prognosestellung und des Therapieansatzes dar.

Schlüsselwörter: Synovia, Knorpelabriebprodukte, degenerative Gelenkerkrankungen, Gelenkknorpel, Arthrosis, Osteochondritis dissecans.

10 REFERENCES

AAEP. (1999)
Guide to veterinary services for horse show, ed 7, Lexington, Ky.
American Association of Equine Practioners.

AIGNER T., VORNEHM S.I., ZEILER G., GEBHARD P.M., KIRCHNER T., and McKENNA L. (1997)
Suppression of gene matrix expression in upper zone chondrocytes of osteoarthritic cartilage.
Arthritis Rheum 40:562-9.

AIGNER T., HEMMEL M., NEUREUTER D., DUJIA G., VON DER MARK K., and BAYLISS M.T. (2001)
Apoptotic cell death is not a widespread phenomenon in normal aging and ostheoarthritic human articular knee cartilage: a study of proliferation, programmed cell death (apoptosis), and a viability of chondrocytes in normal and osteoarthritic human knee cartilage.
Arthritis Rheum 44:1304-12.

AIGNER T., KURZ B., NAOSHI F., FUKUI N., and SANDELL L. (2002)
Roles of chondrocytes in the pathogenesis of osteoarthritis.
Curr Opin Rheum 14:578-84.

ALLEN R.E., BLAKE D.R., and NAZHAT N.B. (1989)
Superoxide radical generation by inflamed human synovium after hypoxia.
Lancet 2:282-3.

AMIEL D., FRANK C., and HARWOOD F. (1984)
Tendons and ligaments: a morphological and biochemical comparison.
J Orthop Res 1:257-265.

BARTHOLOMEW B.A. (1968)
Synovial fluid enzymes.
Univ of Michigan Med Cent J 34:249-51.

BERTONE A.L., and McILWRAITH C.W. (1987)
A review of current concepts in the theraphy of infectious arthritis in the horse.
Proc Am Assoc Equine Pract 32:323-39.

BERTONE A.L., McILWRAITH C.W., and JONES R.L. (1987)
Comparison of various treatments for experimentally induced equine infectious arthritis.
Am J Vet Res 48:519-29.

BERTONE A.L., DAVIS D.M., and COX H.U. (1992)
Arthrotomy versus arthroscopy and partial synovectomy for treatment of experimentally induced infectious arthritis in horses.
Am J Vet Res 53:585-91.

BERTONE A.L., PALMER J.L., and JONES J. (2001)
Synovial fluid cytokines and eicosanoids as markers of joint disease in horses.
Vet Surg 30:528-38.

BILLINGHURST R.C. (2002)
Biomarkers of joint disease.
In: Current Therapy in Equine Medicine, 5th edn, Ed: NE Robinson, WB Saunders Co., Philadelphia, pp 513-20.

BILLINGHURST R.C., BRAMA P.A., and VAN WEEREN P.R. (2004)
Evolution of serum concentrations of biomarkers of skeletal metabolism and results of radiography as indicators of severity of osteochondrosis in foal.
Am J Vet Res 65:143-50.

BIRKELAND R., and HAAKENSTAD L.H. (1968)
Intracapsular bony fragments of the distal tibia of the horse.
J Am Vet Med Assoc 152:1526-29.

BJORK J., KLEINAU S., TENGBIAD A., and SMEDEGARD G. (1989)
Elevated levels of serum hyaluronate and correlation with disease activity in experimental models of arthritis.
Arthritis Rheum 32:306-11.

BLACKBURN W.D., BERNREUTER W.K., and ROMINGER M.(1994)
Arthroscopic evaluation of knee articular cartilage: a comparison with plain radiographs and magnetic resonance imaging.
J Rheumatol 21:675-9.

BLANCO F.J., GUITIAN R., VÁZQUEZ-MARTUL E., TORO F.J., and GALDO F. (2004)
Osteoarthritis chondrocytes die by apoptosis: a possible pathway for osteoarthritis pathology.
Arthritis Rheum 41: 284-9.

BLUNDEN A., DYSON S., MURRAY R., and SCHRAMME M. (2006a)
Histological findings in horses with chronic palmar foot pain and age-matched control horses. Part 1: navicular bone and related structures.
Equine Vet J 38:15-22.

BLUNDEN A., DYSON S., MURRAY R., and SCHRAMME M. (2006b)
Histological findings in horses with chronic palmar foot pain and age-matched control horses. Part 2: deep digital flexor tendon.
Equine Vet J 38:23-27.

BONIFACE R.J., CAIN P.R., and EVANS C.H. (1988)
Articular responses to purified cartilage proteoglycans.
Arthritis Rheum 31:258-66.

BOON D. (1997)
Synovial fluid analysis: a guide for small-animal practitioners.
Vet Med 92:443-51.

BRAMA P.A.J., VAN DE BOOM R., DE GROOT J., KIERS G.H., and VAN WEEREN P.R. (2004)
Collagenase-1 MMP-1 activity in equine synovial fluid: influence of age, joint pathology, exercise and repeated arthrocentesis.
Equine Vet J 36:34-40.

BROMMER H., van WEEREN P.R., and BRAMA P.A.J. (2003)
New approach for quantitative assessment of cartilage degeneration in horses with osteoarthritis.
Am J Vet Res 64:83-7.

BRENDOV E. (1997)
Osteochondrosis in Standardbred trotters: heritability and effects on racing performance. Doctorate Thesis. Swedish University of Agricultural Sciences, Uppsala.

