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CORRELATION OF SPECIFIC SYNOVIAL FLUID BIOMARKERS AND
PROPERTIES WITH THE DEGREE OF ARTICULAR CARTILAGE
DEGENERATION IN HORSES

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# Table of contents

1.0. Abbreviations  

2.0. Introduction  

3.0. Joint biology and pathobiology  
3.1. Classification of joints  
3.2. Joint capsule and perarticular ligaments  
3.3. Synovial membrane  
3.4. Synovial fluid  
3.5. Subchondral bone  
3.6. Articular cartilage  
3.7. Extracellular matrix  
3.8. Collagen  
3.9. Proteoglycans  
3.10. Aggrecan  
3.11. Chondrocytes  
3.12. Glycoproteins  
3.13. Matrix Metalloproteinases  
3.14. Joint mechanics  
3.14.2. Kinetics  
3.14.3. Lubrication  
3.14.3.1. Boundary lubrication  
3.14.3.2. Elastohydrodynamic lubrication  
3.14.3.3. Surface amorphous layer  
3.14.3.4. Boosted lubrication  
3.14.3.5. Additional lubrication systems  

4.0. Pathophysiology of osteoarthritis  
4.1. Pathologic changes  
4.2. Ethiology  
4.3. Role of the synovium  
4.4. Synovial Fluid/HA  
4.5. Matrix Metalloproteinases  
4.6. Sources of pain  
4.7. Formation of osteophytes and enthesiophytes  
4.8. Cartilage repair  

5.0. Clinical evaluation of joint disease  
5.1. Clinical signs  
5.2. Radiography  
5.3. Ultrasonography
5.4. Nuclear scintigraphy 41
5.5. Computed tomography (CT) 41
5.6. Magnetic resonance imaging (MRI) 41
5.7. Synovial fluid changes 42
5.7.1. Total protein (TP) 42
5.7.2. Cytology/Cellcount 43
5.7.3. Synovial fluid viscosity 43
5.7.4. Hyaluronic acid 43
5.8. Biomarkers 43
5.8.1. Matrix Metalloproteinases 44
5.8.2. Cytokines 44
5.8.3. GAG 45
5.8.4. Carboxy propeptide of Type 2 collagen 45
5.8.5. Degradation of collagen Type 2 45
5.8.6. Cartilage oligomeric matrix protein (COMP) 46
5.9. Arthroscopy 46

6.0. Materials and methods 47
6.1. Cartilage analysis 47
6.1.1. Native lesion scoring 47
6.1.2. Lesion scoring after Indian ink staining 50
6.1.3. Computer assisted Grey level analysis 53
6.2. Synovia sample analysis 55
6.2.1. Macroscopic evaluation 55
6.2.2. Evaluation of rheologic behaviour 55
6.2.3. Hyaluronic acid quantity 57
6.2.4. Hyaluronic acid molecular weight distribution 58
6.2.5. Determination of MMP1 and MMP3 quantity 61
6.2.5.1. Matrix Metalloproteinase-1 (MMP-1), Human, Biotrak ELISA System 61
6.2.5.2. Matrix Metalloproteinase-3 (MMP-3), Human, Biotrak ELISA System 63
6.2.6. Total Protein content 64
6.3. Statistical analysis 64

7.0. Results 65
7.1. Cartilage analysis 65
7.1.1. Native lesion scoring 65
7.1.2. Lesion scoring after Indian ink staining 65
7.1.3. Computer assisted Grey level analysis 66
7.1.3.1. Cartilage degeneration index 66
7.1.3.2. Colour degeneration index 66
7.2. Synovia sample analysis 67
7.2.1. Evaluation of rheologic behaviour 67
7.2.2. Hyaluronic acid quantity 74
7.2.3. Hyaluronic acid molecular weight distribution 76
7.2.4. Matrix Metalloproteinases (MMP1 and MMP3) 94
7.2.5. Total Protein content 95
1.0. Abbreviations

Ab  Antibody
CB  Cannon bone
CDI  Cartilage degeneration index
COMP  Cartilage oligomeric matrix protein
CS  Chondroitin sulphate
CT  Computed tomography
Da  Dalton
DJD  Degenerative joint disease
dl  Deciliter
DPB  Deutsche Physikalische Bundesanstalt
DS  Dermatan sulphate
Eta 0  plateau value of the initial viscosity
FG  Fetlock joint
g  Gramm
G value  Elasticity value
G0  plateau value of the final elasticity
G'  Storage modulus (elasticity)
G"  Loss modulus (viscosity)
G  Globular domain
GAG  Glucosaminoglycan
GPC  Gel Permeation Chromatography
h  Hour
HA  Hyaluronic acid/ hyaluronan/ hyaluronate
HABP  Hyaluronan Binding Protein
HG  Coffin joint
HPLC  High Performance Liquid Chromatography
IL  Interleukin
ip  interactive potential
KS  Keratan sulphate
l  Liter
LC  Liquid Chromatography
lg  Logarithm
MC  Metacarpal- or Cannon bone
mc  molecular conformation
md  molecular dimension
mg  Milligram
µg  Microgram
µl  Microliter
µm  Micrometer
mm  Millimeter
ml  Milliliter
MMP  Matrix Metalloproteinases
Mn  Number average molecular weight
MRI Magnetic resonance imaging
Mw  weight average molecular weight
MW  Molecular weight
ng  Nanogramm
OA  Osteoarthritis
OCD Osteochondrosis dissecans
P1  Proximal Phalanx (first Phalanx)
P2  Intermediate Phalanx (second Phalanx)
P3  Distal Phalanx (third Phalanx)
Pa  Pascal
PG  Proteoglycan
PGE Prostaglandin E
PLA Phospholipase
RA  Rheumatoid Arthritis
rpm Rotations per minute
SAL Surface amorphous layer
SAPL Surface active phospholipids
SEC Size Exclusion Chromatography
SF  Synovial fluid
TIMP Tissue inhibitor of metalloproteinases
TGF Transforming growth factor
TNF Tumor necrosis factor
TP  Total protein
UDS Universal Dynamic Spectrometer
VF5: Delution factor 5
WBC White Blood Cell Count

Explanation of sample names:
1FG1 Horse 1 left front fetlock joint
2FG2 Horse 2 right front fetlock joint
1HG3 Horse 1 left hind coffin joint
2HG4 Horse 2 right hind coffin joint
2.0. Introduction

It is well known that joint disease is the most prevalent cause of lameness in horses (BROMMER et al. 2003b; TODHUNTER 1992; VAN DEN BOOM et al. 2005). Degenerative joint disease (DJD) in particular constitutes almost 33 per cent of all equine lamenesses and osteoarthritis (OA) is referred to as the most important (ALWAN et al. 1991). FRISBIE (2005) even estimated that traumatic osteoarthritis makes up 60% of all equine lameness issues, thus making it the leading cause of equine lameness. Therefore OA does obviously not only have a major impact on equine performance (JOUGLIN et al. 2000; TRUMBLE et al. 2001) but is also a fundamental cause of economic loss (JEFFCOTT et al. 1982; TRUMBLE et al. 2001).

Degenerative joint disease such as osteoarthritis may occur in any kind of horse. It is commonly defined as a disease of diarthrodial joints (moveable joints) and characterised by variable degrees of articular cartilage deterioration, sclerosis of the subchondral bone plate and formation of marginal osteophytes (BROMMER et al. 2003b; CHANTLEY et al. 1999; GIBSON et al. 1996; VOLK et al. 2003).

OA can either be caused by strenuous exercise (BRAMA et al. 2000) or results from trauma. It may even occur naturally, further increasing with age, as recently shown in metacarpophalangeal joints of wild horses (BROMMER et al. 2003a; CHANTLEY et al. 1999). In humans a strong relationship between articular cartilage destruction and increasing age is reported (BROMMER et al. 2003a).

The pathogenesis of DJD and the complexity of the disease-mechanism have only lately been approved, but are still not completely understood (TULAMO et al. 1996). Osteoarthritis is regarded as a disease affecting the entire joint. Originating from a variety of different mechanisms and resulting in a progressive degradation of the articular cartilage (GUIDOLIN et al. 2001) OA is a complex disease attributed to alterations in normal biomechanical and biochemical processes (BROMMER et al. 2003b; SHARIF et al. 1995) and leads to soft tissue inflammation and gradual destruction of articular cartilage. In addition it is a cyclical disease with alternating active and remittent periods. This can cause overlapping of classification.

OA is generally classified as primary or secondary. Primary OA result of cumulative micro-damage, whereas secondary OA is caused by reasons of structural abnormalities. In the horse OA affecting the proximal interphalangeal joints and distal tarsal joints is commonly an example of primary OA that slowly develops over time due to continuous repetitive trauma. The equine fetlock joint typically shows the largest number of traumatic and degenerative lesions of the appendicular skeleton (BROMMER et al. 2003b). Secondary OA in horses in turn is similar to traumatic arthritis and is presumably best represented by OCD (osteochondrosis dissecans) lesions (TRUMBLE et al. 2001). Use trauma such as traumatic
capsulitis, synovitis or direct concussion damage to articular cartilage is also considered to be an essential factor in equine DJD aetiology (TULAMO et al. 1996).

Although degenerative joint disease is classically constituted as a non inflammatory condition inflammation of the synovial membrane as well as the joint capsule and therefore joint effusion is frequently noted in clinical cases. The inflamed synovium subsequently functions as a source of inflammatory mediators and enzymes capable of articular cartilage destruction (CHANTLEY et al. 1999; GIBSON et al. 1996).

Currently therapies for OA are only capable of alleviating the symptoms (BELLO et al. 1997; GUIDOLIN et al. 2001) but no preparation has so far definitely slowed down the progression of the contributing factors (GUIDOLIN et al. 2001). The limiting factor is, that by the time when changes in the joint can be appreciated radiographically or by ultrasound the disease has progressed quite far and has already caused severe and irreparable cartilage lesions (BELLO et al. 1997; FULLER et al. 2001; TULAMO et al. 1996). In many cases it has even already lead to specific alterations in the underlying bone such as bone sclerosis or osteophyte formation (TULAMO et al. 1996). Possible future therapies should aim to stop the process before it leads to irreversible changes in the joint. This would require early diagnosis (FULLER et al. 2001; TULAMO et al. 1996). Unfortunately the diagnostic methods are still not sufficient and need considerable improvement (TULAMO et al. 1996). At present diagnosis is based on clinical signs (JOUGLIN et al. 2000; VAN DEN BOOM et al. 2005) and significant improvement in lameness score following intra articular analgesia (TAYLOR et al. 2006) combined with relatively insensitive diagnostic imaging (mainly radiography and/or ultrasound), which can only provide an indication of the actual damage extent (JOUGLIN et al. 2000; VAN DEN BOOM et al. 2005). Recent studies have even revealed extensive cartilage lesions without radiographic findings (TULAMO et al. 1996). Hence radiographic abnormalities do obviously not correlate with the severity of articular cartilage degeneration. Alterations in the cartilage matrix and the subchondral bone can develop despite of radiographic abnormalities, making early diagnosis of OA challenging (FOX, et al. 2001; SALINARDI et al. 2006; TAYLOR et al. 2006) and therefore a major issue in veterinary (JOUGLIN et al. 2000) as well as human medicine. The urgent need for more sensitive and specific techniques for early diagnosis and monitoring of patients suffering from OA has prompted studies into different fields such as arthroscopy (FOX et al. 2001) – nowadays the “Gold standard” diagnostic technique- (TAYLOR et al. 2006), MRI and ultrasound. Although each of these methods has improved our competency to diagnose OA, none is unfortunately potent enough to detect early degeneration or to monitor the progression of OA (FOX et al. 2001). Within the last years many scientists aimed to find diagnostic alternatives that would enable an earlier verification of DJD (FRISBIE 2003).

Irrespective of the underlying cause OA is certainly the result of cumulative aberrant homeostatic and biochemical processes within a joint (VOLK et al. 2003). For that reason the physical properties of synovial fluid are considered to be a fundamental criterion for the integrity of joint homeostasis. Therefore one feasible option for early diagnosis could be
synovia analysis but so far no specific marker could be found that could be particularly used for diagnosis and monitoring of DJD neither in humans nor horses (SCHMIDT-ROHLFING et al. 2002; TULAMO et al. 1996). Molecular markers are protein fragments released into body fluids from cartilage, bone and/or synovium and have the potential to reflect normal and pathologic changes in the articular tissue. A marker of reversible and irreversible stages of OA in these joints would benefit management decisions (TAYLOR et al. 2006). Unfortunately most of the synovial fluid (SF) parameters used at present only provide an indication of the degree of joint inflammation (synovitis). Conventional analysis will therefore not provide information necessary for a specific diagnosis it is more applicable in the diagnosis of infective arthritis (McILWRAITH 2002).

A major difficulty in evaluating the results from diseased synovial fluids is the absence of normal control values (VIITANEN et al. 2000). For the horse normal values for several synovial fluid parameters have been determined but vary very much. Summarized, up to now the established parameters may indicate the severity of synovial inflammation, but do not provide information of the cartilage degradation extent (McILWRAITH 2002). Potential future markers could for example be components of articular cartilage that are released into synovial fluid during the anabolic response to OA, and should be particularly sensitive in detection of early OA (FOX et al. 2001; VIITANEN et al. 2000).

Evaluation of SF rheologic properties (viscoelasticity) may also assist in early detection of joint disease before clinical or radiographic changes have occurred (KORENEK et al. 1992). Hyaluronic acid (HA) comprising 0.14-0.36% of synovial fluid in normal joints is one of its principal components responsible for its rheologic properties (KAWAI et al. 2004). Viscoelasticity is basically dependent on HA quantity and molecular weight (KORENEK et al. 1992; TULAMO et al. 1994). The regulation-mechanism of size and concentration of hyaluronan (hyaluronic acid) within synovial fluid is unknown. A variety of methods are applied to determine either the concentration or the molecular weight of hyaluronan or both. This has lead to a wide range of normal values (TODHUNTER 1996). Nevertheless the quantity and physical state of hyaluronan produced under pathologic conditions is most likely the primary determinant of the nature, the remainder and the composition of the joint fluid (McILWRAITH 2002). In degenerative joint disease the viscoelastic properties are negatively influenced by reduction of HA quantity and/or HA molecular weight resulting in a thinning of the fluid film (MORI et al. 2002). Decreased synovial fluid apparent viscosity resulting from the destruction, depolymerisation and/or reduced concentration of hyaluronan due to DJD may be of tremendous diagnostic value (KAWAI et al. 2004; LUMSDEN et al. 1996).

Matrix metalloproteinases (MMP) are also assessed for their potential to give an indication of the progression of DJD. They are considered to be a major factor in the control of extracellular proteolysis (JOUGLIN et al. 2000). Therefore a connection between the rheologic properties and HA quality of synovial fluid and MMP content may be expected. A connection between the changes of the viscoelastic properties, the deterioration of HA, activation and activity of MMPs and the degree of cartilage destruction could not be found.
yet. So far viscosity and its disease related changes have been described in several studies (KORENEK et al. 1992; LUMSDEN et al. 1996; Renjo 1976) but elasticity which is presumably a parameter of same importance has not been evaluated before, nor have changes in elasticity caused by any kind of disease been described.

The complex of problems outlined above, concerning an early enough diagnosis and consequently treatment of degenerative joint disease, leads to the question:

Is it possible to draw a conclusion from the condition of the SF to the condition of the cartilage or is the synovial clearance too high (POPOPOT et al. 2004) to use synovial fluid quality parameters as a reliable tool in early diagnosis? LINDHOLM et al. 1996 could determine t½ for low molecular weight hyaluronan in the metacarpophalangeal joints to be 9.7h. In addition increased clearance of proteins from SF is reported especially in inflammatory phases of OA (TAYLOR et al. 2006)

The aim of this study was to describe the correlation of the SF parameters and the actual condition of the cartilage surfaces building the equine fetlock and coffin joints. Based on that, the study aimed to evaluate if a diagnosis or at least an estimation of the severity of cartilage defects in horses can be made upon the results of synovia analysis.
3.0. Joint biology and pathophysiology

To understand the changes in synovial fluid due to DJD and their correlation with the degree of cartilage degeneration it is necessary to be familiar with the joint biology and pathophysiology first.

A prerequisite for the function of a synovial joint is the integrity of normal anatomy and cellular function of each of its components. A healthy joint provides an almost frictionless system with an efficiency that is an order of magnitude superior to the best bearing surface in modern engineering. Due to dysfunction of one or more components pathologic states can arise (FRISBIE 2006a). This chapter describes the most important components of synovial joints that might be altered during the course of DJD.

3.1. Classification of joints

Joints are generally classified based on their normal range of motion. Three groups are distinguished (KAHLE et al. 1975):

1) Synarthroses (immovable joints)
Synarthroses are typically found in the skull, where bone plates are connected to each other by fibrous cartilaginous tissue (KAHLE et al. 1975).

2) Amphiarthroses (slightly moveable joints)
Amphiarthroses are defined as joints with a flatted disk of fibrocartilage in between the articulating surfaces, as for example between the vertebrae. The entire structure is surrounded by a fibrous capsule (KAHLE et al. 1975).

3) Diarthroses (moveable joints)
Most joints in the body and especially the appendicular skeleton are diarthrodial joints (KAHLE et al. 1975). The diarthrodial or synovial joints are formed by the extremities of at least two bones. The articulating surfaces are typically covered by a thin layer of hyaline cartilage. These elements and the joint space which contains synovial fluid (SF) are enclosed by a fibrous joint capsule. The joint capsule is composed of the synovial membrane which lines the inside (VAN DEN BOOM 2004a) and the fibrous layer on the outside. Surrounding ligaments and muscles provide stability to synovial joints. These structures are in most cases situated extra capsular (FRISBIE 2006a).
3.2. Joint capsule and periarticular ligaments

As mentioned above the stability of synovial joints is provided by periarticular ligaments, the fibrous joint capsule and surrounding muscles. Joints more proximal in the limb, compared to the more distal ones, are stabilized mainly from muscles. Therefore it is easy to understand that the shape of the joint surface and stability implicated by the joint capsule are more important in the distal limb (FRISBIE 2006a). The joint capsule is assembled of two parts: The fibrous joint capsule continuous with the periosteum or perichondrium and the synovial membrane. The collateral ligaments are usually associated with the fibrous layer whereas intraarticular ligaments are inherently lined by synovial membrane (McILWRAITH 2002). The insertion zone of the fibrous capsule and the articular ligaments into bone is characterized by a special organization. First parallel bundles of collagen turn into fibrocartilaginous stroma and become calcified close to the bone. The collagenous fibers insert the bone cortex in a special manner similar to that of Sharpey’s fibers. This progressive transformation of the joint capsule into ligaments and later mineralized fibrocartilage and bone is essential for the ability of the insertions to distribute applied forces evenly and decreases the risk of pullout injury (MANKIN et al. 1993). Another integral property of the joint capsule is to enable complete range of motion. However this property can be altered by inflammation and fibrosis which may subsequently lead to joint stiffness (McILWRAITH 2002).

3.3. Synovial membrane

The synovial membrane (synovium) lines the inside of the joint cavity. Flat areas of the synovium are intermixed with areas of loose collections of villi (FRISBIE 2006a). These villi are located in specific areas and show diverse morphology. Villi are already present at birth but proliferate in consequence of trauma and other insults. Healthy equine synovial membrane is white to yellowish, but discoloration (pink to brown) occurs secondary to initial trauma (McILWRAITH 2002). The synovium is not uniform e.g. areas predisposed to trauma showing dense connective tissue may be found (CARON 2003). The synovial membrane is made up of two layers:

The intimal layer (intima) regulates the content of the synovia. The synovial cells or synoviocytes of the intima build a one to four cells thick, incomplete layer with no basement membrane. This special formation allows most small molecules to cross the synovial membrane by free diffusion that is only limited by the diameter of intercellular spaces (McILWRAITH 2002). The responsibilities of synoviocytes are phagocytosis, pinocytosis and the protein-secretion of a variety of proteins that in turn influence the synovial membrane
properties and the composition of the synovial fluid. Examples for these proteins are collagen, lubricin, matrix pro-metalloproteinases, interleukins and eicosanoids. The cell types in the synovium have been histologically subdivided into type A and B synoviocytes. Cells responsible for phagocytosis or pinocytosis are defined as type A synoviocytes while those concerned with protein- (FRISBIE 2006a) collagen- (HENDERSON 1985) and hyaluronan secretion (McILWRAITH 2002) have been defined as type B (HENDERSON 1985). Type C cells have also been described. These cells are thought to represent a transition state between A and B and vice versa (ROY et al. 1966)

The subintimal layer (subintima) consists of fibrous, areolar and fatty tissues. The subintima shows a very good blood supply as well as innervation (GRONBLAD et al. 1985). It is supposed that each joint has a dual nerve supply, a combination of specific articular nerves that represent independent articular branches of adjacent peripheral nerves that reach the joint capsule and secondly non specific articular branches that arise from near by muscle nerves (McILWRAITH 2002).

3.4. Synovial Fluid

Synovial fluid is produced by synovial membrane and is characterised as a highly viscous dialysate of plasma (KORENEK et al. 1992; LITTLE et al. 1990). Synovial fluid is involved in a variety of mechanisms in the joint such as lubrication, oxygen and nutrition supply, load bearing, shock absorption (KORENEK et al. 1992; VIITANEN et al. 2000), resistance to compression and reduction of shear stresses (KAWAI et al. 2004). Healthy synovial fluid is pale yellow, clear, free of flocculent debris and does not clot. This property is due to a lack of clotting factors such as e.g. fibrinogen (McILWRAITH 2002). SF contains cells, hyaluronan, electrolytes, glucose, proteins and enzymes. Physiological values for all these components of horse-SF have been reported (KORENEK et al. 1992). SF TP (Total protein) concentration is approximately 25-35% of the plasma protein concentration of the same individual. The normal value for horses has been defined to be 1.81 +/- 0.27 g/dl. Synovial fluid comprises neutrophils, lymphocytes and large mononuclear cells, but the percentage of neutrophils is normally below 10%. Quantitative and qualitative changes can indicate the degree of synovitis (McILWRAITH 2002). Plasma constituents less than 10 kDa in size such as glucose, oxygen, carbon dioxide and proteins can pass through the endothelium of the subintima and influence the composition of the synovial fluid, large molecules on the other hand are excluded (FRISBIE 2006a). Thus the intercellular space between the synoviocytes acts as an important filter like permeability barrier (McILWRAITH 2002). Hyaluronan which is added to the synovial fluid by the synoviocytes is considered to be fundamental in the exclusion of large molecules from the synovial fluid. Therefore it is interwoven into the SF composition and lubrication mechanism of joints (TODHUNTER 1996).
Hyaluronan is a high molecular weight component of SF (KORENEK et al. 1992) and articular cartilage. It is a specialized glycosaminoglycan, a polysaccharide composed of repeating N-acetylglucosamine with glucuronic acid units (LITTLE et al. 1990; ROWLEY et al. 1982; SAARI et al. 1989).

HA is especially unique among the GAGs because it is non sulphated and naturally exists independent of a protein (LITTLE et al. 1990). In addition it is doubtlessly the largest MW constituent of SF (TULAMO et al. 1994). Hyaluronan imparts a lot of unique properties into synovial fluid (McILWRAITH 2002) and is playing a prominent role in all mechanisms of the joint. It is actively synthesised by the synoviocytes and it decisively dictates the viscoelastic properties of the synovial fluid (GUIDOLIN et al. 2001). The high viscosity is based on the fact that HA molecules form a special temporary network (DE SMEDT et al. 1993). Thus the viscosity of synovial fluid is directly dependent on the hyaluronan content and represents an indicator of the quantity, quality or degree of polymerization of Hyaluronan (PERSSON et al. 1971). A high molecular weight HA is also most likely to be important for joint- lubrication and prevention of friction (KAWAI et al. 2004). Synovial fluid viscosity may be reduced due to a decrease in Hyaluronate concentration, degree of polymerization or aggregation which in turn is thought to be fundamental for functional lubrication. Measurement of viscosity may therefore not only provide valuable information on the quality of the hyaluronate but also the functional properties of SF (KORENEK et al. 1992). Physiological values for HA concentration as well as molecular weight have often been described but differ significantly. POPOT et al. (2004) reported SF mean HA concentration to be 328 +/- 112 µg/ml with a mean range of 221 +/- 37 to 481 +/- 149, whereas ROWLEY et al. (1992) reported that the mean value for hyaluronic acid concentration in synovia of healthy horses was 127mg/100ml. According to LINDHOLM et al. (1996) Hyaluronan in healthy joints has a high molecular weight (MW) about 3*10⁶ Daltons. This is in agreement with TULAMO et al. (1994) who reported that the MW in control joints ranged from 2 to 3 *10⁶ (mean 2.5 +10⁶) whereas POPOT et al. (2004) for example estimated the range of HA MW between 1 and 10*10⁶
directly depending on the number of repeating disaccharide units. HA is important in maintaining the structural and functional characteristics of cartilage in which it is permanently connected to proteoglycans (PG). In addition HA is reported to contribute to the formation of a so called amorphous layer which is about 0.6 μm thick and covers the articular surface of the cartilage. This surface amorphous layer (SAL) contributes to the boundary lubrication mechanism (see lubrication) under extreme loading conditions. SAL also supports protection of the tissue from penetration through inflammatory cells and lytic enzymes (GUIDOLIN et al. 2001). Recently it was found that HA provides an array of other physiologic and pathologic mechanisms, through modulation of chemotactic, proliferative and phagocytotic response to various inflammatory cells, regulation of oxidative damage and inhibition of PG release from cartilage (NITZAN et al. 2001; POPOT et al. 2004; TULAMO et al. 1994).

3.5. Subchondral bone

The shape and stability of the articular cartilage are provided by the subchondral bone plate, together with the epiphyseal bone (FRISBIE 2006a). From a histologic and biochemical point of view subchondral bone is similar to other parts of bone, but anyways the organization of the subchondral plate is special. It is thinner compared to cortical bone in other locations and its haversian systems are typically oriented parallel to the joint surface and not the long axis of the bone (CARON 2003). It has been shown that the subchondral bone plate is about 10 times more deformable than the cortical shaft (MANKIN et al. 1993). Osteoarthritis in humans is associated with remodeling or stiffening of the subchondral bone plate and is considered to gradually alter joint function (RADIN et al. 1970a). The organization of subchondral cancellous bone even varies between joints, due to exposure to predominant biochemical forces and adaptation to exercise (CARON 2003).

3.6. Articular cartilage

Articular cartilage constitutes the joint surface and plays a major role in joint function. Cartilage is a distinct connective tissue with elastic properties capable of dissipating the compressive load associated with weight bearing of the joint (PLATT et al. 1994). Together with synovial fluid, articular cartilage accounts for the nearly frictionless system of the synovial joint (FRISBIE 2006a). The typical articular cartilage of synovial joints is the hyaline cartilage (McILWRAITH 2002). It is generally opaque white in color, with thinner areas that appear pink at which the underlying subchondral bone shines through (FRISBIE 2006a). Adult articular cartilage of diarthrodial joints consists of chondrocytes surrounded by
extracellular matrix which is composed of collagen, proteoglycans, noncollagenous proteins and water (PALMER et al. 1995), it is avascular and has neither blood nor lymph supply (McILWRAITH 2002). The thickness of articular cartilage which is usually in the 1-4 mm range varies depending on the joint, location (related to the degree of weight bearing) and age (FRISBIE 2006a). The surface seems to be completely smooth and only with electron microscope scanning could be shown to be gently undulating. This is due to small impressions that appear to correlate with the superficial chondrocytes (MANKIN et al. 1993). Healthy articular cartilages provide elastic smooth surfaces with high surface energy and therefore would generate increased friction when lubrication is altered. Increased friction plays a fundamental role in causing changes in the joint, which may all lead to deteriorating degenerative joint disease (NITZAN et al. 2001). In mature individuals the articular cartilage is separated from the vascular spaces of the subchondral bone by the bone endplate (the so called subchondral plate) (McILWRAITH 2002). This in addition with the missing vascular-, lymphatic- and nerve-supply leads to a diffusion-dependency to provide proper nutrition and waste removal (FRISBIE 2006a). Maroudas (1972) calculated that the depth to which the diffusion dependent nutrition can work is limited to cartilage thickness of approximately 6 mm. Thus diffusion would be sufficient for nutrition of almost all cartilage layers but the deepest. Joint movement though is thought to probably facilitate diffusion (McILWRAITH 2002).

The amount of chondrocytes in the articular cartilage extracellular matrix, typically ranges between 1%-12% of the overall cartilage volume (TODHUNTER 1996). Chondrocytes function by anaerobic glycolysis (McILWRAITH 2002). The extracellular matrix consists of different collagen types, proteoglycans and water. Water makes up the biggest part of the extracellular matrix (about 70% of the wet weight in adults and even close to 80% in young and immature cartilage). On a dry weight basis the extracellular matrix comprises about 50% collagen, 35% proteoglycan, 10% glycoprotein, 3% minerals, 1% lipids and 1% miscellaneous substances (TODHUNTER et al. 1996).

Cartilage can be divided into four zones:

1) The superficial (tangential) zone implies the highest amount of chondrocytes. They are flattened and oriented with the long axis of the cell parallel to the cartilage surface. The collagen with a diameter of about 31 nm is compared to deeper layers more densely packed as well and the orientation of the fibers is again parallel with the cartilage surface. This enables preclusion of large proteins e.g. Hyaluronan (FRISBIE 2006a). However, on the other hand permeability of the articular surface is remarkably higher than in the middle and deep zones (NAKA et al. 2005). A special part of the tangential layer is the “lamina splendens” which is reported to be an acellular collagen layer with fibril diameters that differ clearly from those of the cellular portion of the tangential zone (FRISBIE 2006a).
2) The intermediate (transitional) zone contains larger and more ovoid to round chondrocytes (FRISBIE 2006a) arranged in single or pairs (MclLWRAITH 2002). The collagen fibrils with a diameter between 40 and 100 nm appear to be oriented in a more random fashion.  
3) The deep (radiate) zone has the biggest chondrocytes (FRISBIE 2006a) arranged in vertical columns and the long axis of the cells is oriented perpendicular to the cartilage surface. The collagen fibrils there typically have the largest diameter, are orientated perpendicular to the cartilage surface and cross the calcified zone before they enter the subchondral bone (MclLWRAITH 2002).  
4) The calcified zone comprises mineralized chondrocytes in various degeneration stages (MclLWRAITH 2002) and matrix. The tidemark is defined as the connecting zone between the noncalcified and calcified cartilage and represents a transition seen on histologic sections (FRISBIE 2006a). 

Formerly, articular cartilage was defined as shock absorber. However, recent force studies have revealed that the major shock absorbers of the joint are bone and periarticular soft tissue. Cartilage itself provides shock absorption only to a little percentage (RADIN et al. 1970b). The condition of the articular cartilage typically defines joint health and degeneration whereas the slow and poor healing capacity of articular cartilage generally influences the degree and progression of joint disease (FRISBIE 2006a). Due to cartilage being aneural, cartilage lesions are usually non-painful. Therefore only the nerves of the underlying bone and periarticular soft tissues provide information on joint condition (CARON 2003). 

3.7. Extracellular Matrix 

The extracellular matrix consists to the most part of a 3-dimensional network of cross-linked collagen type 2 fibrils. In this network chondrocytes are entrapped. The fibrils are hydrophilic proteoglycan (PG) aggregates that are physiologically negatively charged (BRAMA et al. 2000, NAKA et al. 2005). The excellent mechanical properties of articular cartilage are based on interaction between the collagen network, PG and water (NAKA et al. 2005). The typical compressive stiffness which is a function of the polyanionic character of the matrix glucosaminoglycans (GAG) enables the articular cartilage to withstand load. GAGs are covalently linked to a proteoglycan core protein. The GAGs, chondroitin sulphate and keratan sulphate with their sulphate and carboxyl groups attract and trap water, causing an expansion of the cartilage matrix. Tightly packed type 2, 9 and 11 collagen fibrils counteract this expansion which provides the articular cartilage with the capability to resist compressive forces (PALMER et al. 1995). This property depends on the integrity of the extracellular matrix (PLATT et al. 1994). The balance between tension in the collagen network and the osmotic swelling pressure of the proteoglycans is essential for normal joint function. It
provides the special biomechanical characteristics of the articular cartilage (BRAMA et al. 2000). Changes in proteoglycan and GAG structure, amount or compressive stiffness add to cartilage damage (PALMER et al. 1995). While in newborn individuals turnover of the collagen component of the extracellular matrix is relatively high it clearly decreases rapidly afterwards. In this juvenile period essential functional adaptation takes place. The typical specifications essential to adequately withstand the locally varying biomechanical challenges in collagen characteristics are formed in different sites of the joint (BRAMA et al. 2000). Articular cartilage has been shown to adapt to the conditions to which it is subjected on a regular basis. In comparison to the less loaded tissue high stress levels lead to increased PG content and consequently stiffness of cartilage. Joint loading is therefore obviously one of the main factors affecting the biological and physical mechanisms and contributes to maintenance of functionally normal articular cartilage (VIITANEN et al. 2000).