BUCKWALTER J.A., and MANKIN H.J. (1998)
Articular cartilage: Degeneration and osteoarthritis, repair, regeneration, and transplantation.
AAOS Instructional Course Lectures 47: 487-504.

BURR D.B. (2003)
Subchondral bone in the pathogenesis of osteoarthritis. Mechanical aspects.
In: Osteoarthritis, 2nd Edn. Eds: KD Brandt ; M Doherty and LS Lohmander, Oxford University press, Oxford. pp 125-33.

CABAUD P.A., and WRÖBLEWSKI F. (1958)
Colorimetric measurement of lactic dehydrogenase activity of body fluids.
Am J Clin Path 30:234-6.

CARLSTEN J., SANDGREN B., and DALIN G. (1993)
Development of osteochondrosis in the tarsocrural joint and osteochondral fragments in the fetlock joints of Standardbred trotters. I. A. Radiological survey.
Equine Vet J Suppl 16:42-7.

CARROL G. (1989)
Measurement of sulphated glycosaminoglycans and proteoglycan fragments in arthritic synovial fluid.
Ann Rheum Dis 48:1-24.

CASSCELLS S.W. (1980)
The place of arthroscopy in the diagnosis and treatment of internal derangement of the knee.
Clin Orthop 151:135-42.

CHAN W.P., LANG P., and STVENS M.P. (1991)
Osteoarthritis of the knee: Comparison of radiography, CT, and MR imaging to assess extent and severity.
Am J Roentgenol 157:799.

CHEUNG H.S., RYAN L.M., KOZIN F., and McCARTY D.J. (1980)
Identification of collagen subtypes in synovial fluid sediments from arthritic patients.
Am J Med 68:73-9.

CHRISMAN O.D., FESSEL J.M., and SOUTHWICK W.O. (1965)
Experimental production of synovitis and marginal articular exostoses in the knee joints o dogs.
Yale J Biol Med 37:409-12.

CHUNG H.T., PAE H.O., CHOI B.M., BILLAR T.R., and KIM Y.M. (2001)
Nitrous oxide as a bioregulator of apoptosis.
Biochem Biophys Res Comm 282:1075-9.

CLEGG P.D., and MOBASHERI A. (2003)
Chondrocyte apoptosis, inflammatory mediators and equine osteoarthritis.
Vet J 166:3-4.

CLEGG P.D., BURKE R.M., COUGHLIN A.R., RIGGS C.M., and CARTER S.D. (1997a)
Characterization of equine matrix metalloproteinases 2 and 9; and identification of the cellular source of these enzymes in joints.
Equine Vet J 29:335-42.

CLEGG P.D., COUGHLIN A.R., RIGGS C.M., and CARTER S.D. (1997b)
Matrix metalloproteinases 2 and 9 in equine synovial fluids.
Equine Vet J 29:343-48.

COHEN A.S. (1967)
Synovial fluid.
In: Laboratory Diagnostic Procedures in the Rheumatic Disease.
Little, Brown and Company, Boston, MA. pp 2-50.

COLLES C., and HICKMAN J. (1977)
The arterial supply of the navicular bone and its variations in navicular disease.
Equine Vet J 9:150-154

COPPER C. (1994)
Osteoarthritis Epidemiology. In: Rheumatology. Eds. Klippel J, Dieppe P.
Mosby, London, pp 7.3.1-7.3.4.

CURTISS P.H. (1964)
Changes produced in the synovial membrane and synovial fluid by disease.
J Bone & joint Surg 46A:873-88.

DABAREINER R.M., SULLINS K.E., and WHITE II N.A. (1993)
Progression of femoropatellar osteochondrosis in nine young horses. Clinical, radiographic and arthroscopic findings.
Vet Surg 22:515-23.

DIK K.J., EZERINK E.E., and VAN WEERE P.R. (1999)
Radiographic development of osteochondral abnormalities, in the hock and stifle of Dutch Warmblood foals, from age 1 to 11 months.
Equine Vet J Suppl 31:9-15.

DODGE G.R., and POOLE A.R. (1989)
Immunohistochemical detection and immunohistochemical analysis of type II collagen degradation in human normal, rheumatoid, and osteoarthritic articular cartilage and explants of bovine articular cartilage cultured with interleukin 1.
J Clin Invest 83:647-61.

DONABÉDIAN M. (2006)
Les affections de type ostéochondrotique: effet d´une croissance rapide liée à des apports nutritionnels élevés, mécanisme endocrine, et outils de detection précoces chez le cheval. Doctorate Thesis. University of Limoges.

DYSON S., MURRAY R., and SCHRAMME M. (2005)
Lameness associated with foot pain: results of magnetic resonance imaging in 199 horses (January 2001- Dcember 2003) and response to treatment.
Equine Vet J 37:113-121.

DYSON S., MURRAY R., BLUNDEN T., and SCHRAMME M. (2006)
Current concepts of navicular disease.
Equine Vet Educ 18(1):45-56.

ELLISON R. (1988)
The cytologic examination of synovial fluid.
Semin Vet Med Surg (Small Anim) 3:133-9.

ERBACHER M. (1993)
Nachweis von Knorpelabriebprodukten in der Synovia von Fesselgelenken bei Pferden – eine klinische, röntgenologische, arthroskopische und labordiagnostische Studie.
Veterinary Medicine Thesis, Hannover

EVANS C.H. (1992)
Response of synovium to mechanical injury.
In. Fineman G.A.M., Noyes F.R. (eds): Biology and biomechanics of the traumatized synovial joint. Rosemont IL, American Academy of Orthopedic Surgery, pp 17-26.

EVANS C.H., and TEW W.P. (1981)
Isolation of biological materials by use of erbium III-induced magnetic susceptobilities.
Science 213:653-4.

EVANS C.H., BOWEN E.R., BOWEN J., TEW W.P., and WESTCOTT VC (1980)
Synovial fluid analysis by ferrography.
Biochemical and Biophysical Methods, 1980 Jan-Feb;2(1):11-8.

EVANS C.H., MEARS D.C., and MCKNIGHT J.L. (1981a)
A preliminary ferrographic survey of wear particles in human synovial fluid.
Arthritis Rheum 24:912-918.

EVANS C.H., MEARS D.C., and COSGROVE J.L. (1981b)
Release of neural proteinases from mononuclear phagocites and synovial cells in response to cartilaginous wear particles in vitro.
Biochem Biophys Acta 677:287-94.

EVANS C.H., MEARS D.C., and STANITSKI C.L. (1982)
Ferrographic analysis of wear in human joints.
The journal of bone and joint surgery 64(5):572-78.

EYRE D.R.. WU J.-J., and WOODS P. (1992)
Cartilage-specific collagens: structural studies. In: Articular Cartilage and Osteoarthritis. Ed. K. Kuettner. Raven Press New York, pp. 119-31.

FARNDALE R.W., BUTTLE D.J., and BARRET A.J. (1986)
Improved quantification and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue.
Biochem. Biophys. Acta 883:173-177.

FELSON D. (1988)
Epidemiology of hip and knee osteoarthritis.
Epidemiol Rev. 10: 1-28.

FERNANDEZ F., GRINDEM C., and LIPOWITZ A. (1983)
Synovial fluid analysis:preparation of smears for cytologic examination of canine synovial fluid.
J Am Anim Hosp Assoc 19:727-34.

FIFE R.S., and BRANDT K.D. (1993)
Extracellular matrix of cartilage:glycoproteins. In. Woessner JF, Howell DS (Eds): Joint Cartilage Degradation: basic and clinical aspects. New York, Marcel Dekker pp139-158.

FIFE R.S., BRANDT K.D., and BRAUNSTEIN E.M. (1991)
Relationship between arthroscopic evidence of joint space narrowing in early osteoarthritis of the knee.
Arthritis Rheum 69:302-12.

FRISBIE D.D., KAUCAK C.E., TROTTER G.W., POWERS B.E., WALTON R.M., and McILWRAITH (1997)
Effects of triamcinolone acetonide on an in vivo equine osteochondral fragment exercise model.
Equine Vet J 29:349-59.

FRISBIE D.D., RAY C.S., IONESCU M., POOLE A.R., CHAPMAN P.L., and McILWRAITH C.W. (1999)
Measurement of synovial fluid and serum concentrations of the 846 epitope of chondroitin sulphate and of carbory propeptides of type II procollagen for diagnosis of osteochondral fragmentation in horses.
Am J Vet Res 60(3):306-9.

GADHER S.J., EYERE D.R., and DUANCE V.C. (1988)
Susceptibility of cartilage collagens type II, III, X and XI o human synovial collagenases and neuthrophil elastase.
Eur J Biochem 175:1-7.

GIBSON N., CARMICHAEL S., and LI A. (1999)
Value of direct smears of synovial fluid in the diagnosis of canine joint disease.
Vet Rec 144:463-5.

GOLDBERG R.L., and RUBIN A.S. (1989)
Serum hyaluronate as a marker for disease severity in the lactobacillus casei model of arthritis in the rat.
J Rheumatol 16:92-6.

GOLDBERG R.L., HUFF J.P., LENZ M.E., GLICKMAN P., KARTZ R., and THONAR E.J.-M.A. (1991)
Elevated plasma levels of hyaluronate in patients with osteoarthritis and rheumatoids arthritis.
Arthr Rheum 34:799-807.

GREENWALD R.A., MOY W.W., and SEIBOLD J. (1978)
Functional properties of cartilage proteoglycans.
Sem Arth Rheum 8:53-67.

HAGUE B.A., HONNAS C.M., SIMPSON R.B., and PELOSO JG. (1997)
Evaluation of skin bacterial flora before and after aseptic preparation of clipped and nonclipped arthrocentesis sites in horses.
Vet Surg. 26: 121-25.

HAMBLIN M.G., and STACHOWIAK G.W. (1993)
Comparison of boundary fractal dimensions from projected and sectioned particle images. Part I – Technique evaluation.
J Comput Assist Microsc 5:291-300.

HARDINGHAM T., and BAYLISS M. (1990)
Proteoglycans of articular cartilage: Changes in aging and in joint disease.
Semin. Arthr. Rheum. 20, 12-33.

HARDY R., and WALLACE L. (1974)
Arthrocentesis and synovial membrane biopsy.
Vet Clin North Am Small Anim Pract 4: 449-62.

HASHIMOTO S., OCHS R.L., KOMIYA S., and LOTS M. (1998a)
Linkage of chondrocyte apoptosis and cartilage degradation in human Osteoarthritis.
Arthritis Rheum 41: 1632-8.

HASHIMOTO S., OCHS R.L., ROSEN F., QUACH J., MCCABE G., SOLAN J., SEEGMILLER J.E., TERKELTAUB R., and LOTZ M. (1998b)
Chondrocyte-derived apoptotic bodies and calcification of articular cartilage.
Proc Natl Acad Sci USA 95: 3094-9.

HASHIMOTO S., TAKAHASHI K., AMIEL D., COUTTS R., and LOTZ M. (1998c)
Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis.
Arthritis Rheum 41:1266-74.