3.8. Collagen

Collagen is the component of articular cartilage that builds the network in which all other matrix molecules are constrained. As described previously the architecture of the collagen differs depending on its location or depth within the cartilage. The configuration of articular cartilage collagen is described as arcade which is supportive in understanding the functional properties of the cartilage (FRISBIE 2006a) and the three-dimensional framework or skeleton of the cartilage matrix (CARON 2003). Collagen protein monomers consist of triple helices of polypeptide alpha chains and are arranged to form fibrils. The 7 recognized types of collagen in articular cartilage are defined by various arrangements of these alpha chains. Articular cartilage is primarily made up of collagen types II and IX (FOX et al. 2001). There are also small amounts of types VI, IX, XI, XII and XIV collagen recognized in the cartilage. These minor collagens contribute to formation and stability of the type 2 fibril network (McILWRAITH 2002). Chondrocytes produce Type II collagen which constitutes 90% to 95% of the total collagen content (VACHON et al. 1990). This Collagen is produced at clearly lower levels in adult horses than in younger ones which may influence the naturally occurring changes in joints of older horses (McILWRAITH 2002). Type II Collagen consists of three equal amino acid chains combined to a triple helix, is less soluble, comprises a higher content of hydroxiprolin residues and is more glycosylated compared to type I Collagen. Further collagen type II collagen is organized in fibrils (TODHUNTER et al. 1994) that are constructed in such a way that the start of each fibril is offset of the following by 25% stagger (FRISBIE 2006a). Fibrils vary in size throughout the matrix. They are larger in the middle and deep zones of the matrix, to be able to fulfill regional biochemical demands (CARON 2003). Collagen is also attributed to the counteraction of tensile stresses at the joint surface (FRISBIE 2006a; ISRAEL et al. 1991). In
adult articular cartilage this property remains in the superficial layers. It is not as critical in the
deeper layers of normal cartilage, but in case of superficial erosion the collagen of these
deeper layers becomes vulnerable to destruction. In immature cartilage the deeper layers also
show specific tensile properties, but this is gradually increased with maturation. The tensile
properties are altered with enzymatic degradation of hydroxyprolin cross links. The collagens
special molecular structure enables the typical high resistance towards tension at a maximum
expansibility of 5% (LIEBICH 1993).
Collagen turnover times within articular cartilage have been estimated to be 120 years in the
dog and approximately 350 years in adult humans (TODHUNTER 1996). Turnover of the
proteoglycan portion of the extracellular matrix is much faster than collagen it has been
estimated to be approximately 300 days in dogs and rabbits (FRISBIE 2006a). The slow
turnover time of collagen in contrast to that of proteoglycan is presumably due to the
structural features (McILWRAITH 2002).

3.9. Proteoglycans

Proteoglycans are the other major acellular component of the articular cartilage matrix that
fills the spaces between the collagen fibers. Compared to collagen that’s amount is highest in
the upper most layer of the articular cartilage and falls significantly with increasing depth the
proteoglycan content shows a trend in opposite direction (McILWRAITH 2002). The main
components of the cartilage extracellular matrix are proteoglycan aggregates embedded
within the collagen network. Proteoglycan monomers consist of 1 or more GAG chains
covalently attached to a protein core. The GAG chains are composed of repeating
disaccharide units and a protein linkage region. They are negatively charged which forces
them to repel one another when attracted to the protein core. The profound anionic charge
resulting from the large number of GAG molecules leads to an osmotic gradient within
articular cartilage that causes the typically extremely hydrophilic properties of the tissue
(FOX et al. 2001). The most frequent types of GAG in proteoglycan molecules that form
articular cartilage are Chondroitin sulphate (CS), Keratan sulphate (KS) and dermatan
sulphate (DS) (FOX et al. 2001; PALMER et al. 1995).
Keratan sulphate is an essential constituent of aggrecan (the largest aggregating proteoglycan)
in the extracellular matrix of hyaline, fibrous and elastic cartilage. CS is formed of a repeating
disaccharide unit and is a major constituent of aggrecan as well.
Individuals are born with a joint without the specific topographical varieties characteristic of
mature articular cartilage. In horses the biochemical heterogeneity of PG as well as collagen
was formed for the most part within the first few months following the onset of weight
bearing. Dependent on the magnitude plus the duration of loading proteoglycan synthesis is
enhanced. In vitro it appears that high and continuously applied loads might be potentially
damaging, whereas moderate cyclic load supports proteoglycan synthesis by stimulating chondrocyte metabolism (BRAMA et al. 2000). However environmental changes like pH-, oxygen-, loading- and movement-changes seem to have no effect on the synthesis of specific GAGs. On the other hand cartilage thickness was described to have an effect on KS concentration with higher levels being detected in the deep regions of thicker cartilage (FULLER et al. 1996; PALMER et al. 1995) and areas with diminished hydration and oxygenation. Higher contents of KS can be typically found in weight bearing areas (FULLER et al. 1996). Keratansulphate matrix concentrations also increase and become more variable in size throughout the aging process. The diffusion of KS from the cartilage matrix to the joint fluid is less restricted than that of the larger CS chains due to its relatively small size. However it is shielded from enzymatic attack due to steric hindrance of the large CS chains (PALMER et al. 1995).

PG molecules are noncovalently attached to hyaluronic acid (FOX et al. 2001) to form PG aggregates (FOX et al. 2001; PALMER et al. 1995). Aggrecan is considered the most important PG monomer in articular cartilage (FOX et al. 2001). Average proteoglycan units in articular cartilage have a molecular mass of approximately 3 million Daltons and contain about 100 chondroitin sulfate side chains and another 100 keratan sulfate side chains (McILWRAITH 2002). To form the proteoglycan aggregate aggrecan, over 100 of the proteoglycan monomers are bound to a Hyaluronan, which has a molecular mass of over 200 million Daltons (McILWRAITH 2002). Aggrecan makes up about 85% of the proteoglycans which in turn make up the extracellular matrix and it provides the cartilage resistance to compressive forces (FRISBIE 2006a; ISRAEL et al. 1991). About 5% of the cartilage proteoglycans fall upon the small proteoglycans biglycan, decorin and fibromodulin (FRISBIE 2006a).

Proteoglycans fulfill different regulatory tasks in the cartilage. Proteoglycan function depends on enmeshment of the proteoglycans by the collagenous framework and specific interaction between the two components. As the proteoglycans are entrapped within the collagen fibers, the proteoglycans are hindered from expanding, so the matrix acts like a fine sieve-like structure through which molecules attempt to pass. The proteoglycans avoid the diffusion of large, uncharged solutes but do not interfere in the passage of small, uncharged solutes. The molecular size and conformation of the solute is also an important factor. Removal of proteoglycans results in increased diffusion of large molecules into the tissue matrix, which leads to the conclusion that proteoglycan removal may increase the diffusion of large molecules from the tissue matrix as well (McILWRAITH 2002).

PG may also contribute to cartilage protection. PGs are bound in the collagen fibres with one part of them probably exposed at the surface. Based on their hydrophilic properties, they could thereby decrease the shear rate and therefore assist lubrication (NAKA et al. 2005).

In healthy joints cartilage matrix turnover facilitates release of PG fragments through anabolic and catabolic pathways (FOX et al. 2001). The proteoglycan fragments are released into the synovial fluid and ultimately into the serum by the action of proteases (FULLER et al. 1996).
In normal articular cartilage the dynamic balance between synthesis and degradation of proteoglycans is regulated by chondrocytes. In OA disease this regulation is altered and the loss of PG exceeds the rate of synthesis. This in turn results in a tissue with altered mechanical properties that has reduced capability to withstand mechanical forces (VIITANEN et al. 2000).

3.10. Aggrecan

Aggrecan molecules generally consist of two components: The core protein, which is the "backbone" of aggrecan and the glycosaminoglycans that are attached radially to the core protein. Aggrecan monomers are typically organized as huge aggregates around a Hyaluronan molecule (FRISBIE 2006a). The linear core protein is sectioned by three globular domains (G). The first of which is defined as G1. It is situated at the amino-terminal site of the molecule and represents the part at which the proteoglycan attaches to Hyaluronan. The specific roles of G2 and G3 domains remain unclear however. The G3 domain is found only in about every third aggrecan monomer in adult cartilage, for that reason it is considered not to play a determining role in the function of extracellular matrix. The region between the second and third globular domain is characterised by glycosaminoglycan chains of different length and composition which are attached radially to the core protein. Immediately next to the G2 domain is a keratan sulfate rich region. Further in the periphery of the core protein is the chondroitin sulfate region, which comprises up to 100 chondroitin sulfate chains, again attached radially to the core protein (CARON 2003). The main GAGs that form aggrecan are chondroitin-4-sulfate, chondroitin-6-sulfate and keratan sulfate. Striking is an increase of chondroitin-6-sulfate compared to chondroitin-4-sulfate as the cartilage turns older (FRISBIE 2006a).

A major function of aggrecan in cartilage is to retard the rate of stretch and alignment caused by a suddenly applied tensile load. This mechanism is also necessary to protect the cartilage collagen network during physiologic situations (SCHMIDT et al. 1990).

3.11. Chondrocytes

The chondrocytes typically exist in a relative hypoxic and acidic environment, with an extracellular pH ranging between 7.1 and 7.2 (CARON 2003). Despite of their relatively little number, chondrocytes play a major role in production, organization and maintenance of the extracellular matrix. Chondrocytes not only synthesize extracellular matrix components but also proteinases responsible for the degradation of the extracellular matrix. Chondrocytes are
situated inside a capsule in the matrix, named lacuna the whole structure is surrounded by a periarticular or pericellular rim. This combination of elements represents a structural and functional entity called a chondron. Chondrons have the inherent ability to adapt to variations in biochemical and biomechanical processes due to changes in extracellular matrix synthesis and destruction (FOX et al. 2001). The chondrocytes morphology and metabolism vary with their depth within the cartilage. For example the presence of lacunae represents a microenvironment around the chondrocytes that is more established in the deeper layers (FRISBIE 2006a). In healthy articular cartilage they maintain the dynamic balance between synthesis and degradation of proteoglycans (VIITANEN et al. 2000). The relative rates of matrix synthesis and degradation are adjusted to achieve net growth, remodeling, or equilibrium at each stage of growth, development and maturation. The unique interaction between chondrocytes and the surrounding matrix may enable to sense changes in the matrix composition. This may also include transmission of mechanical signals due to changes in matrix tension or compression. Forces to which chondrocytes are exposed may influence their shape and dictate alterations in cellular biochemistry and matrix metabolism. It is well accepted that the metabolic activity of chondrocytes is influenced by mechanical forces whereas the specific mechanisms of mechanical signal transduction in articular cartilage still remain unknown (McILWRAITH 2002). It seems that at low levels of mechanical stress chondrocyte metabolism tends more towards the catabolic processes, whereas at physiologic levels, anabolic processes are pronounced and a balance of metabolism is achieved (FRISBIE 2006a). Research in sheep stifle joints has indicated that different areas of articular cartilage exposed to different mechanical stresses show a phenotypically distinct chondrocyte population (McILWRAITH 2002).

In degenerative joint disease the loss of PG may exceed the synthesis rate and therefore cause development of a tissue with altered mechanical properties that has reduced ability to withstand mechanical loads (VIITANEN et al. 2000).

3.12. Glycoproteins

Noncollagenous, nonproteoglycan glycoproteins represent a small but important portion of articular cartilage. Link protein for example is one of these glycoproteins whose function has been discovered. For the most part proteoglycans (85%) form large aggregates (aggrecan) through noncovalent attachment to the core protein stabilized by a link protein. Other glycoproteins of cartilage are for example chondronectin (assumed to contribute to adhesion of chondrocytes to type 2 collagen surfaces), fibronectin (enables adherence of cells to molecules and surfaces), cartilage oligomeric matrix protein (COMP which has been characterized from human articular cartilage), thrombospondin and anchorin C-2 (McILWRAITH 2002) (which is suggested to possibly act as a mechanoreceptor, informing
chondrocytes about changes in stress to which the matrix is exposed) (CARON 2003) as well as cartilage-derived growth factor (McILWRAITH 2002). Further decorin and fibromodulin inhibit fibrillogenesis of type 2 collagen which is thought to regulate the size of collagen fibrils in the matrix (SCOTT 1988). COMP is most prevalent in the proliferative layer of growth cartilage where it is expected to influence cell growth (CARON 2003).

3.13. Matrix Metalloproteinases

MMPs are a group of zinc dependent endopeptidases that contribute to the turnover of the extracellular matrix. At least 20 MMPs have been identified so far (SALINARDI et al. 2006; VOLK et al. 2003). MMPs are classified in 3 groups: Stromolysin, collagenase and gelatinase (JOUGLIN et al. 2000). CARON 2003 mentioned a 4th type: membrane type MMPs. According to Tumble et al. (2001) most important of these endopeptidases are collagenase 1 (MMP1) collagenase 3 (MMP13) stromolysin 1 (MMP3) and gelatinases (MMP2 and 9). Secretion of MMPs is reported to be strictly regulated and tissue specific. The synovial membrane as well as chondrocytes are supposed to be source of several of these enzymes (TRUMBLE et al. 2001). CLEGG et al. (1997) suggest that the enzymes probably originate from the synovial membrane type A cells. The MMPs are produced as latent proenzymes that are thought to be activated through extracellular proteolytic action to turn into fully active metalloenzymes. The activated enzymes are capable of destroying all matrix components of cartilage (TRUMBLE et al. 2001).

MMPs are usually responsible for normal development and turnover of articular cartilage extracellular matrix (SALINARDI et al. 2006). SF from healthy joints of juvenile dogs comprises significantly higher MMP levels than does SF from adult dogs (VOLK et al. 2003). It has been suggested that in clinically normal maturing dogs the reduced metabolic activity results from termination of growth and along with that the decrease in cartilage remodelling is represented by a gradual decrease in MMP activity (VOLK et al. 2003).

The activity of the MMP is consorted by specific inhibitors: tissue inhibitors of metalloproteinases (TIMP). To abolish MMP activity the N-terminal domain of the TIMP competitively binds to the active site on the MMP enzyme through 1:1 non covalent complex. TIMP2 is believed to be the most prevalent and most important TIMP synthesized by fibroblasts, macrophages and endothelial cells and has the potential to inhibit most MMP. In the healthy joint a good balance between MMP and TIMP activities are necessary for normal turnover and degradation of the cartilage matrix. Imbalances between MMP and TIMP synthesis are an essential event in the alteration of MMP from physiological to pathological processes (SALINARDI et al. 2006; VOLK et al. 2003).
Human articular cartilage affected with OA was proven to show elevated MMP synthesis without corresponding increase in the TIMP expression. Due to that it is considered that unregulated MMP activity may cause massive matrix destruction. MMP1, which is capable of degrading the collagen component of the extracellular matrix is one of the most important among the MMPs due to its expression by various different cell types such as chondrocytes, synoviocytes fibroblasts keratinocytes, endothelial cells monocytes and macrophages. MMP1 expression is influenced by a variety of inflammatory cytokines including tumor necrosis factor and interleukin 1 (SALINARDI et al. 2006). Another important factor is its large range of activity: The triple helices of collagen types 2, 9 and 10 are vulnerable by the cleaving activities of collagenase (MMP1) and stromolysin (MMP3) (FOX et al. 2001).

There are lots of other factors that play a role in joint function such as e.g. Tumor Necrosis Factor α (TNF α) or Interleukin (IL) which have not been taken into account within the scope of this study and therefore are not described here.

3.14. Joint mechanics

Joint function is dependent on three mechanics: Kinematics, kinetics and joint lubrication.


Kinematics are influenced by the geometry of joints. The motion that occurs in joints can be defined as translation, rolling and sliding.

3.14.2. Kinetics

Kinetics take the muscle forces pulling across a joint, the superimposed body weight, the force of the floor or surface pushing back against the joint in question and the force felt by the cartilage in any given load-bearing situation into account (FRISBIE 2006a).

In an unloaded joint the opposing articular surfaces are not completely congruent, however under physiologic loading, because the cartilage is soft, deformation causes increase of
contact area and also increases joint conformity (McILWRAITH 2002). This may be a physiological reason why cartilage tends to be somewhat thicker in less congruous joints (ARMSTRONG et al. 1977). The adaptation of the shape of loaded cartilage may also help to form and retain boundary lubrication. As articular cartilage directly under load is compressed, the surrounding areas are subjected to transfer tensile strains. These forces tend to redistribute fluid away from the compressed area and into the stretched regions. Because of swelling pressure the collagen network of the articular cartilage is under pressure and tensile stress even when unloaded. During compression the concentration of the organic material and the charge density increase because the interstitial fluid is forced to flow from the matrix. A new equilibrium is reached when the charge density, collagen tension and applied load are in balance. During prolonged periods of stationary loading, fluid is slowly redistributed within the cartilage until an equilibrium position is reached, at which stage the increased concentration of fixed charge density is counterbalanced by the increased osmotic swelling pressure of proteoglycan. When fluid motion ceases, all external load is borne by the solid extracellular matrix. Intermittent pressure created by the interaction of the opposing articular surfaces is needed to pump fluid through the cartilage for nutrition and removal of metabolic products (McILWRAITH 2002). Indeed the capacity for considerable elastic deformation permits normal cartilage to withstand compressive stresses considerably greater than those of body weight alone. Nonetheless, cartilage is subjected to mechanical breakdown after supraphysiologic stresses. Loads exceeding 25kg/mm² are reported to result in matrix damage (CARON 2003).

3.14.3. Lubrication

Synovial joints contain two systems that require lubrication, the soft tissue system (sliding of synovial membrane on itself or other tissues) and a cartilage on cartilage system (HILBERT et al. 1984; McILWRAITH 2002).

3.14.3.1. Boundary lubrication:

Lubrication of the synovial membrane is for the most part by boundary lubrication (RADIN et al. 1971). This is a key function because the main part of the frictional resistance in joint movement is caused by synovial membrane and the fibrous joint capsule (MANKIN 1993). According to DELAHAY (2001) boundary lubrication counteracts against adhesion and aberration of two surfaces and is not dependent on the physical properties of the lubricant or contacting surfaces. HILBERT et al. (1984) on the other hand report that the lubrication
properties of synovial fluid in the soft tissue system are however dependent on the concentration and molecular size of hyaluronic acid. CARON (2003) also explained that the lubrication of the soft tissue system is considered to be dependent on HA molecules in the synovial fluid. The HA molecules are thought to adhere to the synovial membrane and keep the moving surfaces apart (CARON 2003; HILBERT et al. 1984). This is in agreement with RIBITSCH (1977) who found that adhesion of the long Hyaluronan molecules to the cartilage surface enables the development of a layer with minimal surface shear force and friction. McILWRAITH (2002) wrote that it was concluded that lubrication by fluid film was influenced by the viscosity of the lubricant. The viscosity of the synovial fluid was considered to be essential in reducing friction at low loads of articular surfaces.

Besides Hyaluronan, Lubricin is thought to be a major boundary lubricant of diarthrodial joints (DELAHAY 2001). Lubricin (proteoglycan 4) is a heavy glycosylated mucinous glycoprotein produced by synovial fibroblasts (ELSAID et al. 2005) and has been isolated from the load bearing fractions of synovial fluid (NITZAN et al. 2001). It is considered the lubricant responsible for boundary lubrication (ELSAID et al. 2005; LITTLE et al. 1990) and is a water soluble carrier of surface active phospholipids (SAPL) (NITZAN et al. 2001). A significant correlation is reported to exist between friction coefficients and Lubricin content in SF (ELSAID et al. 2005).

Boundary lubrication is the main mechanism decreasing friction between soft tissue and cartilage, but on the other hand is considered to be relatively ineffective at creating a frictionless surface between articular cartilage surfaces even at physiological loads (DELAHAY 2001). In contrast HILBERT et al. (1984) wrote that under low loading conditions the glycoprotein molecules are thought to lubricate the cartilage on cartilage system in a fashion comparable to that of the soft tissue system. It was also described that boundary lubrication operates at low loads because the lubricant would be sheared off the articular cartilage at higher loads which would cause the lubrication mechanism to fail (RADIN et al. 1972). A completely different view is offered by HILLS et al. 1998 who reported that SAPLs are the main load bearing lubricants in the joint and that improved lubrication is accounted to their unique ability to lubricate under high loading conditions. NITZAN et al. 2001 also reported that SAPLs serve as the main boundary lubricant and that HA plays a fundamental but indirect role in the boundary lubrication process by preventing lysis of SAPL. They also conclude that under load the boundary lubrication system is able to adapt itself constantly by a process of remodelling.

3.14.3.2. Elastohydrodynamic lubrication:

Elastohydrodynamic lubrication which is considered as best representing lubrication of the articular cartilage functions based on the attraction of water by aggrecan molecules at the porous articular surface. When load or pressure is applied to the surface, water is squeezed
from the cartilage and interposed between the articular surfaces. Joint movement leads to creation of a fluid wedge. Later the fluid which was squeezed from the cartilage in order to separate the surfaces is resorbed as the load decreases. Fluid film mechanisms are presumed to provide a low friction condition for the articular cartilage at physiologic loads (DELAHAY 2001). CARON (2003) and HILBERT et al. (1984) also wrote that under high loads a watery fluid is squeezed form the cartilage and this fluid produces a film which keeps the surfaces apart. RADIN et al. (1972) reported that in this system the cartilage surfaces are held apart by a fluid film consisting of joint fluid and interstitial fluid derived from the articular cartilage itself. DELAHAY (2001) suggested that with the release of compressive force, the cartilage expands and water is sucked back into the matrix.
The squeeze film effect is probably facilitated by the undulation of the cartilage surface and the elasticity of the cartilage, which may lower friction itself (RADIN et al. 1972).
RIBITSCH et al. (1977) also reported about a lubrication mechanism named “Depot lubrication” were additional synovial fluid, which had diffused into the cartilage surface, is pressed into the joint cavity in support of the regular synovia film.

3.14.3.3. Surface amorphous layer (SAL):

Microscopic analyses allow presumption of the presence of a layer covering the articular surface. Histological analyses indicated the presence of proteoglycans in this special superficial layer. The hydration of the surface layer and presence of proteoglycan within it supports lubrication (NAKA et al. 2005).
Results from KAWAI et al. 2004 even suggest that the lubrication of joints might be dependent on this superficial amorphous cartilage layer rather than on the synovial fluid.
SAL is made up of equal quantities of lipid, protein and sulphated GAG, proteoglycans and hyaluronic acid protein complexes, which are believed to originate from synovial fluid. There is also a very small amount of hydroxyproline which could be of cartilage or synovial fluid origin in the SAL. Protein and lipid might as well be of cellular origin. Healthy SAL comprises 10-20 % lipid and 20-50 % protein. The surface of normal cartilage is coated by this amorphous electron dense layer. The layer is about 0.2 µm - 2 µm thin, and lacks of collagen and cells. The surface amorphous layer is believed to extend directly from the superficial tangential zone of the articular cartilage. It seems discontinuous and shows variable thickness rather than being a distinct layer (GRAINDORGE et al. 2006).
The amount of proteoglycans on the articular surface appears to be lower than in the deep zone. Therefore the reduced amount of PG could facilitate exudation of water onto the surface during the initial phase of compression while in the deepest region water could be retained for longer times because of the high content of PG (NAKA et al. 2005). Independent of the viscosity of the lubricant the superficial layer plays an essential role in proper lubrication and this cannot be achieved by physical action of the cartilage alone (MORI et al. 2002).
3.14.3.4. Boosted lubrication:

Some scientists suggest that the plugging of the cartilage pores with Hyaluronate when a squeeze film is present on the surface may support hydrostatic lubrication (McILWRAITH 2002) because only low molecular components of the synovial fluid are pressed into the cartilage surface which leads to an increased Hyaluronan concentration in the synovial fluid itself (RIBITSCH 1977).

3.14.3.5. Additional lubrication systems:

When a sudden perpendicular force is applied to each of the two bearing surfaces the viscous synovial fluid provides pressure that is directed against the force and therefore keeps the bearing surfaces apart (RIBITSCH 1977).
4.0. Pathophysiology of osteoarthritis

Osteoarthritis is a disorder of moveable joints (FRISBIE 2006a) and is a slowly progressing disease. It is characterized by altered balance between synthesis and degradation of articular cartilage constituents (FOX et al. 2001; FUJITA et al. 2005; GIBSON et al. 1996; VOLK et al. 2003). Degenerative joint disease typically causes degeneration and loss of articular cartilage, changes of the subchondral bone plate and is accompanied by insufficient cartilage repair (GIBSON et al. 1996; McILWRAITH 2002). The sliding properties of a joint are thereby impaired, resulting in increased joint friction (ISRAEL et al. 1991).

Although DJD is defined as a non inflammatory disease, inflammation of the synovial membrane and the joint capsule as well as joint effusion typically go along with clinical cases (GIBSON et al. 1996; McILWRAITH 2002). Inflammatory mediators and enzymes capable of considerable articular cartilage destruction are expressed by inflamed synovium (GIBSON et al. 1996). The degeneration of the articular cartilage is displayed by local splitting and fragmentation (fibrillation) (McILWRAITH 2002). Finally the disease leads to subsequent degeneration of joint cartilage, remodelling of the underlying subchondral bone plate, formation of osteophytes and different degrees of synovitis (FOX et al. 2001; FUJITA et al. 2005; GIBSON et al. 1996; VOLK et al. 2003).

Typical cytological markers of inflammation are scant in OA. However the presence of inflammatory mediators is a sufficient sign of inflammation in the disease process (SALINARDI et al. 2006). Due to the cyclical character of the disease with alternating periods of activity and inactivity, overlapping of classification of cases can occur (TRUMBLE et al. 2001). OA is a complex disease with a variety of co-acting biochemical and biomechanical factors of influence contributing to its pathogenesis (SALINARDI et al. 2006; TRUMBLE et al. 2001). It is therefore not a simple disease but represents the response of joint tissues to a variety of different causes (CARON 2003). Chondrocytes and synoviocytes respond to inflammatory process due to acute trauma or chronic repetitive micro trauma by producing proteolytic enzymes and cytokines which in turn enzymatically and chemically affect the extracellular matrix. The intensity of the inflammatory process determines the severity of lameness and other clinical signs (BROMMER et al. 2004).

Human OA is commonly classified as primary and secondary (CHANTLEY et al. 1999; McILWRAITH 2002; TRUMBLE et al. 2001): Primary in case of unidentified causes (gradually developing disease of old people) and secondary when evidence for an etiologic factor can be provided (McILWRAITH 2002). In horses OA in the proximal interphalangeal joint and distal tarsal joints are typical examples of primary OA that develop gradually due to repetitive trauma (TRUMBLE et al. 2001). The clinical form of primary OA is likely to represent an exaggerated or accelerated form of age related asymptomatic cartilage fibrillation which is detected frequently amongst humans. It is also suggested that age related cartilage
lesions might develop which do not typically progress to clinical disease and are therefore considered nonprogressive lesions. The nonprogressive changes are incidental cartilage alterations encountered at necropsy. Age related OA processes can even be found in wild horses. It is likely that nonprogressive cartilage alterations become progressive when other factors, such as trauma from racing are superimposed on the joint. It is also credible that racing and training exacerbate and accelerate naturally occurring age related degeneration. However upon necropsy evidence for articular cartilage lesions has been found in horses with no history of lameness (CHANTLEY et al. 1999). In man a strong relationship between articular cartilage degeneration and increasing age is reported. This has been associated with high levels of nonenzymatic glycation products such as pentosidine cross links in the articular cartilage which are known to accumulate with age and therefore thought to be leading to a stiffer and more brittle collagen network of the extracellular cartilage matrix (BROMMER et al. 2003a). Primary OA of the human knee is characterised by important structural modifications of the articular cartilage. Articular cartilage degeneration includes the appearance of abnormal histomorphic features such as increased surface roughness cleft formation and substantial disaggregation of the amorphous protective layer covering the articular surface. In human and animal models of OA cartilage biopsies from osteoarthritic patients showed a significant cell density reduction, more pronounced in the region proximal to the articular surface. Additionally a sort of a metabolic switching of the surviving chondrocytes towards a more catabolic activity as indicated by the changes in the ultrastructure of the cells was observed. (GUIDOLIN et al. 2001).

Indicative for microdamage and loosening of the collagen network is the significant reduction of crosslinks and increase in water content. Usually covalent crosslinks such as Hydroxyproline crosslinks form strong bonds between collagen fibres. An increasing number of crosslinks therefore enhances the strength of the tissue. Disruption of crosslinks leads to damage of the structure of collagen network and in turn to leakage of PG aggregates and increased susceptibility for further damage (BRAMA et al. 2000). In equine articular cartilage an age related increase in pentosidine cross links is reported although absolute numbers seem to be much lower as in man. However TRUMBLE et al. (2001) also showed that the median age of horses increased with subjective histological score categories. They further wrote that grooves or wear lines were reported as frequent suggestive findings in mature equine and human joints but not in joints of children or foals. Therefore OA may in principal as well be a naturally occurring age related disorder in horses (BROMMER et al. 2003a).

Secondary OA in horses is alike traumatic arthritis and is prevalently caused by OCD fragmentation (TRUMBLE et al. 2001). Many authors describe specific mechanisms being the cause for the pathogenesis of osteoarthritis. One mechanism might be essentially defective cartilage resulting in abnormal biochemical properties. These altered properties cause production of a Type II collagen unable to withstand even normal joint loading, thus the cartilage might already fail under normal loads.
Another mechanism is based on fundamental changes in the subchondral bone, which in turn lead to a stiffer or less flexible bone cartilage unit that is predisposed for failure (FRISBIE 2006a). Because articular cartilage is too thin to be a really effective shock absorber, impact loading is absorbed by periarticular soft tissue and muscles in combination with subchondral bone. Subchondral bone is believed to be a major shock attenuator. It can happen that normal mechanical stress causes microfractures of the subchondral and epiphyseal trabecular bone. However, when the frequency at which these fractures occur exceeds the rate at which healing and remodelling can take place the joint is prone to damage. Bone accretion caused by healing of these microfractures leads to increased density of the subchondral plate and adjacent trabeculae, with an associated reduced ability to absorb physiological loads. The consequently increased bone stiffness causes a condition at which the bone-cartilage unit is not able to deform normally under load and therefore experiences supraphysiological stress which in turn causes further mechanical damage (CARON 2003). Increased stiffness of the subchondral bone is believed to result in shear stress in the cartilage and might initiate cartilage damage or contribute to its progression. The relationship between subchondral bone sclerosis and articular cartilage degeneration seems to be significant considering the median bone mineral density increase with subjective histological score (CHANTLEY et al. 1999).

The third proposed mechanism revolves around the exposure of normal cartilage to abnormal forces. Abnormal forces can exceed the normal metabolic repair mechanisms in the articular cartilage and finally cause its failure (FRISBIE 2006a). The resulting injuries lead to metabolic alterations of chondrocytes, and in turn to the release of proteolytic enzymes that cause cartilage degradation and breakdown of the proteoglycan network. Cartilage is characterised by its remarkable resistance to shear force but is on the other hand relatively susceptible to repetitive impact trauma (CARON 2003). Biomechanical factors are commonly believed to be of great importance regarding the pathogenesis due to the small surface area, large range of motion and impact of full body weight. It has been suggested that repeated overextension results in impact trauma of the proximodorsal articular margin of P1 (proximal/first phalanx) at the distal end of MC (metacarpal- or cannonbone) and may therefore play a fundamental role in the high incidence of joint pathologies in the fetlock joint (BROMMER et al. 2003a). CHANTLEY et al. (1999) also reported that repeated overextension of the joint, occurring at high speed might result in impaction of the proximodorsal margin of P1 upon the distal end of the third metacarpal bone. This might also be one reason for the fetlock joint suffering the largest number of traumatic and degenerative lesions of all joints of the appendicular skeleton (BROMMER et al. 2003a; CHANTLEY et al. 1999; JOUGLIN et al. 2000). Other possible explanations might be its relatively small surface area and the compared to other joints wide range of motion (CHANTLEY et al. 1999). In the equine fetlock joint degenerative cartilage lesions are found at various sites though most of the pathological signs are situated at the articular cartilage surface of the first phalanx. It has also been shown, that osteochondral fragmentation and OA changes are more often encountered at the proximodorsal articular margin and less at the proximopalmar/plantar aspect (BROMMER et
al. 2003a). At the dorsal and proximal palmar/plantar margins the medial side has been reported to be affected more frequently (BROMMER et al. 2003a; CHANTLEY et al. 1999). This obvious difference can probably be explained by differences in loading. Chondropathies at the palmar/plantar surface of P1 on the other hand are thought to be connected to the attachment of several ligaments, e.g. the short and cruciate sesamoidean ligaments (BROMMER et al. 2003a).

It is likely that the major part of cases of equine joint disease affects several if not most joint tissues like the subchondral bone, articular cartilage, synovial fluid, synovium, ligamentous joint capsule and stabilizers of the joint such as ligaments, tendons and muscles. Clinical disease is usually characterised by some involvement of several joint tissues depending on the degree of pathology. Each of these components has its particular capacity of repair (FRISBIE 2006a). The integrity of all these components and in particular collagen and PG is essential. However the collagen component of cartilage in comparison to the PG component has a very limited capacity of repair and remodelling due to its inherent extremely low turnover rate. Therefore alterations within the articular collagen network play an important role in the pathophysiology of degenerative joint disease (BRAMA et al. 2000).

4.1. Pathologic changes

The mechanical factors to which articular cartilage is normally exposed in vivo are most likely insufficient to destroy tissue directly but once the integrity of the matrix is impaired biochemically, the way for direct mechanical damage may be paved. However impact loads can result in shear stress that is sufficient for breaking collagenous cross links. This substructural damage in turn provokes chondrocyte enzyme production and enables access of catabolic enzymes into the matrix.