HEINEGARD D., INEROT S., WISLANDER J., and LINDBLAD G. (1985)
A method for the quantification of cartilage proteoglycan structure liberated to the synovial fluid during developing degenerative joint disease.
Scand J Clin Lab Invest 45:421-7.

HENDERSON B., and PETTIPHER E.R. (1985)
The synovial lining cell: biology and pathobiology.
Semin Arthritis Rheum 15:1-32.

HEPPLEMANN B., PFEFFER A., SCHAIBLE H.G., and SCHMIDT R.F. (1986)
Effects of acetylsalicylic acid and indomethacin on single groups III and IV sensory units from acutely inflamed joints.
Pain. Sep;26(3):337-51.

HERTSCH B. (1983)
Die röntgenologische Diagnose der Podotrochlose. In. PF Knezevic: Orthopädie bei Huf- und Klauentieren.
Verlag Schlütersche, Hannover, 12-17.

HERTSCH B. (1991)
Der orthopädische Beschlag bei der chronischen Stralbein- und Hufrollenerkrankung des Pferdes.
Prakt Tierarzt 72:65-68.

HERTSCH B., and HÖPPNER S. (1999)
Zur Diagnostik des Podothrochlose-Syndroms in der Praxis.
Pferheilkunde 15: 293-308.

HERTSCH B., WISSDORF H., and ZELLER R. (1982)
Die „sogenannten“ Gefäßlöcher des Strahlbeines und ihre Beziehung zum Hufgelenk – Ein Beitrag zur Pathogenese der Hufrollenerkrankung.
Tierärztl Prax 10:365-79.

HO G. (1993)
How best to drain an infected joint. Will we ever know for certain?
J Rheumatol 20:2001-3.

HOFFMAN K.D., POOL R.R., and PASCOE J.R. (1984)
Degenerative joint disease of the proximal interphalangeal joints of the forelimbs of two young horses.
Equine Vet J 16:138-40.

HOLLANDER J.L., JESSAR R.A., and McCARTY D.J. (1961)
Synovianalysis: an aid in arthritis diagnosis.
Bull Rheumat Dis, 12:263-4.

HÖPPNER S. (1993)
Vergleichende Studie über die Hufgelenkanästhesie und die direkte Druckmessung im Hufgelenk als diagnostische Kriterien bei der Differenzierung des Podotrochlose-Syndroms beim Pferd.
Thesis, Tierärztl Hochsch Hannover

HOROWITZ T. (1948)
Bone and cartilage debris in the synovial membrane.
J Bone Joint Surg (Am) 30A:579-88.

HOWELL D.S., and PELLETIER J.-P. (1993)
Etiophatogenesis of osteoarthritis.
In. McCarthy D.J. (ed): arthritis and Allied conditions. A Textbook of Rheumatology. Philadelphia, Lea & Febiger, pp1723-34.

HUNZINKER E.B., and TYLER J.A. (2003)
Articular cartilage repair.
In: Osteoarthritis, 2nd Edn. Eds. K.D. Brandt, M. Doherty and L.S. Lohmander, Oxford University Press, Oxford, pp 93-101.

IKE R.W. (1993)
The role of arthroscopy in the differential diagnosis of osteoarthritis of the knee.
Rheum Dis Clin North Am 19:673-96.

JOUGLIN M., ROBERT C., VALETTE J.P., QUINTIN-COLONNA F., and DENOIX J.M. (2000)
Metalloproteinases and tumor necrosis factor-alpha activities in synovial fluids of horses: correlation with articular cartilage alterations.
Vet Res 31(5):507-15.

KANE A.J., MCILWRAITH C.W., and PARK R.D. (2003)
Radiographic changes in Thoroughbred yearlings. Part 2:associations with racing performance.
Equine Vet J 35:366-74.

KAWCAK C.E., FRISBIE D.D., McILWRAITH C.W., TROTTER G.W., GILLETE S., POWERS B.E., and WALTON R. (1997)
The effect of intravenous administration of sodium hyaluronate on carpal joints in exercising horses after arthroscopic surgery and osteochondral fragmentation.
Am J Vet Res 58:1132-40.

KELLGREN J.H. (1983)
Pain in osteoarthritis.
J Rheumatol 9(Suppl):108-9.

KIDD J.A., FULLER C., and BARR A.R.S. (2001)
Osteoarthritis in the horse.
Equine Vet Educ 13:160-168.

KIRK T.B., and STACHOWIAK G.W. (1991)
Fractal computer image analysis applied to wear particles from arthritic and asymptomatic human joints.
J Orthop Rheumatol 4:13-30.

KORENEK N.L., UNDREWS F.M., MADDUX J.M., SANDERS W.L., and FAULK D.L. (1992)
Determination of total protein concentration and viscosity of synovial fluid from the tibiotarsal joints of horses.
Am J Vet Research 53(5):781-4.

KUETTNER K.E., and THONAR E.J.M.A. (2001)
Osteoarthritis and related disordes. Cartilage integrity and homeostasis.
In: Rheumatology, 2nd edn. Eds: J.H. Klippel and P.A. Dieppe, Mosby, London pp 6.1-6.16.

KUSTER M.S., PODSIADLO P., and STACHOWIAK G.W. (1998)
Shape of wear particles found in human knee joints and their relationship to osteoarthritis.
British J Rheumatol 37:978-84.

LANGOIS B., BLOUIN C., and PERROCHEAU M. (2006)
Influence of radiographic osteochondrosis status at 16-18 months, on racing performance in French trotters.
Pferdeheilkunde 22:461-4.