Already a single but major impact can cause articular cartilage damage. Sometimes it might then resemble chondromalacia and not be progressive, but in most cases, mechanical forces are believed to destroy cartilage indirectly following insult to the subchondral bone, synovial membrane or chondrocytes. While excessive mechanical forces can cause articular cartilage loss, removal of all mechanical stimulation is not good either since it results in atrophy. Normal function of healthy cartilage is maintained by some intermediate level. It is assumed that a horse in a good physical shape may also be able to tolerate joint loads better due to better muscle tone and increased joint nutrition due to physical activity. Another theory characterises OA as an enhanced process of remodelling of the articular ends of the bone manifested by an advancement of the tide mark and thickening of the calcified bed with subsequent decreased thickness of noncalcified cartilage. Cartilage can further be lost through advanced endochondral ossification characterised by reduplication of the tide mark, focal vascular resorption and ossification of the calcified layer. Extension of the calcified cartilage
zone towards the articular surface would result in a decreased cartilage height and such lead to impaired distribution of stress in cartilage and bone (McILWRAITH 2002). The first noticeable alterations in OA include increased water content of articular cartilage and reduced proteoglycan content as well as structural alteration of the proteoglycans, which is believed to result in loss of restraining capacity of the collagen network. The first visible changes are loss of the cartilages normal luster and consistency, it becomes yellow and soft. The formation of typical blisters is a common early change as well and is followed by pitting and superficial fraying of the cartilage. The initial OA-changes of high motion joints are usually acute synovitis and capsulitis. The occurrence of cartilaginous wear particles entails an increased production of prostaglandin E2, cytokines and metalloproteinases. The proteoglycans released into synovial fluid further contribute to the development of synovitis. In more advanced stages of the disease increased amounts of denatured collagen and a decreased collagen content indicating a clearly degraded collagen network can be encountered (VAN DEN BOOM et al. 2005). The essential histologic lesion is a progressive disruption of the articular cartilage parallel to the planes of the collagenous matrix fibrils. When the disruption only extends to the tangential layer the process is called flaking. Processes extending further to the radiate layer are referred to as fibrillation. Early stage fibrillation is typically represented as discoloration or thinning at the gross pathologic level. Fibrillation into the intermediate layer may be manifested by superficial erosions. A sequence of focal oedema and localized fibrillation is referred to as Blisters. If fibrillation even extends through the radiate layer, vertical clefts may be formed associated with full thickness fragmentation and loss of articular cartilage. Thinning of the cartilage is defined as an even decrease in its thickness. Wearing is a superficial erosion which is typically manifested as partial thickness loss of cartilage. Histologically so called “wear lines” are a kind of fibrillation that extends to varying depth, with deep ones appearing similar to deep erosions. Wear lines are encountered frequently in articular cartilage as lines running in the direction of joint motion (McILWRAITH 2002). Wear lines have been defined as the earliest macroscopic signs of cartilage degeneration indicating degenerative joint disease (BROMMER et al. 2003a). Ulcerations are focal defects in cartilage that are believed to follow blister formation. Erosion is defined as full thickness loss of cartilage. It may be either localized or wide spread. Eburnation is the polished sclerotic appearance that develops when the subchondral bone emerges. When the cartilage is continuously exposed to wear the next stage of degeneration - grooving may develop. The extent of necrosis among chondrocytes in fibrillated cartilage is variable. As a reactive response chondrons develop from other still viable chondrocytes. The changes of the articular cartilage are additionally accompanied by marginal ripping, osteophytosis and subchondral bone sclerosis (resulting from reactive new bone formation in the subchondral end plates). Subchondral cyst formation has been reported as a typical but secondary change. Inflammatory changes in the synovial membrane and fibrous joint capsule also go along with the disease, causing villous hypertrophy and joint capsule fibrosis.
The degree of pathologic changes and the clinical signs do not correlate necessarily. Quite contrary fibrillation in articular cartilage can even develop without any clinical signs and even in clinically affected joints, the correlation between the degree of lameness and the cartilage degeneration extend is not obligatory. Degenerative cartilage lesions are not considered to be painful if they are confined to the cartilage and do not extend to subchondral bone (McILWRAITH 2002).

As already mentioned above it was shown that with increased severity in degenerative joint disease the spread of cartilage degeneration seems to follow a specific pattern. In the fetlock joint initial cartilage degeneration is encountered at the medial dorsal margin of P1 and from there extends to the lateral margin. With further progressing degeneration changes appear at the medial and finally also the lateral dorsal margin as well as the lateral central fovea (BROMMER et al. 2004). A reason for this may be the differences in loading. A clear indication that asymmetrical weight bearing can strongly influence the 3-dimensional behaviour of the fetlock joint is the medial to lateral distribution pattern. It is considered to have important clinical implications (BROMMER et al. 2003a).

Biochemical heterogeneity of the extracellular matrix is fundamental to withstand local biomechanical requirements. Exercise induced changes though, may result in a vanishing physiological, biochemical heterogeneity between both sites. While PG is reported to respond rapidly to a biomechanically changing environment and consequently to exercise, collagen framework of the articular cartilage is produced during early growth after maturation it is hard to be changed by external factors (BRAMA et al. 2000).

4.2. Ethiology

Especially the hock joints of standardbreds, quarter horses and jumping horses are subjected to particular demands. Additional adversarial factors such as conformational defects, bad, wrong or insufficient shoeing can even exacerbate insults. Ringbone is typically seen in horses whose hind limbs are exposes to a lot of jarring motion (quarter horses).

A particularly severe traumatic insult which often leads to DJD is rupture of collateral ligaments especially when associated with luxation or subluxation.

Horses with OCD are often prone to secondary DJD.

OA can also result from infectious arthritis when the septic process has not been treated successfully before cartilage degeneration was induced.

In regions exposed to eminent concussion considerable damage may as well occur directly to the cartilage. Ulcerative lesions for example have been suggested to develop following direct concussion and when the joint is overextended (McILWRAITH 2002). Immediate damage could be inflicted to the cartilage with one single insult without chance of repair, such as an articular fracture caused by a traumatic event.
Another mechanism that may lead to immediate catastrophic failure is abnormal mechanical load due to mechanical instability, microdamage or a single traumatic event. Insults in the form of microdamage are thought to accumulate over time, leading to failure of the tissue when the reparative effort fails (FRISBIE 2006a).

Therefore a quick and thorough diagnosis of acute articular injury is essential to avoid single articular trauma to lead to progressive degenerative joint disease if not identified or treated appropriately or early enough. HARDY et al. (1998) suggest that therapeutic efforts may even be more successful in restoring joint health after acute injury if gross changes have occurred.

An important step in the pathogenesis of a more insidious osteoarthritic entry could be cyclic fatigue damage to the collagen network, however. Fatigue or damage in the collagen network could cause deleterious physical forces leading to injury and metabolic changes.

Besides fractures other primary damage of the subchondral bone may also lead to secondary damage of the articular cartilage resulting either from loss of supports or release of cytokines. Another theory is that abnormal movement may cause synovial fluid turbulence and therefore joint lubrication defects, which result in increased friction.

Age related degenerative changes typically occur in unloaded parts of the joint rather than in loaded. It is proposed that with the redistribution of load onto formerly unloaded cartilage, parts of the joints that over time have become deficient in proteoglycans come to bear considerable stress. Because of the lack of use these areas are not sufficiently suited to these loads and break already under physiological stress. The change from non weight bearing to weight bearing should be considered in varying training programs and change in work, because training is probably the best way to bring cartilage into the optimum condition to resist wear (McILWRAITH 2002).

Acute synovitis can lead to a prolonged inflammation and this in turn can result in degradation of joint cartilage and furthermore in a degenerative joint disease. In the case of underlying inflammation the breakdown of articular cartilage matrix is caused primarily by enzymatic degradation. The impaired ability of this biochemically affected cartilage to withstand repeated even physiological loading and mechanical trauma over the course of the inflammation most probably entails cartilage fibrillation and possibly erosive lesions. Cartilage damage of course worsens the prognosis for a full recovery. In an OA joint, inflammatory mediators such as IL1 (Interleukin 1), PGE2 (Prostaglandin E), TNF-alpa (Tumor Necrosis Factor α), degradative enzymes as MMP and degradative enzymes are produced exceeding metabolic homeostasis (MARTTINEN et al. 2006).
4.3. Role of the Synovium

The synovium plays a major role in the central pathophysiological event of cartilage matrix depletion (CARON 2003). It is regulates inflammatory cells and peripheral mediators that interfere in metabolic processes, especially through releasing catabolic substances which in turn aect the joint (FRISBIE 2006a). Most forms of joint disease are accompanied by synovitis which predominates in acute articular inflammation. Inflamed synovial cells synthesize mediators such as Interleukin 1 (IL1) and Prostaglandin E2 (PGE2) which in turn activate adjacent synoviocytes and shift the balance between chondrocyte matrix synthesis and degradation towards a destructive pathway (HARDY et al. 1998). Inflammation of the synovial membrane is like any other inflammation characterised by increased blood flow and capillary permeability, thus enabling influx of circulating neutrophils and plasma proteins into the joint. The reported significant correlation between SF GAG concentration and SF white blood cell count (WBC) may be accounted to the neutrophil migration into the inflamed joint and the subsequent release of cytokines proteolytic enzymes and eicosanoids that alter both articular cartilage matrix and synovial membrane ground substance. Activation of synoviocytes and chondrocytes by inflammatory mediators is assumed to result in an enhancement of chondrocyte synthetic and degradative products (PALMER et al. 2005). Synovitis further leads to a diminished concentration and molecular size of HA (HILBERT et al. 1984). Arthritic tissues continue to release greater quantities of inflammatory mediators even in the chronic stages of the diseases (GIBSON et al. 1996).

Primary synovitis or capsulitis is thought to originate from biochemical damage, based on a repetitive trauma and discrete incidents. Severe traumata effect joint stability and thus cause joint-wide abnormal biomechanical forces. In response to injury the joint capsule forms fibrous repair tissue which can lead to decreased range of motion. It is obvious that this may have long term effects on the use of the joint and result in overall abnormal biochemical forces in the joint tissue. Generally abnormalities in the joint can also cause secondary synovitis and capsulitis. Histologic abnormalities found in the synovial membrane of OA are usually oedema, hyperplasia of the intimal cell lining, hypervascularity, cellular infiltration of inflammatory cells and fibrosis of the subintima (FRISBIE 2006a).

4.4. Synovial Fluid/HA

It is nothing new that due to pathological processes such as OA and rheumatoid arthritis (RA) the molecular weight and concentration of the HA in the synovial fluid can be considerably reduced. The reduced HA concentration is caused by the accumulation of liquid released from the inflamed synovial vessels in the joint cavity (GUIDOLIN et al. 2001). On the other hand breakdown of HA in pathologically altered joints by hyaluronidases or superoxide radicals
may also play a role in the decrease of the total concentration of HA (PALMER et al. 1995). Additionally pathologic synovial fluid seems to contain cleaved HA with various chain lengths (KAWAI et al. 2004). Structural state of the hyaluronic acid molecules is essential for the rheologic properties of the synovial fluid (REJNÖ 1976). Destruction, depolymerization and/or reduced concentration may therefore influence flow behaviour (KAWAI et al. 2004; REJNÖ 1976) resulting in a reduced viscoelasticity of the fluid and increased susceptibility of cartilage to damage (GUIDOLIN et al. 2001; KAWAI et al. 2004; MORI et al. 2002). It is assumed that the higher the molecular weight and concentration of HA the higher the synovias viscosity and the greater its lubrication effect (KAWAI et al. 2004). Therefore one could conclude that alteration of concentration of HA in synovia caused by any functional abnormality of the synovial membrane leads to a defect in the lubricating and shock absorbing capacity of the synovia (ROWLEY et al. 1982). In addition HA in its degraded form is likely to be unable to inhibit phospholipase 2 (PLA2) activity and thus SAPL (Surface active phospholipids) become accessible to hydrolysis (NITZAN et al. 2001).

According to HILBERT et al. (1984) the mean HA concentration is 1.26 g/l +/- 0.26 in healthy joints and 0.58 g/l +/- 0.16 in arthritic joints. Other typical alterations in the properties of synovial fluid that occur in response to synovitis, degenerative joint disease, or infectious arthritis are increased synovial fluid volume, total protein concentration, and nucleated cell count (LUMSDEN et al. 1996). An accumulation of breakdown products of PG and GAG can also be found in the synovial fluid caused by increased synthesis and degradation of cartilage matrix by chondrocytes and cleavage of PG and GAG into fragments by several proteases (FUJITA et al. 2005).

### 4.5. Matrix Metalloproteinases

MMPs are considered to play an important role in cartilage matrix degeneration (FUJITA et al. 2005; VAN DEN BOOM et al. 2005). They are structurally and functionally related (FUJITA et al. 2005) and able to digest all main components of the extracellular matrix (CARON 2003). Collagenase (MMP 1, 8, 13) cleaves the interstitial collagen triple helix. Gelatinase (MMP 2, 9) acts on unwound collagen and stromolysin degrades proteoglycans (MARTTINEN et al. 2006; VAN DEN BOOM et al. 2005). In addition MMPs support immune response by activating inflammatory mediators such as growth factors, cytokines and chemokines and probably even evolve anti-inflammatory or defensive characteristics (MARTTINEN et al. 2006).

MMPs are produced in an inactive “pro”-form (FRISBIE 2006a; MARTTINEN et al. 2006) and stored in secretory granules of neutrophils (MARTTINEN et al. 2006). Their degRADING effect on extracellular cartilage matrix is caused by an increase in biologically
active forms (FUJITA et al. 2005). Latency is attributed to an intramolecular complex formation between a single cysteine residue in the propeptide domain and the essential zinc atom in the catalytic domain. Activation occurs due to a detachment of the cysteine residue from the complex (McILWRAITH 2002). Activation of MMP is considered to be a fundamental factor in extracellular proteolysis (JOUGLIN et al. 2000). MMPs are activated at a physiologic pH (McILWRAITH 2002) through proteolytic cleavage. Able of such cleavage are Trypsin, Chymotrypsin, Plasmin, Kallikrein, Cathepsin B, and even certain MMPs themselves (FRISBIE 2006a). The presumed scenario is that joint insult leads to synovitis caused by the release of proinflammatory agents (such as IL1α and β and tumor necrosis factor (TNF) α) from synovial membrane and chondrocytes. These cytokines are secreted in excess of the concentrations typically secreted for normal metabolic homeostasis in the joint and work synergistically. Both IL1 and TNF induce PG increase the rate of PG degradation and/or decrease synthesis by the chondrocytes therefore resulting in articular cartilage depletion. This is partially accomplished in through MMP and PGE2 stimulation (TRUMBLE et al. 2001). In OA MMPs are responsible for proteoglycan breakdown in the advanced stages of disease (VAN DEN BOOM et al. 2005). Increasing concentrations of MMPs could be correlated to areas of histologic disorders. MMP activity is generally related to the condition of the articular cartilage, but at the same time seems to be a better measure for active processes than cartilage status in chronic cases (MARTTINEN et al. 2006; VAN DEN BOOM et al. 2005).

The most prevalent MMPs found in connection with osteoarthritis so far include collagenase 1 (MMP1), collagenase 2 (MMP 8), collagenase 3 (MMP 13), stromelysin 1 (MMP 3) and two gelatinases (MMP 2 and MMP 9) (FRISBIE 2006a).

MMP 3 and 9 are synthesised in the deeper cartilage layers whereas MMP9 appears early in the course of disease and MMP3 biphasic in early and advanced disease. Since their expression is related to the depths of the chondrocyte it is stated that their appearance is related to the nature and extend of the cartilage lesion (McILWRAITH 2002).

MMP3 is thought to play a major role in cartilage matrix destruction because it is capable of degrading a number of cartilage components and is also involved in the activation of other MMPs (FUJITA et al. 2005).

Interstitial or tissue collagenase (MMP 1) is able to cleave all three collagen chains of the triple helix of intact Type II collagen molecules at a specific amino acid sequence (FRISBIE 2006a). It is also capable of cleaving collagen Types I, III, VII, VIII and X. Interstitial collagenase is synthesized by macrophages, fibroblasts, synovial cells, osteoblasts, chondrocytes and endothelial cells (McILWRAITH 2002) and therefore especially important. MMP1 synthesis is augmented by several different inflammatory cytokines involved in the development of OA such as tumor necrosis factor and interleukin 1 (SALINARDI et al. 2006). MMP1 is considered to play a role in homeostasis and early joint disease in horses. It is synthesised primarily from the synovial lining cells of patients with OA but has been found in the superficial layers of articular cartilage at increased concentrations in humans suffering
from OA. MMP1 has compared to other MMPs a low level of expression in articular cartilage though. MMP1 was so far found to be expressed only in synovial membrane samples of horses with acute trauma and clinical signs of disease. Therefore MMP1 may contribute to cartilage catabolism mostly during the inflammatory phases. It has been concluded that MMP1 may be focal and related to the degree of local inflammation. This enzyme could later diffuse into the synovial fluid and eventually enter the superficial layer of articular cartilage where its catabolic effects could then predominate if the cartilage had been mechanically damaged (TRUMBLE et al. 2001).

Collagenase 2 or MMP 8 has only recently been appreciated to have activity in equine cartilage.

Collagenase 1 (MMP 13) has been found to be even more aggressive in Type II collagen destruction than MMP 1. Additionally it has been suggested in the same studies that the excessive cleavage of Type II collagen in OA cartilage is mediated primarily by collagenases other than MMP1. Others have demonstrated increased ratios of MMP 1 over MMP 13 in OA patients (VAN DEN BOOM et al. 2005).

MMP2 which is upregulated by interleukin 1 and tumor necrosis factor (McILWRAITH 2002) may degrade Type I and III collagen directly (MARTTINEN et al. 2006).

Stromelysin (MMP 3) is activated by plasmin or other proteinases that activate collagen (McILWRAITH 2002). This MMP has been studied most notably for its capability of breaking down proteoglycans, partially degraded collagen and other minor cartilage proteins (FRISBIE 2006a). It is additionally considered to trigger activation of procollagenase to collagenase, though collagenase and possibly other MMPs can also be activated by plasmin (McILWRAITH 2002). Stromolysin has been described as the key enzyme in the pathogenesis of OA because of its multifaceted activity. It may play an important role in aggrecan and collagen degradation and it may potentate the activation of MMP1, 8, 9, 13. Enhanced amounts of MMP3 mRNA have also been identified in synovial membranes (TRUMBLE et al. 2001). In humans increased stromolysin and collagenase levels have been demonstrated in SF in OA and post traumatic joint disease (CLEGG et al. 1997).

The gelatinases comprise a variety of substrates, including partially degraded Type II collagen and Types X and XI collagen and elastin (CARON 2003). They also show a significant activity against fibronectin and collagens Type IV, V, VII, X and XI (McILWRAITH 2002). High activity of gelatinases was detected in SF drawn from joints of dogs with DJD (VOLK et al. 2003). Elevated activity of the collagenase MMP1 and of gelatinase Type MMP have been found in association with equine arthritis (MARTTINEN et al. 2006). Like collagenase membrane Type 1 MMP is also capable of digesting fibrillar collagen and a number of other matrix components.

In healthy cartilage the activity of MMPs is controlled by inhibitors. Most important among these inhibitors are the TIMPS (tissue inhibitor of matrix metallo proteinase). Imbalance between MMP and TIMP expression are considered to be a key event in the transition of MMP from physiological to pathological stages (MARTTINEN et al. 2006; Tumble et al.
2001; SALINARDI et al. 2006) and is according to CARON et al. 2003 an important determinant of the rate of matrix degeneration. Cartilage from humans with OA has been shown to have elevated MMP expression without associated increase in the TIMP levels. Based on these findings it is hypothesized that unregulated MMP activity may lead to excessive matrix degeneration. In both dogs and humans elevated cytokine activity has been found to be associated with increased levels of MMP and TIMP in SF and cartilage but the relative ratio of MMP: TIMP was found to be decreased (SALINARDI et al. 2006).

Other proteinases involved in cartilage destruction are for example serine proteinases, cystein proteinases and aspartic proteinases (McILWRAITH 2002). Correlation between arthroscopically detected cartilage damage and the activity of serine proteinases and TIMP1 in SF obtained from human knees was found (VOLK et al. 2003).

Again there are many other factors involved in the complex course of degenerative joint disease which have not been taken into account in this study.

4.6. Sources of pain

Through mechanical stimuli, such as stretching chemical mediators are activate and stimulate sensory nerves whereas mediators such as kinins and neuropeptides seem to stimulate pain fibres directly. However, the correlation between clinical signs and disease severity is evidently poor (FRISBIE 2006a).

4.7. Formation of osteophytes and enthesiophytes

Osteophyte formation is a characteristic proliferative component of OA. Reasons for the formation of osteophytes include aging, mechanical instability, response to synovitis and stretching of the synovial membrane insertion or other forces to any soft tissue attachment. Not to be mistaken for osteophytes are enthesiophytes which are bony proliferations at ligament-, tendon-, or joint capsule-insertions into bone.

Bony proliferations typically follow two patterns of growth in DJD. The junction of the synovial membrane, the perichondrium and the periosteum is prone to the formation of marginal osteophytes as a characteristic response of the tissue. The bony proliferations developing within the joint capsule and ligamentous insertions to the periosteum are considered to develop due to tearing and stretching of the attachments. A bit controversial is however the origin of the osteophytes that are formed at the marginal transition zone. They are considered to control the initial pathologic lesion by extending the surface area of the
articulart cartilage and at the same time reduce concussion and limit motion of the joint. It is hypothesised that this osteophyte-formation is induced by release of GAGs from the cartilage matrix. Osteophytes can also be caused by synovitis. It seems likely that osteophytes can be produced by multipotential cells as a reaction to a variety of substances that are secreted or released into inflamed and degenerated joints. Direct trauma or instability have also been suggested to play a role in the pathogenesis of marginal osteophytes. However osteophytes sometimes develop at the transition zones despite of any detectable cartilage damage. So correlation between articular cartilage degeneration and osteophyte formation is obviously poor (McILWRAITH 2002).

4.8. Cartilage repair

The limited potential of cartilage for repair and healing is evident (McILWRAITH 2002). HUNTER stated in 1995 that “Cartilage once destroyed never heals” (HUNTER 1995)! Articular cartilage potential to response to tissue damage is limited mainly due to inability of natural repair as well as to produce tissue with the morphologic, biochemical and biomechanical properties of healthy articular cartilage. There are many factors influencing the repair and remodelling abilities of articular cartilage such as the depth of the injury, defect size, location and relation to weight bearing or non weight bearing areas and the age of the animal (McILWRAITH 2002). In general defects up to a certain size range are difficult to identify grossly one year after they developed, whereas larger defects show good initial healing but degenerate within a years time. Partial thickness defects are thought to have some minor capacity for healing as well, but usually they seem neither to be progressive nor to largely influence joint function and are therefore not the focus of most cartilage repair procedures (HURTIG et al. 1988) The repair is facilitated by increased GAG and collagen synthesis, however it is never fully effective (McILWRAITH 2002). In contrast it was reported by GUIDOLIN et al. (2001) that the capacity of spontaneous repair of adult articular cartilage is limited to full thickness defects whereas partial thickness lesions do not heal spontaneously. This is analogous to what was found for the clefts and fissures encountered during early stages of human OA.

Limited repair, necessary to replace dead cells and damaged matrix at the margins of the wound, is provided by the adjacent tissue. The healing process of such defects is facilitated by ingrowth of subchondral fibrous tissue that may undergo metaplasia to form fibrocartilage (McILWRAITH 2002).

An altered subchondral bone plate and tidemark may create a stiffness gradient and cause shear stresses of the junction of the repair tissue and underlying bone. This again can lead to the degradation of the repair fibrocartilage followed by exposure of the bone. This chain of mechanical failures has been shown experimentally and clinically in the horse. Therefore
fibrocartilage repair typically found in normal full-thickness lesions is biomechanically insufficient as a replacement bearing surface and has been proven to be prone to mechanical failure. Two mechanisms might be responsible for the lack of durability. The first is faulty biochemical composition of the old matrix and the second incomplete remodelling of the interface between old and repaired cartilage. Another theory is that it might also be due to augmented stress in the regenerated tissue because of abnormal remodelling of the subchondral bone plate and calcified cartilage layer.

Partial thickness lesions on the other hand may be debrided of any surface fibrillation without progressive deeper debridement. Histologically intrinsic and extrinsic repair mechanisms are described for articular cartilage (McILWRAITH 2002).

Intrinsic repair emanates directly from the cartilage. Thus intrinsic repair depends on the limited ability of chondrocytes to proliferate and repair the damage (FRISBIE 2006a) and on the ineffective increase in collagen and proteoglycan production. The repair tissue contains Type 1 rather than Type II collagen (McILWRAITH 2002).

Extrinsic repair is a repair mechanism based on cells and other factors contributing to the repair process from sources other than the chondrocytes (FRISBIE 2006a). Mesenchymal elements from the subchondral bone contribute to the formation of new connective tissue that may undergo some metaplastic change to form cartilage (McILWRAITH 2002).

Matrix flow is another mechanism contributing to cartilage repair by forming cartilage lips from the perimeter of the lesion that “flow” towards the centre of the defect. It is still believed that large defects most likely obtain most of their reparative capacity from extrinsic sources. In experiments it has been shown that defects heal through influx of granulation tissue, which is composed of Types I and III collagen and a little bit by proteoglycan or aggrecan in the first 6 weeks. The granulation tissue is later slowly replaced by Type II collagen and aggrecan by chondrocyte like cells. In the optimum case a tissue similar to hyaline tissue is formed but the biomechanical and biochemical properties of this tissue do not approximate the properties of the original tissue. However it has been shown in humans that the quality of the repair tissue is not necessarily directly correlated to the functional utilizability of the patient (FRISBIE 2006b).

The healing response may not be as inadequate in immature animals or non-weight bearing defects. This is concluded from the fact that young horses after surgery for Osteochondrosis dissecans show impressive healing response which might be related to increased chondrocytic capacity for mitosis and matrix synthesis and further the presence of intracartilaginous vascularisation. It is hypothesized that mature chondrocytes produce a less organized matrix than younger and that the proteoglycan synthesis by chondrocytes might change with advanced age (McILWRAITH 2002).

For all these reasons relatively light damage may have severe consequences (BRAMA et al. 2000).
5.0. Clinical evaluation of joint disease

Synovial fluid analysis, cytology and radiography combined with lameness examinations make up the current basis for diagnosis of joint disease (JOUGLIN et al. 2000; PALMER et al. 1995; TAYLOR et al. 2006; VAN DEN BOOM et al. 2005). More sensitive markers are required for proper detection of radiographically silent cartilage lesions and to distinguish the degree of synovitis as well as between early and late stages (PALMER et al. 1995). The clinical signs of OA are similar to those of traumatic synovitis and capsulitis. Thus identification of OA based on radiographs or other diagnostic imaging methods is challenging. However though localisation of the problem may be confirmed by nerve blocks or intrasynovial anaesthesia the diagnostic techniques are not sufficient.

5.1. Clinical signs

The clinical signs vary with the type and degree of degeneration and are related to the amount of acute inflammation. In high-motion joints with acute synovitis typically lameness, heat, swelling, and pain on flexion are encountered. In more chronic stages joint enlargement is often combined with deposition of fibrous tissue which results in decreased motion, some acute inflammatory signs may as well persist. In low motion joints, the most ostentatious signs found are joint enlargement and increased lameness with flexion (McILWRAITH 2002). Limited range of motion is a typical characteristic of equine joint disease and presumably caused by a combination of reasons including guarding from pain, synovial effusion, oedema and progressive periarticular fibrosis. Effusion is a common feature in DJD and is manifested as distinct distension of joint pouches. Increased protein content in the synovial space, based on increased permeability of the capillary endothelium and augmentation of the intercellular spaces of the synovium, leads to a progressive increase of the colloid osmotic pressure and synovial fluid volume. Mild effusion even promotes nutrient exchange in the joint but significant effusion leads to gradually elevated intraarticular pressure and finally destabilization of the joint and resulting pain, stiffness and reduced range of motion (CARON 2003). Though volume of synovial fluid is increased in most cases of acute synovitis, in some cases of DJD it may be decreased and possibly manifested as a dry joint (McILWRAITH 2002).

5.2. Radiography

Radiography is the traditional means of assessing structural changes due to osteoarthritis. It has many advantages such as availability, convenience, relative safety and economy. Initially joint space narrowing, subchondral sclerosis and osteophytosis associated with OA can be appreciated radiographically. With time subchondral lysis, osteochondral fragmentation and eventually ankylosis may develop and become presentable. Unfortunately, although
radiography has some clear merit in demonstrating changes in bone associated with established osteoarthritis, it definitely lacks sensitivity and its applicability in identifying patients with incipient or focal lesions is controversial. It is well known that radiographically silent but performance limiting lesions may occur in horses (CARON 2003).

Macroscopically, cartilage degeneration is manifested as fibrilliation, erosion and wearlines. The radiological alterations accompanying OA are appreciable only in advanced stages of disease and furthermore they are, as mentioned above, only poorly related to the clinical signs (CARON 2003; FOX, et al. 2001; SALINARDI et al. 2006; TAYLOR et al. 2006; VAN DEN BOOM et al. 2005). McILWRAITH (2002) also agrees that considerable articular cartilage damage can be present along with a normal radiographic appearance of the joint. By the time radiographic changes become verifiable cartilage destruction is well established (BELLO et al. 1997; FULLER et al. 2001; McILWRAITH 2002; TULAMO et al. 1996; VAN DEN BOOM et al. 2005). Considering the very limited repair potential of mature articular cartilage this implies that therapeutic possibilities are limited (VAN DEN BOOM et al. 2005) at this point in the course of the disease. Accurate evaluation of radiographic findings is also hampered by difficulties in exactly duplicating conditions from one radiographic examination to the other (CARON 2003).

Fig. 2: Radiographic image of an osteoarthritic joint

5.3. Ultrasonography

Ultrasonography is another diagnostic tool that has gradually been used more and more lately. The principal benefit is its capability to demonstrate soft tissue changes. It also enables identification and localization of synovial or other fluid accumulations, thickened tissue, injured intraarticular or periarticular ligaments, as well as periarticular osteophytes, enthesophytes, osteochondral fragments and abnormalities in cartilage and subchondral bone. Limitations of ultrasonography result from the volume of tissue overlying the joint of interest being too voluminous or for specific structures or having a shape or orientation that is not conductive to ultrasonographic evaluation (DENOIX 1996).

Fig. 3: Sonographic image of an osteoarthritic joint
5.4. Nuclear scintigraphy

Nuclear scintigraphy offers information on the actual bone metabolism. The technique is based on bone seeking isotopes that accumulate for instance in the subchondral bones of osteoarthritic joints (CARON 2003). Distribution of the radiopharmaceutical for nuclear imaging is increased in tissue with augmented blood flow or osteoblastic activity. It is influenced by various disease processes and therefore changes may be detectable prior to radiographic changes (McILWRAITH 2002). Usually the most intense uptake of radiopharmaceutical is observed in the subchondral bone and at the osteochondral junctions of osteophytes. However variations in the uptake distribution may occur (CARON 2003). Scintigraphy seems to be useful in predicting the progression of osteoarthritis and it apparently may prove useful in diagnosis of preclinical joint disease stages, before of radiographic abnormalities appear (CARON 2003; SHARIF et al. 1995). Regardless scintigraphy images may be normal even in osteoarthritic joints once the rate of bone turnover returns to normal. As disadvantages of scintigraphy the lack of a cartilage specific agent relatively, poor resolution and specificity clearly have to be adduced (CARON 2003). Its sensitivity is high though (McILWRAITH 2002), due to the typical increased bone turnover as reaction to most insults (CARON 2003).

5.5. Computed tomography (CT)

CT can be particularly useful combined with radiographic studies. It is an excellent tool for the definition of stress induced subchondral bone sclerosis and other subchondral bone lesions, as well as for the determination of the subchondral bone sclerosis degree (McILWRAITH 2002).

Fig. 4: 3-Dimensional reconstruction of CT images

5.6. Magnetic resonance imaging (MRI)

MRI images give a very good soft tissue and intraarticular detail information on basis of sectional images (McILWRAITH 2002). It has a number of evidentiary advantages like non invasive high resolution and three dimensional illustration of all regions of interest. Additionally MRI nowadays provides much better contrast between articular cartilage and
surrounding tissue than was previously possible and hence makes changes at the tissue level that typically accompany osteoarthritis (e.g. increased water content and matrix swelling), better detectable. It facilitates evaluation of moderate articular cartilage lesions with considerable sensitivity and specificity as well as characterization of the amount and within certain limits quality of synovial fluid. It is also an adequate, sensitive way of delineating osteophytes and subchondral bone sclerosis. However the detection of subtle defects requires relatively sophisticated equipment and like other imaging techniques specific experience for proper image acquisition and interpretation. Widespread use of MRI in horses is unfortunately limited for economical reasons and the configuration of the tunnels of the currently available devices (CARON 2003).

5.7. Synovial fluid changes

Conventional analysis will not provide information about the degree of articular cartilage degeneration, however as mentioned before it does give an indication of the degree of inflammation and metabolic derangement (CARON 2003). Diffuse haemorrhage indicates an acute traumatic situation. Dark yellow or pale amber samples in turn represent previous haemorrhage and are most often an indication of chronic traumatic arthritis. Opacity and flocculent material in the sample are usually associated with synovitis. These changes are generally not very distinct in chronic degenerative joint disease (McILWRAITH 2002). Inflammation of the synovium usually goes along with increased blood flow and capillary permeability thus causing influx of neutrophils and plasma proteins into the joint (McILWRAITH 2002; PALMER et al. 1995). Acute articular inflammation is associated with variable degrees of neutrophilic leukocytosis increased protein concentration and decreased mucin precipitate quality (HARDY et al. 1998).