LATIMER F.G., KANEPS A.T., and PASQUINI C. (2000)
Stifle disease in the horses.
Compendium continuing education practicing veterinarian 22(4):381-90.

LEWIS J.L., DELORIA L.B., OYEN-TIESMA M., THOMPSON R.C., ERICSON M., and OEGEMA T.R. (2003)
Cell death after cartilage impact occurs around the matrix cracks.
J Orthop Res 21:881-7.

LOHMANDER L.S., and FELSON D.T. (1997)
Defining the role of molecular markers to monitor disease, intervention, and cartilage breakdown in osteoarthritis.
J Rheumatol 24:782-5.

LOTZ M., HASHIMOTO S., and KÜHN K. (1999)
Mechanism of chondrocyte apoptosis.
Osteoarthritis and Cartilage 7: 389-91

MACGREGOR C. (1984)
Studies on the pathology and treatment of navicular disease.
PhD Theisis, University of Edinburgh.

MACWILLIAMS P.S., and FRIEDRICHS K.R. (2003)
Laboratory evaluation and interpretation of synovial fluid.
Vet Clin Small Anim 33:153-78.

MADISON J.B., SOMMER M., and SPENCER P.A. (1991).
Reletions among synovial membrane histopathologic findings, synovial fluid cytologic findings, and bacterial culture results in horses with suspected infectious arthritis.64 cases (1979-1987).
J Am Vet Med Assoc. 198: 1655-1661.

MAYNE R. (1989)
Cartilage collagens. What is their function and are they involved in articular disease?
Arthr Rheum 32, 241-6.

MCDEVITT C.A., and MUIR H. (1976)
Biochemical change in the cartilage of the knee in experimental and natural osteoarthritis in the dog.
J Bone Joint Surg 58B:94-101.

MCILWRAITH C.W. (1982)
Current concepts in equine degenerative joint disease.
J Am Vet Med Assoc 180:239-50.

MCILWRAITH C.W. (1983)
Treatment of infectious arthritis.
Vet Clin North Am (Large animal Practice) 5:363-379.

MCILWRAITH C.W. (1996)
General pathobiology of the joint and response to injury.
In: *Joint disease in the horse*, Eds: C.W. McIlwraith and G.W. Trotter, W.B. Saunders Co., Philadelphia.pp 40-70.

MCILWRAITH C.W. (2002)
Disease of joints, tendons, ligaments and related structures.
In: Adams Lameness in Horses, 5th edn Ed. T.S. Stashak pp 459-479.

MCILWRAITH C.W. (2005)
Use of synovial fluid and serum biomarkers in equine bone and joint disease: a review
Equine Vet. Journal 37(5):473-482.

MCILWRAITH C.W., and TROTTER G.W. (1996)
Joint disease of the horse. Eds: Saunders Co., Philadelphia.

MCILWRAITH C.W., and VACHON A.M. (1988)
Review of pathogenesis and treatment of degenerative joint disease.
Equine Vet J S6:3-11.

MCILWRAITH C.W., and van SICKLE D.C. (1981)
Experimentally induced arthritis of the equine carpus: histologic and histochemical changes in the articular cartilage.
Am J Vet Res 43:209-17.

MEARS D.C., HANLEY E.N., RUTKOWSKI R., and WESTCOTT V.C. (1978)
Ferrography: its application to the study of human joint wear.
Wear 50:115-25.

MILLER E.J. (1980)
The collagen of joints.
In: The joints and synovial fluid. Ed. L. Sokoloff, Academic Press San Diego pp205-242.

MORREL V., and QUINN T.M. (2004)
Cartilage injury by ramp compression near gel diffusion rate.
J. Orthop. Res. 22:141-151.

MOW V.C., and HUNG C.T. (2003)
Mechanical properties of normal and osteoarthritic articular cartilage and the mechanobiology of chondrocytes.
In: Osteoarthritis, 2nd edn. Eds. K.D. Brandt, M. Doherty and L.S. Lohmander, Oxford University Press, Oxford. pp102-112.

MUIR H. (1977)
Molecular approach to the understanding of osteoarthritis.
Ann Rheum Dis 36:199-208.

MUIRDEN K.D. (1970)
Giant cells, cartilage and bone fragments within rheumatoid synovial membrane.
Aust Ann Med 2:105-110.

NAGASE H., and WOESSNER (1998)
Role of endogenous proteinases in the degradation of cartilage matrix.
In. Woessner JF, Howell DS (Eds): Joint cartilage degradation: Basis and clinical aspects. New York, Marcel Dekker pp 159-85.

MURRELL G.A.C., JANG D., and WILLIAMS R.G. (1995)
Nitric oxide activated metalloproteinase enzymes in articular cartilage.
Biochem Biophys Res Commun 206:15-21.

NILSSON G. (1973)
Lameness and pathologic changes in the distal joints and phalanges of the standardbred horse.
Acta Vet Scand 44 (Suppl):83-96.

NILSSON B., and OLSSON S.E. (1983)
Radiologic and phato-anatomic changes in the distal joints and the phalanges of the standardbred horses.
Acta Vet Scand 44(Suppl):108-12.

OSTBLOM L., LUND C., and MELSEN F. (1982)
Histological study of navicular bone disease.
Equine Vet J 14:199-202.

OXSPRING G.E. (1935)
The radiology of navicular disease, with observation on its pathology.
Vet Res 15:1433-47.

PAIMELLA L., HEISKANEN A., KURKI P., HELVE T., and LEIRISALO-REPO M. (1991)
Serum hyaluronate level as a predictor of radiologic progression in early rhematoid arthritis.
Arthr Rheum 34:815-21.

PALMER J.L., and BERTONE A.L. (1994)
Joint structure, biochemistry and biochemical disequilibrium in synovitis and equine joint disease.
Equine Vet J 26:263-77.

PATWARI P., FAY J., COOK M.N., BADGER A.M., KERIN A.J., LARK M.W., and GRODZINSKY A.J. (2001)
In vitro models for investigation of the effects of acute mechanical injury on cartilage.
Clin Orthop Rel Res 391S, S61-71.

PATWARI P., CHUBINSKAYA S., HAKIMIYAN A., KUMAR B., COLE A.A., KUETTNER K.E., RUEGER D.C., and GRODZINSKY A.J. (2003)
Injurious compression of adult human donor cartilage explants: investigation of anabolic and catabolic processes.
Trans. Orthop. Res. Soc. 49:0695.

PERSSON L.(1996)
On the synovia in horses. A clinical and experimental study.
Acta Vet Scand. (Suppl. 16), 38-41.

PEDERSEN N. (1978)
Synovial fluid collection and analysis.
Vet Clin North Am Small Anim Pract 8:495-9.

PODSIADLO P., and STACHOWIAK G.W. (1995)
Median-sigma filter for SEM wear particle images.
J Comput Assist Microsc 7:67-82.

POOL R.R. (1996)
Pathologic manifestations of joint disease in the athletic horse.
In: *Joint disease in the horse*, Eds: C.W. McIlwraith and G.W. Trotter, W.B. Saunders Co., Philadelphia.pp 87-104.

POOL R.R., and MEAGHER D.M. (1990)
Pathologic findings and pathogenesis of racetrack injuries.
Vet Clin N Am:Large animal practice 6:1-30.

POOL R.R., MEAGHER D., and STOVES S. (1989)
Pathophysiology of navicular syndrome.
Vet Clin N Am: Equine Pract 5:109-29.

POOLE A.R. (1993)
Cartilage in heath and disease.
In: Arthritis and allied conditions. A Textbook of Reumathology. Ed. D.J.. McCarty, Lea & Febiger, Philadelphiy pp 279-333.

POOLE A.R., IONESCU M., SWAM A., and DIEPPE P.A. (1986)
Changes in cartilage metabolism in arthritis as measured by release of proteoglycan structures into the synovial fluid.
Ann Rheum Dis 45:491-7.

POOLE A.R., IONESCU M., SWAM A., and DIEPPE P.A. (1994)
Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of glycosaminoglycan epitopeson fragments of the cartilage proteoglycan aggrecan: implications for pathogenesis.
J Clin Invest 94:25-33.

RADIN E.L. (1999)
Subchondral bone changes and cartilage damage.
Equine Vet J 31:94-5.

RADIN E.L., and ROSE R.M. (1986)
Role of subchondral bone in the initiation and progression of cartilage damage.
Clin Orthop Rel Res 213:34-40.

RATCLIFFE A., DOHERTY M., MAINI R.N., and HARDINGHAM T.E. (1988)
Increased concentrations of proteoglycan components in the synovial fluids of patients with acute but not chronic joint disease.
Ann Rheum Dis 47:826-32.

RAY C.S., POOLE A.R., and McILWRAITH C.W. (1996)
Use of synovial fluid and serum markers in articular disease.
In. Joint disease in the horse. Ed: C.W. McIlwraith and G.W. Trotter, W.B. Saunders Co., Philadelphia. pp 203-16.

REITMAN S., and FRANKEL S. (1957)
A colorimetric method for the detection of serum glutamic oxalacetic and glutamic pyruvic trasaminases.
Am J Clin Path 28:55-63.

RIGGS C.M. (2006).
Osteochondral injury and joint disease in the athletic horse.
Equine Vet Educ 18(2): 100-12.

RIJKENHUIZEN A.B.M. (2006)
Navicular disease: a review of what's new.
Equine Vet J 38:82-8.
RIJKENHUIZEN A.B.M., NEMETH F., DIK K. and GOEDEGEBURE S. (1989)
The arterial supply of the navicular bone in adult horses with navicular disease.
Equine Vet J 21:418-24.

ROPES M.W., and BAUER W. (1953)
Synovial fluid changes in joint disease.
Harvard University Press, Cambridge, MA.

ROPES M.W. (1957)
Examination of synovial fluid.
Bull Rheumat Dis 7:21-22.

SANDER T. (1990)
Synoviauntersuchungen - im Besonderen die Bestimmung der Hyaluronsäure bei an Corpora libera im Talokruralgelenk erkrankten und artroskopisch behandelten Pferden.
Vet med Diss., Hannover.

SAPOLSKY A.I., HOWELL D.E.S., and WOESSNER J.F. (1974)
Neutral proteases and cathepsin-D in human articular cartilage.
J Clin Invest 53:1044-53.

SAPOLSKY A.I., KEISER H., HOWELL D.E.S., and WOESSNER J.F. (1976)
Metalloproteinase of human articular cartilage that digest cartilage proteoglycans at neutral and acid pH.
J Clin Invest 58:1031-1041.

SASAKI K., HATTORI T., and FUJISAWA T. (1998)
Nitric oxide mediates interleukin-1-induced gene expression of matrix metalloproteinases and basic fibrosblast growth factor in cutured rabbit articular chondrocytes.
J Biochem 123:431-39.

SCHENK R.K., EGGLI P.S., and HUNZIKER E.B. (1986)
Articular cartilage morphology.
In: Joint disease in the horse, Eds: McIIwraith C.W. and Trotter G.W. Saunders Co., Philadelphia. 1996 pp 10.