5.7.1. Total protein (TP)

An increase of total protein content in synovial fluid with joint inflammation is observed, it gradually approaches that of plasma. The levels of different protein fractions are similar to those in serum. Synovial fluid is considered not normal when total protein content is higher than 2.5 g/dl. Above 4 g/dl the inflammation is referred to as severe. Non infective inflammatory conditions commonly go along with concentrations below this level (McILWRAITH 2002; VIITANEN et al. 2001). However assessment of TP levels is not considered to be a very reliable tool. According to VIITANEN et al. (2001) TP values are higher in diseased animals but that difference could be shown to be statistically significant only in the DIP (distal interphalangeal) joint. Further GAG and total protein content already varies markedly between different joints in normal horses (VIITANEN et al. 2001).
5.7.2. Cytology/ Cellcount

The cell count may vary tremendously in traumatic arthritis and OA depending on the amount of active synovitis present (McILWRAITH 2002). Increase in cell count and TP concentration in chronic OA is usually not considerable, thus it does not make sense to use cytological evaluation of synovial fluid routinely in the diagnosis of OA (CARON 2003).

5.7.3. Synovial fluid viscosity

A common finding in horses with joint disease is reduced viscosity particularly in horses with active synovitis. It is attributed to a reduced concentration, or depolymerisation of synovial fluid hyaluronan (TULAMO et al. 1996).

5.7.4. Hyaluronic acid

Normal synovia has a mean hyaluronan concentration approximately 50% higher than that of synovia from joints with synovitis (SAARI et al. 1989). The decrease of total concentration of HA, as typically seen in pathologic joints, may result from breakdown of released HA by hyaluronidases or superoxide radicals (PALMER et al. 1995). The molecular weight of HA is found to be significantly reduced in SF from arthritic patients as well (LITTLE et al. 1990). However, according to SAARI et al. (1989), the variability between healthy horses is sufficient, that the difference is not significant. The mucin clot test is described to be a relatively simple, semi-quantitative test for measurement of hyaluronan quantity and quality, but is not very sensitive (CARON 2003). In contrast TULAMO et al. (1996) were able to show a significant correlation between HA concentration and molecular weight and the degree of arthritis and previously even with radiographic changes (TULAMO et al. 1994).

5.8. Biomarkers

Biomarkers are molecular products of tissue turnover. Measuring their levels in healthy and diseased cartilage and bone might have the potential to achieve early diagnosis and improve the understanding of OA pathophysiology (FRISBIE 2003). In the past years scientists searched for and developed biochemical as well as immunologic markers to identify and quantify breakdown products of the articular cartilage. The underlying basic principle of markers is that cartilage degeneration involves disruption of the collagen framework and loss of proteoglycan, the breakdown products are consequently released into the synovial fluid and ultimately the serum (McILWRAITH 2002). Direct or indirect factors can assault the matrix molecules of these tissues and lead to a destruction and loss of macromolecules (FRISBIE 2003). Therefore direct and indirect markers for diagnosis of DJD should be distinguished. PG and related molecules, epitopes of chondroitin sulphate and Collagen type 2 c-propeptide are examples of direct markers (FUJITA et al. 2005). Other potential molecules determined to
be products of OA are e.g. KS, aggrecan fragments, cartilage matrix glycoproteins and cartilage oligomeric matrix protein (FOX et al. 2001). Potential markers for the anabolic response particularly to early OA are specific types of CS, link protein, and collagen 10 (FOX et al. 2001).

Indirect markers of cartilage metabolism include proinflammatory cytokines such as IL1 and 6 (FOX et al. 2001; FUJITA et al. 2005), TNF (FUJITA et al. 2005) and matrix metalloproteinases related to the mechanisms of cartilage degradation (e.g. stromolysin-MMP3 and collagenase-MMP1) as well as tissue inhibitors of matrix metalloproteinase (FOX et al. 2001).

Although biomarkers may play a specific role in diagnosis and monitoring of joint disease, it is assumed that a combination of markers will be necessary, especially since many factors act together (FRISBIE 2003).

5.8.1. Matrix Metalloproteinases

Some of the most important mediators of articular cartilage and synovial membrane destruction are the MMPs that degrade the proteoglycan core protein close to its attachment site to hyaluronate (PALMER et al. 1995). MMP9 monomer seems to be particularly strongly correlated to the severity of cartilage alterations (JOUGLIN et al. 2000). MMP1 presumably influences the homeostasis and plays a role in early joint disease in horses as well. It has been reported to be synthesized primarily by synovial lining cells of patients with OA but lately increased concentrations could also be identified in the superficial layers of articular cartilage in humans suffering from OA. According to TRUMBLE et al. (2001) the expression of MMP1 seems to be low in normal articular cartilage which agrees with their observation that MMP1 was expressed only in synovial membrane samples of horses with acute arthritis and clinical signs of disease. Therefore they concluded that MMP1 may be involved in cartilage catabolism mainly during the inflammatory stage. Hence synovial fluid MMPs may be used as a marker of alterations that occur in the joints associated with acute OA (JOUGLIN et al. 2000).

5.8.2. Cytokines

IL 1 alpha and beta and TNF alpha are produced by a variety of cells such as inflammatory cells, synoviocytes and chondrocytes and have been found at high concentrations in patients suffering from OA. TNF alpha is accepted as an essential mediator of acute inflammation during joint disease whereas IL1 beta has only recently been reported to be the most prevalent cytokine in OA joints and to play a major role in synovitis (TRUMBLE et al. 2001).
5.8.3. GAG

One of the first biochemical changes observed in OA is a loss of GAG from articular cartilage (VAN DEN BOOM et al. 2005). In people, horses and rabbits an increased concentration of synovial fluid GAG has already been reported to occur in association with traumatic or osteoarthritic synovitis. One theory is that cleaved proteoglycan fragments with attached GAG side chains may diffuse out of the synovial tissue matrix into the synovial fluid. Increase in GAG synthesis following the inflammatory stimulus but without the ability of the GAG to be retained in the matrix may also cause increased synovial fluid GAG contents (PALMER et al. 1995). Therefore a possible way to monitor breakdown of articular cartilage could be determination of the levels of different specific degraded proteoglycan molecules like GAG in SF and sera (ALWAN et al. 1991). In humans it was observed that the concentration of the whole or fragmented sulphated GAG in SF and plasma is positively correlated with the degree of active cartilage degradation in osteoarthritic joints (LITTLE et al. 1990) but it must be taken into account that concentrations of GAG and total protein already vary significantly between joints in normal horses (VIITANEN et al. 2001). VAN DEN BOOM et al. 2004 could not show a correlation between the SF GAG content and the degree of cartilage degradation. Two examples for GAGs useful for diagnosis and estimation of the degree of DJD could be CS and KS. Chondroitin sulphate is the major glucosaminoglycan (GAG) of aggrecan and therefore a useful tool to mark aggrecan synthesis (POOLE et al. 1994). Keratan sulphate is another GAG on the proteoglycan molecules of aggrecan. In some previous studies elevations in serum levels of KS could be shown to be associated with osteoarthritis (POOLE et al. 1994) but the lack of correlation of synovial fluid KS levels with the degree of cartilage damage (BELLO et al. 1997) leads to a questionable value of KS as a marker for OA (FRISBIE 2003).

5.8.4. Carboxy propeptide of type II collagen

Levels carboxy propeptide of type II collagen were found to be significantly higher in synovial fluid from joints with osteoarthritis. Therefore it may be a useful measure of the anabolic process of type 2 collagen synthesis (FRISBIE 2003).

5.8.5. Degradation of collagen type II

Another marker of potential benefit in monitoring osteoarthritis is the degradation of collagen type II. Antibodies to identify cleaved or denatured type II collagen fragments have been developed. In synovial fluid and serum samples of horses with OA significant elevations in the levels of degraded type II collagen could be shown (FRISBIE 2003).
5.8.6. Cartilage oligomeric matrix protein (COMP)

Cartilage oligomeric matrix protein is referred to as substantial non-collagenous protein constituent of cartilage. Expression of COMP in synoviocytes was reported to be upregulated in OA. It was formerly thought to be cartilage-specific, but recently has been localized in tendons and synovium. However, serum and synovial fluid contents of COMP are demonstrably increased in people with OA. A positive correlation was found between COMP levels and radiographic appearance of OA, progression of radiographic alterations (FRISBIE 2003) and results of nuclear scintigraphy (SHARIF et al. 1995). In contrast VIITANEN et al. (2001) found that COMP levels did not differ in horses with radiographic changes and in disease.

5.9. Arthroscopy

Arthroscopic examination of joints, the gold standard in diagnosis of OA (TAYLOR et al. 2006), facilitates evaluation of the nonosseous surfaces of the joint, including synovium and its villi (with increasing chronicity of the OA disease process the villi commonly become thicker and denser), articular cartilage, intraarticular ligaments and menisci. Arthroscopy enables assessment of cartilage fibrillation, erosion and wear lines. The magnification effect of the arthroscope is a useful support for evaluation. However examination of the whole joint remains a problematic part of any arthroscopic surgery. In many cases additional damage is created (McILWRAITH 2002). The downside of arthroscopic evaluations is the evident lack of correlation of clinical signs with arthroscopic findings and radiographic appearance (BROMMER et al. 2004; KANNEGIETER et al. 1990).

Fig. 5: Arthroscopic view of a cartilage defect
6.0. Materials and methods

Within the scope of the study 60 legs from 17 polish draught-horses (16 mares and one gelding) were used for synovial fluid and cartilage sample collection. The ages of the horses included in the study ranged from three to twelve years (mean age 7.12 years). Prior to slaughter the horses were observed in the walk and showed no evidence of severe lameness, which could have possibly indicated severe acute trauma or septic arthritis. The horses were slaughtered in a licensed slaughterhouse in Bagnolo in Piano, Italy. All samples were collected within three hours after death. A post mortem examination of the amputated limbs revealed that the horses used in this study showed no signs of joint inflammation—neither visual nor palpable.

Synovial fluid samples were drawn through puncture of the dorsal pouch of the distal interphalangeal joints (of all four limbs), the palmar and respectively the plantar pouch of the metacarpal- and metatarsophalangeal joints, and the dorsal pouch of the intercarpal joints (for calibration purposes). To rule out severe acute trauma or septic arthritis the synovia was first macroscopically assessed for normal amount, colour, transparency, viscosity and consistency as well as presence of foreign particles or if it showed signs of coagulation. The samples were centrifuged and the supernatant filled into pyogenfree cryo tubes and stored first at -196°C in liquid Nitrogen and then at -80°C until further processing (assessment of viscosity-, elasticity-, hyaluronic acid quantity and molecular weight distribution as well as evaluation of MMP concentration). About 0.5 ml synovial fluid were filled into sterile EDTA tubes without centrifugation for total protein analysis, cytology and white blood cell count determination.

The limbs from which the synovial fluid samples were taken had been amputated in the carpal joint respectively tarsal joint and stored in dry-ice (-80°C) for the duration of the transport and later at -20°C until further processing, as described in BROMMER et al. (2003a, 2003b).

6.1. Cartilage analysis

After thawing the limbs for two days at room temperature the metacarpophalangeal and distal interphalangeal joints, from which synovial fluid had been drawn, were dissected. Three different methods were performed to determine the degree of cartilage degeneration most efficiently: Native lesion scoring, lesion scoring after Indian ink staining and computer assisted Grey level analysis.

6.1.1. Native lesion scoring

The cartilage was assessed for the presence of macroscopic changes following a modified lesion score developed by JOUGLIN et al. (2000). The cartilage surfaces were assessed by two independent veterinarians and the mean value resulting of the two independent assessments was calculated.
Lesion score (JOUGLIN et al. 2000):

0  Normal

Fig. 6: Cannon bone/ JOUGLIN lesion score 0

1  Minor: One disorder without cartilage changes

Fig. 7: Proximal surface of proximal phalanx (P1)/ JOUGLIN lesion score 1
2 Mild: One disorder with superficial cartilage alterations

Fig. 8: Cannon bone/ JOUGLIN lesion score 2

3 Moderate: One or two disorders with cartilage fibrillation and softening

Fig. 9: Proximal surface of P1/ JOUGLIN lesion score 3

4 Numerous or severe changes: One or several disorders with deep or full thickness cartilage lesions

Fig. 10: Cannon bone/ JOUGLIN lesion score 4
6.1.2. Lesion scoring after Indian ink staining

A method capable of detecting OA originated cartilage damage over a whole joint surface is Indian ink staining. Indian ink uptake into a healthy/intact cartilage surface is avoided by an unaffected proteoglycan rich matrix. On the other hand Indian ink particles show a high affinity towards articular cartilage with surface fibrillation and cartilage erosion. Hence the intensity of Indian ink staining depends on the reduction in the proteoglycan content of the cartilage surface. This technique is scarce if cartilage ulceration already extends to the level of the underlying subchondral bone, since Indian ink does not stain bone. This limits the usefulness of the method to the evaluation of initial stages of cartilage damage (BROMMER et al. 2003b).

Following BROMMER et al. (2003a, 2003b) and CHANTLEY et al. (1999) the cartilage surfaces were stained with Indian ink to make even very small lesions or superficial cartilage fibrillation visible. The Indian ink stained articular surfaces were macroscopically scored, this time following a lesion score by CHANTLEY et al. (1999).

The “Indian ink” used was a black (17), opaque, non-fading, lightfast, reproducible, “Pelikan® drawing ink A, for paper and board by the registered trademark of Pelikan®, (D-30177 Hannover, Germany). The cartilage surfaces were again assessed by two independent veterinarians and the mean value resulting of the two independent assessments was calculated.

Lesion score (CHANTLEY et al. 1999):

0  Either no stain uptake by the cartilage, or a fine line stain, 1 mm or less in length and width.

Fig. 11: Cannon bone/ CHANTLEY lesion score 0
1. Either a fine line stain more than 1 mm long, or grey staining up to 2 mm in length and width.

Fig. 12: Distal surface of middle phalanx (P2)/ CHANTLEY lesion score 1

2. Either more than one strongly stained line with lines possibly overlapping and up to 5 mm in length, or an area stained grey/ black up to 3 mm wide and 2 mm long.

Fig. 13: Distal surface of P2/ CHANTLEY lesion score 2
3 Either strong, black stain 2-4 mm long and 3-5 mm wide, or more than one black stained area (1-4 mm in length and up to 5 mm in width) separated by either black or grey lines or no stain. Wear lines poorly stained and more than 1.5 mm apart.

Fig. 14: Proximal surface of PI/CHANTLEY lesion score 3

4 Either the presence of a cartilage flap (defined as an attached flap of articular cartilage that did not appear to be ossified) and black or grey staining of the surrounding cartilage, or black stain more than 4 mm in length and width. The stained surface may appear irregular. Wear lines black stain more than 0.5-1 mm apart.

Fig. 15: Cannon bone/CHANTLEY lesion score 4
The presence of an ossicle (defined as a rounded, apparently, ossified fragment attached to the parent bone by fibrous tissue) or osteophyte (defined as bony exostoses attached to the parent bone) and/or grey or black staining of the surrounding cartilage.

Additionally all cartilage lesions were described thoroughly, including lesions due to dissection (such as cuts) to be able to distinguish between artificial and degenerative or traumatic lesions later. Unfortunately the lesions were not described in all joints from the very beginning, because it was only noticed with increasing routine that this might be useful.

6.1.3. Computer assisted Grey level analysis

The cartilage degeneration index (CDI) has been shown to be a reliable tool for the quantitative assessment of articular cartilage damage across the entire joint surface. The CDI index can range from 0-100% and it represents the amount of articular cartilage that is stained with Indain ink. Correlation with a semi-quantitative scoring technique described by CHANTLEY et al. (1999) was reported. A macroscopic score 0 corresponded with a CDI mean value of 2.5\(\pm\) 2.8% and a macroscopic score of 5 corresponded with a mean value of 38.1\(\pm\) 7.9%. The CDI reflects cartilage damage. No other features of OA, such as changes in the subchondral bone or joint capsule are determined (BROMMER et al. 2003b).