SCHNEIDER R.K., BRAMLAGE L.R., and MOORE R.M. (1992)
A retrospective study of 192 horses affected with septic arthritis/tenosynovitis.
Equine Vet J 24:436-42.

SCHOSSIER N. (1986)
Arthrotische Veränderung im Fesselgelenk des Pferdes und ihre Beziehung zu mikroskopisch nachweisbarem Knorpelabrieb in der Synovialflüssigkeit.
Veterinary Medicine Thesis, Hannover.

SCHÖTT E. (1989)
Direkte Druckmessung im Hufgelenk bei Pferden – ein Beitrag zur Pathogenese und Therapie von Strahlbein- und Hufgelenkerkrankungen.
Veeterinary Medicine Thesis, Hannover.

SCHOUGAARD H., FALK RONNE J., and PHILIPSSON J. (1990)
A radiographic survey of tiobiotarsal osteochondrosis in a slected population of trotting horses in Denmark and its possible genetic significance.
Equine Vet J 22(4):288-9.

SCHUBERT M., and HAMERMAN D. (1968)
A primer on connective tissue biochemistry.
Lea & Febiger, Philadelphia 166-8.

SCOTT D., SEIFERT W.W., and WESTCOTT V.C. (1974)
The particles of wear.
Sci Amer 230(5):88-97.

SOMMER A.J. (1954)
The determination of acid and alkaline phosphatise using p-nitrophenyl phosphate as substrate.
Am J Med Tech 20:244-53.

STOCK K.F. (2004)
Radiographic findings in the limbs of Hanoverian Warmblood horses: genetic analysis and relationships with performance in sports.
Doctorate Thesis. Tierärzliche Hochschule Hannover.

STOCKWELL R.A. (1971)
The interrelationship of cell density and cartilage thickness in
mammalian articular cartilage.
J Anat 109: 411-21.

STOGAARD-JORGENSEN H., PROSCHOWSKY H., and FALK-RONNE J. (1074)
The significance of routine radiographic fidings with respect to subsequent racing performance and longevity in Standardbred Trotters.
Equine Vet J 29:55-9.

SWEET M.B., THONAR E.J., IMMELMAN A.R., and SOLOMON L. (1977)
Biochemical changes in progressive osteoarthrosis.
Ann Rheum Dis 36:387-98.

TEW W.P. (1980)
Synovial fluid particle analysis in equine joint disease.
Mod Vet Pract Dec 61(12): 993-7.

TEW W.P., and HACKETT R.P. (1981)
Identification of cartilage wear fragments in synovial fluid from equine joints.
Arthritis Rheum. 24(11):1419-1424.

THIABAULT M., POOLE A.R., and BUSCHMANN M.D. (2002)
Cyclic compression of cartilage/bone explants in vitro leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments.
J Orthop Res 20:1265-1273.

THONAR E.J., LENZ M.E., MASUDA K., and MANICOURT D.H. (1999)
Body fluid markers of cartilage metabolism.
In: Dynamics of bone and cartilage metabolism.
Eds: M.J. Seibel, S.P. Robins and J.P. Bilezikian, Academic Press, San Diego. pp 453-464.

TODHUNTER R.A. (1996)
Anatomy and physiology of synovial joints.
In: *Joint Diseases in the Horse*, Eds: C.W. McIlwraith and G.W. Trotter, W.B.Saunders Co., Philadelphia. pp 1-29.

TODHUNTER R.J., and LUST G. (1990)
Pathophysiology of synovitis: clinical signs and examination in horses.
Comp Cont Educ Pract Vet 12:980.

TODHUNTER R.J., and LUST G. (1992)
Synovial joint anoatomy, biology and pathobiology.
In: Equine Surgery. Ed: JA Auer. W.B. Saunders, Philadelphia, pp 844-66.

TORZILLI P.A., GRIGLENE R., BORELLI J., and HELFET D.L. (1999)
Effect of impact load on articular cartilage: cell metabolism and viability, and matrix water content.
J Biomech Engl 121:433-41.

TREADWELL B.V., PAVIA M., and TOWLE C.A. (1991)
Cartilage synthesis of the serine protease inhibitor PAI-1: support for the involvement of serine proteases in cartilage remodelling.
J Orthop Res 9:309-16.

TROTTER G.W., and MCILWRAITH C.W. (1996)
Advances in equine arthrosopy.
Vet Clin N Am: Equine Pract 12:261.

TURNER T.A., and ANDERSON B. (1996)
Lameness of the distal phalangeal joint.
Equine Pract. 18:15-19.

VAN DEN BOOM R., BRAMA P.A.J., KIERS T.H., DE GROOT J., BARNEVELD A., and VAN WEEREN P.R. (2004a).
The influence of repeated arthrocentesis and exercise on matrix metalloproteinases and tumor necrosis factor alpha activities in normal equine joints.
Equine Vet J 36:155-9.

VAN DE BOOM R., BRAMA P.A.J., KIERS T.H., DE GROOT J., and VAN WEEREN P.R. (2004b)
Assessment of the effects of age in joint disease on hydroxyproline and glycosaminoglycan concentrations in synovial fluid from the metacarpophalangeal joint of horses.
Am J Vet Res 65:296-302.

VAN DE BOOM R., VAN DER HARST M.R., BROMMER H., BRAMA P.A.J., BARNEVELD A., VAN WEEREN P.R., and DE GROOT J. (2005)
Relationship between synovial fluid levels of glycosaminoglycans, hydroxyproline, and general MMP activity and the presence and severity of articular cartilage change in proximal articular surface of Pl.
Equine Vet J 37:19-25.

VAN PELT R.W. (1962)
Properties of equine synovial fluid.
J Am Vet Med Assoc 141:1051-61.

VAN PELT R.W. (1967)
Characteristics of normal equine tarsal synovial fluid.
Can J Comp Med Vet Sci 31:342-7.

VAN PELT R.W. (1971)
Monoarticular idiopathic septic arthritis in horses.
J Am Vet Med Assoc 158:1658-1673.

VAN PELT R.W. (1974)
Interpretation of synovial fluid in the horse.
J Am Vet Med Assoc 165:91-95.

VAN PELT R.W., and RILEY W.F. (1969)
Clinicopathologic fidings and therapy in septic arthritis in foals.
J Am Vet Med Assoc 155:1467-80.

VAN PELT R.W., TILLOTSON P.J., and GERTSEN K.E. (1970)
Intra-articularin jection of betamethason in arthritis in horses.
J Am Vet Med Assoc 156:1589-99.

VAN PELT R.W., TILLOTSON P.J., GERTSEN K.E., and GALLAGHER K.F. (1971)
Effects of intra-articular injection of flumethasone suspention in joint disease in horses.
J Am Vet Med Assoc 159:739-53.

VAN WEEREN P.R. and BRAMA P.A.J. (2003)
Equine joint disease in the light of new developments in articular cartilage research.
Pferdeheilkunde 19:336-44.

VASAN N. (1980)
Proteoglycans in normal and severely osteoarthritic cartilage.
Biochem J 187:781-7.

VERSCHOOTEN F., and DEMOOR A. (1982)
Subchondral cystic and related lesions affecting the equine pedal bone and stifle.
Equine Vet J 14:47-54.

VON RECHENBERG B., McILWRAITH C.W., AKENS M.K., FRISBIE D.D., LEUTENEGGER C., and AUER J.A. (2000)
Spontaneous production of nitrous oxide (NO), prostaglandin (PGE2) and neutral metalloproteinases in media of explant cultures of equine synovial membrane and articular cartilage from normal and osteoarthritic joints.
Equine Vet J 32:140-150.

WERB Z. (1989)
Proteinases and matrix degradation.
In. Kelley WN, Harris ED, Ruddy S, Sledge CB (Eds): Textbook of rheumatology, ed.3, Philadelphia, WB Saunders, pp 300-21.

WERB Z. (1992)
The biological role of metalloproteinasis and their inhibitors.
In Kuettner K, Schleyerbach R, Peyron JG, Hascall VC (Eds): Articular cartilage and osteoarthritis. New York, Raven Press, pp 295-304.

WEST M., POSKE R.M., BLOCK A.B., PILZ C.G., and ZIMMERMAN H.J. (1963)
Enzyme activity in synovial fluid.
J. Lab. & Clin. Med. 62:175-183.

WILLARD M. (1989)
Fluid accumulation disorders. In: Small animal clinical diagnosis by laboratory methods. 1st Edition. Willard M, Tvedten H, Turnwald G, editors. WB Saunders. Philadelphia p.299-42.

WOESSNER J.F. (1991)
Serum hyaluronan, a status report from the joint.
Arthr Rheum 34:927-30.

WOOD P.H.N. (1977)
The challenge of arthritis and rheumatism.
London: British League Against Rheumatism

WRIGHT I., KIDD L., and THORP B. (1998)
Gross, histological and histomorphometric features of the navicular bone and related structures in the horse.
Equine Vet J 30:220-34.

YENIA S.R., and DUNCAN H. (1975)
Synovial fluid analysis.
Clin Orthop 107:11-24.

ZUTHER M., and HERTSCH B. (2004)
Zur Differenzierung des Podotrochlose-Syndroms mit Hilfe von diagnostischen Anästhesien und der Druckmessung in Hufgelenk und Bursa podotrochlearis.
Pferdeheilkunde 20: 525-532.

11 ACKNOWLEDGMENTS

First of all I would like to thank God, fort the gift that is my life, for always giving me more than I deserve;

My mother, Fátima and sister, Lara, for all the love and unconditional support;

My husband, friend and colleague, Pádua, for always being beside me, helping me in every way that he can;

My whole family, uncles, aunts and cousins, even far away they were always present;

My friends, new friends, old friends, so important in every moment;

Prof. Dr. Hertsch, for this great opportunity, for making this dream come true, for the example of veterinarian that I will remember in every working day of my life and also for the great pleasant moments outside the clinic;

The whole team of veterinarians at the Clinic for Horses of the Freie Universität Berlin, for the patience, for dividing their experience with us;

All my colleagues, specially Anastasios Moschos (Polo) and Christoph Klaus, for the friendship that makes this world a little easier;

All secretaries and clinical staff, thank you for everything!

I could also never forget special people that helped me in the very begging: Prof. Dr. Nilberto, Prof. Dr. Manassés Claudino Fonteles, my friend Evani, so important in the first interview that made all this possible and my friend Thais, because I would not have come here without her precious advices.

This study was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – "bolsista CAPES – Brasília/Brasil") and DAAD (Deutscher Akademischer Austauschdienst / German Academic Exchange Service), I would like to express my gratitude to these institutions for the decisive support.

12 SELBSTSTÄNDIGKEITSERKLÄRUNG

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt und nur die erwähnten Quelle und Hilfen verwendet habe.

Die Arbeit ist erstmalig und nur an der Freie Universität Berlin eingereicht worden.

Ticiana Meireles Sousa