The cartilage degeneration index values can be subdivided into 3 groups (BROMMER et al. 2004):

1) Minor lesions: CDI< 25%
2) Moderate lesions: 25%< CDI < 45%
3) Severe lesions: CDI> 45%

It is already known that the intensity of Indian ink uptake by the articular cartilage depends on the reduction of its proteoglycan content. However some Indian ink uptake occurs regardless of degradation. In an earlier study 2-10% staining of non-degenerated cartilage was shown. In the study by BROMMER et al. (2003b) an error of approximately 10% was found.
Pictures for computer assisted grey level analysis were taken with a digital camera principally following the instructions at BROMMER et al. (2003b). The cartilage surfaces were photographed before and after “Indian ink” staining to prove any kind of mechanical cartilage damage. The digital camera used was an Olympus Camedia C-765 Ultra Zoom, 4.0 Megapixel, 10 times optical zoom with an Olympus lens and AF Zoom 6.3-63mm 1:2.8-3.7. According to BROMMER et al. (2003b) a maximum of 10 pictures from non stained specimens were taken without refreshing the bath solution. In stained specimens BROMMER et al. (2003b) observed a significant mean grey level increase after the sixth photograph.

Computer assisted grey level analysis was performed using “Adobe Photoshop 6.0” following a modified version of BROMMER et al. (2003b). First the cartilage surface was “cut out” and the pictures were turned into black and white images. A “histogram” was drawn up to evaluate the pixel count and the mean grey value of the whole cartilage surface. The mean grey value was used to determine the “boundary values” for healthy and damaged cartilage. If necessary the “boundary values” were adjusted manually adding or subtracting the standard deviation value to achieve best possible correlation with the subjective impression of the cartilage surface condition. To calculate the Cartilage Degeneration Index (CDI) the amount of pixels marked as damaged was determined carrying out a “histogram” again and calculating their percentage compared to the whole surface.

\[
\text{CDI} = \frac{100 \times \text{Pixel count of degenerated cartilage areas}}{\text{Pixel count whole cartilage surface}}
\]

Additionally the pixel count and mean grey level of major cartilage lesions were assessed. A colour degeneration index (CODI) was determined calculating the ratio of the mean grey value of healthy cartilage areas and the mean grey value of damaged areas.

\[
\text{CODI} = 100 \times \frac{\text{Pixel count degenerated cartilage areas} - \text{Pixel count healthy cartilage areas}}{\text{Pixel count healthy cartilage areas}}
\]

Fig. 17: Unstained 18.5%
Fig. 18: Stained
Fig. 19: CDI: 48.8%; CODI:
6.2. **Synovia sample analysis**

6.2.1. **Macroscopic evaluation**

First the synovial fluid was macroscopically examined to rule out a severe septic arthritis or acute trauma. 5 different colours of synovial fluid were distinguished:

![Fig. 20: Synovial fluid colours](image)

1= pale  
2= light yellow  
3= yellow  
4= dark yellow  
5= bloody  

To evaluate the transparency the legibility of the scale on the syringes was assessed:

No clouding= 0 (perfectly legible)  
Slight clouding= + (legible but not clearly)  
Severe clouding= ++ (not legible)  

Further viscosity, consistency, signs of coagulation and the existence of foreign particles were evaluated:

0= no foreign particles  
+= some foreign particles  
+++ = many or big foreign particles  

6.2.2. **Evaluation of rheologic behaviour**

Viscosity and elasticity were determined using a Rotational Cone and Plate Viscosimeter: Universal Dynamic Spectrometer, UDS 200 (A. Paar Physica GmbH, Graz)
The UDS has a frequency range from 0.001 – 100 Hz and a strain range from 0.1-10. A detailed description is to be found in the instrument’s manual.

Standards used for calibration: 2 calibration oils from DPB (Deutsche Physikalische Bundesanstalt, Berlin) (At 20°C and at 3.6 mPa.s as well as 100mPa.s)

Viscosity was measured applying increasing shear rate and measuring the actual shear stress. The results are depicted in so called flow curves. This test imitates the synovial fluid behaviour in the joint at different joint motion velocities. The strain test imitates the synovial fluid behaviour in the joint at different applied degrees of deformation – range of joint motion at constant speed. The values for \( \eta_{0} \), the inclination and the inflection point calculated from these curves are important values for the characterisation of viscosity behaviour.

Viscoelasticity was measured using 2 strategies. The first strategy was a strain test (strain sweep or deformation sweep at constant frequency). The strain test shows at which deformation/ angle a joint can be moved without destroying the synovial fluid’s structure (entanglement). The strain applied varies from \( 10^{-2} \) to \( 10^{2} \) resulting in a shear rate from 0.1-100s\(^{-1}\) at a constant frequency of 1 Hz. The deformation values at which the constant \( \eta_{0} \) declines (inflection point) depict the end of the linear viscoelastic range and the onset of network entanglement due to shear stress.

The second strategy was the so called frequency test (frequency sweep). The frequency is increase until the test sample reacts like an elastic solid. In a frequency test the applied deformation is so small, that the molecule’s entanglement is not influenced. It is a kind of mechanical spectroscopy representing the fluid’s response to constant deformation at different frequencies. It imitates the behaviour of synovial fluid in the joint at different deformation speeds at a small range of motion. This test provides the basic information about the viscoelastic properties of fluids. The oscillation frequency varied from \( 10^{-2} \) to \( 10^{0} \) Hz at a constant strain of 1. The results are depicted in a so called frequency curve. The values for the point of intersection and \( G_{0} \) calculated from these curves are important values for the characterisation of viscoelasticity.

To characterise and describe the viscoelastic properties of synovial fluid samples the typical parameters of the flow curves as mentioned above need to be calculated.

\( \eta_{0} \) (Pa*s): The plateau value of the initial viscosity presented in a viscosity curve.

Inclination: Inclination of the viscosity curve

Both parameters were calculated from a Carreaux fit of the viscosity curves.

Inflection Point (s\(^{-1}\)): The point at which the sample starts to flow is obtained from a strain sweep.

Point of intersection: From the frequency sweep one obtains an intersection point between viscous and elastic components (DE SMEDT et al. 1993).

\( G_{0} \): \( G_{0} \) is the plateau value of the final elasticity.
6.2.3. Hyaluronic acid quantity

For Hyaluronic acid quantity analysis, an immunoassay using Hyaluronan binding Protein (HABP) was performed, supervised by Univ. Prof. Dr. Andreas Rössler at the Institute for Systematic Physiology, Medical University Graz, Harrachgasse 21, 8010 Graz following the lab protocol of this Institute.

Materials and chemical compounds:

Assay Puffer:
6.06 g TRIS
5.00 g Pig Albumin (A-2764 Sigma)
0.5 g Bovine Albumin (G-5009 Sigma)
0.5 g Natriumacid
8.0 mg Diethylentriaminpentacetic Acid (DTPA- Sigma D-6518)
9.0 g Natriumchlorid
100 μl Tween 20 (P-1379)
dissolved in 900ml concentrated HCL to a PH of 7.7 and filled up to 1000 ml.

Wash solution:
76 g (60.8) TRIS
113 g (90.4) Natriumchlorid
6.3 g (5.04) Natriumazid
2.50 g (2.00) Tween 20
dissolved in 800 ml concentrated HCL to a PH of 7.7 and filled up to 101.

Coating Puffer:
4.2005 g Natriumhydrogencarbonat
dissolved in 900 ml distilled water with NaOH to a PH of 9.6 and filled up to 1000 ml.

Enhancement:
19.3 mg tri-n-Octylphosphinoxid (TOPO T-4760)
4.0 mg 2-Naphtoyltrifluoroaceton
1.0 g Triton X 100
3.20 g Kaliumhydrogenphthalat
dissolved in 900 ml distilled water and with 100%-Acetic acid to a PH of 3.2.
Mixed for 24 hours and filled up to 1000 ml.
HA Standards:

<table>
<thead>
<tr>
<th>HA- Stand.</th>
<th>Assay Puffer</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>10 µl+</td>
<td>1090 µl</td>
<td>500 ng/ml</td>
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</tr>
<tr>
<td>300 µl+</td>
<td>1200 µl</td>
<td>5 ng/ml</td>
</tr>
<tr>
<td>0 µl+</td>
<td>500 µl</td>
<td>0 ng/ml</td>
</tr>
</tbody>
</table>

500 µl of each standard are incubated into tubes with 500 µl binding protein HABP.

At first the Microtiterplates were coated with HA. Thus 1 ml (1.1 mg/ml) HA in 43 ml coating puffer (ph=9.6) was diluted to 25 µg/ml. The coating was performed using a Multipipette and 200 µl/ Well. Afterwards the plates were sealed and incubated in the fridge at 4° for 24 hours.

The second step was the “Competition” using HABP -Biotine. The samples were thawed and the competition with HABP- Biotine (c=0.25 µg/ml), for about 65 hours at 4°, carried out. 30 µl HABP were diluted with 12 ml assay puffer and 250 µl of this dilution were mixed with synovial fluid. The samples were incubated over night at 4°. After mixing the samples really thoroughly the HA-coated sample tubes were washed, dried and the samples and standards transferred into the coated tubes. The tubes were sealed again and incubated for another 24 hours at 4°.

The last step was marking and measuring the samples.

The tubes were washed again and 200 µl Europium marked Streptavidin (1:1000) were added. The tubes were sealed again and incubated for another 90 minutes at 4°. Then the tubes were washed one last time and 200 µl of enhancement solution were added and dilution mixed very thoroughly. The samples were measured using a Multilabel Counter.

6.2.4. Hyaluronic acid molecular weight distribution

Gel Permeation Chromatography (GPC):

Application of GPC for analysis/characterisation of polysaccharides is supposed to provide information about actually excluded volume of each fraction with details on interactive potential (ip), molecular dimension (md) and molecular conformation (mc) contribution if appropriate detection systems are coupled to GPC. Excluded volume and sphere equivalent radius of excluded volume of polysaccharide fractions may be obtained by means of triple detection coupled to previous GPC. Such an experimental setup consists of a GPC section with 2-5 columns of different pore sizes in series, followed by a detection section with one or more detectors in series and a numerical data processing section where parameters of interest are computed from initially detected elution profiles obtained by different detectors. GPC separation of polysaccharides is typically achieved by aqueous eluents with defined salt content at isocratic conditions and with constant flow rates between 0.3 and 1.0 ml/min. Manually, or via autosampler, applied volumes of sample solution range between 50 and 300
μl with typical concentrations between 0.1 and 0.3%. If selected fractions are transferred to subsequent offline analyses, such as staining/complexation for instance, a fraction collector is the terminal component of such a GPC-multiple-detection system. Combined processing of elution profiles from mass and scattering detectors provides absolute molecular weight for each GPC-separated polysaccharide fraction and enables the establishment of an absolute molecular weight calibration function for the utilized GPC system.

As an initial result of an GPC experiment with mass/scattering/viscosity detection, molecular weight distribution is obtained in a twofold form: distribution of mass fractions and distribution of molar fractions. Although distribution of mass fractions and distribution of molar fractions are strictly correlated by a simple transformation operation, the differences in terms of dominant components are quite drastic for broad distribution in particular: low molecular components dominate molecular weight distribution of molar fractions and high molecular components dominate molecular weight distribution of mass fractions. Molecular weight averages may be computed as representatives of obtained molecular weight distribution. Number average molecular weight (Mn) and weight average molecular weight (Mw) are computed. The ratio of Mw and Mn is assigned as polydispersity, an indicator of heterogeneity. Processing of molecular weight distribution and intrinsic viscosity elution profile from GPC provides distributions of excluded volumes: once again, and analogous to molecular weight distribution, distribution of mass fractions as well as distribution of molar fractions of excluded volumes may be obtained. A simple transformation provides sphere equivalent radii of investigated polysaccharides form their excluded volume. Finally for the case of known molar mass and excluded volume for each polysaccharide fraction, packing density may simply be obtained as the ratio. Even slight changes in these distributions due to technological processing or applied modifications strongly influence materials properties and thus observation of these distributions provides good indicators for appropriate/inappropriate utilization of the fractions.

The excluded volume for each component is controlled by a fine-tuning mechanism at the molecular level discussed below:

Conformation: molecular symmetries such as helices; beta-sheets; branching pattern; external cross links; oxidation status of constituting building blocks; presence of compatibility structures such as N-acetyl-group; packing density of polymer coils.

Dimension: classified by parameters such as molecular weight/degree of polymerization/excluded volume; transition states between geometric molecular dimensions and coherence lengths of supermolecular structure.

Interactive properties: observed by phenomena such as aggregation/association or gel-formation and quantified in terms such as visco-elastic properties and capability to react onto applied stress (HUBER et al. 2004).

Molecular weight mean values either are computed as moments of molecular weight distribution or result from specific experimental approaches.
\[
\bar{M}_n = \frac{\sum_i n_i M_i}{\sum n_i} \\
\bar{M}_w = \frac{\sum_i n_i M_i^2}{\sum n_i M_i} \\
\bar{M}_z = \frac{\sum_i n_i M_i^3}{\sum n_i M_i^2} \\
dp = \frac{M}{M_{\text{unimer}}} \\
\bar{dp}_n \\
\bar{dp}_w \\
\bar{dp}_z
\]

Polydispersity: \( \frac{M_w}{M_n} \)

Mn: Number average molecular weight
Mw: Weight average molecular weight
Mz: Z-average molecular weight
dp: Degree of polymerisation
Polydispersity: \( \frac{M_w}{M_n} \)

Degree of Polymerization Distribution (dpD)

Fig. 21: Degree of Polymerization Distribution (dpD) (HUBER 2008)
6.2.5. Determination of MMP1 and MMP3 quantity

MMP1 and 3 content were analyzed by Prof. Dr. Almuth Einspanier at the University Leipzig, Faculty of Veterinary Medicine, Institute for Veterinary Physiology and Chemistry, An den Tierkliniken 19, 04103 Leipzig. MMP1 and 3 content was assessed by Biotrak ELISA System following the lab protocol of this Institute.

6.2.5.1. Matrix Metalloproteinase-1 (MMP-1), Human, Biotrak ELISA System:

Establishment of pipette pattern (Blank, Standard, samples)

Reagent production (all reagents need to be at temperature of 20-25°C!)

Assaypuffer (AP): The content of the bottle is transferred into a 100 ml cylinder and diluted with 100 ml of distilled water then it is shaken thoroughly.

Antiserum: 11 ml AP-2 are added and the fluid is carefully shaken again, foaming needs to be avoided!
Peroxidase: 11 ml AP-1 are added and the fluid is carefully shaken again, foaming needs to be avoided!

Washpuffer: The content is transferred into a 1 l cylinder and filled up to 750 ml with distilled water, the fluid is then shook thoroughly.

Establishment of calibration pattern (in PP-Tubes)
Stokssolution (SL): 200 ng/ml

<table>
<thead>
<tr>
<th>St.</th>
<th>SL (μl)</th>
<th>AP-2 (μl)</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>500</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>500</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>500</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>500</td>
<td>12.5</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>500</td>
<td>6.25</td>
</tr>
</tbody>
</table>

St. 1-5 are used for the ELISA, they are supposed to be produced maximally one hour prior to assay!

Plate pipetting (Doublesamples)

<table>
<thead>
<tr>
<th>Blank</th>
<th>100 μl AP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>100 μl</td>
</tr>
<tr>
<td>Sample</td>
<td>100 μl</td>
</tr>
</tbody>
</table>

The plate is covered and incubated for two hours at 20-25°C. The plate is emptied, rinsed 4 times and beaten onto a tissue.

100 μl antiserum are pipetted into each well.

The plate is covered and incubated for two hours at 20-25°C. The plate is emptied, rinsed 4 times and beaten onto a tissue.

100 μl POD are pipetted into each well.

The plate is covered and incubated for one hour at 20-25°C. The plate is emptied, rinsed 4 times and beaten onto a tissue.

100 μl Substrate (TMB) are pipetted into each well.

The plate is covered and incubated for 30 min at Room temperature (RT, 15-30°C) preferably on a shaker.

The reaction is stopped with 100 μl (1M) sulphuric acid.

The measurement is performed at 450 nm OD.
6.2.5.2. Matrix Metalloproteinase-3 (MMP-3), Human, Biotrak ELISA System:

Establishment of pipette pattern (Blank, Standard, samples)

Reagent production (all reagents need to be at temperature of 20-27°C, except Conjugates. Conjugates are reproduced within 15 minutes and stored in the fridge. Assay Puffers are thawed over night. Only distilled or deionised water is used for the puffers).

Assaypuffer (AP): The content of the bottle is transferred into a 100 ml cylinder (the cylinder needs to be rinsed several times with distilled water) and diluted with 100 ml of distilled water then it is shaken thoroughly.

Standard: 1 ml AP is added and the fluid carefully mixed until the content is dissolved completely, energetic movements and foaming need to be avoided!

Peroxidase Conjugat: 12 ml AP-1 are added and the fluid carefully shaken again, foaming needs to be avoided!

Washpuffer: The content is transferred into a 500 ml Cylinder (the cylinder needs to be rinsed several times with distilled water) and filled up to 500 ml with distilled water the fluid is then shaken thoroughly.

Reproduced Assay- and Washpuffer are stored at 2-8°C, Standard and Conjugat at 15 to 30°C

Establishment of calibration pattern (in PP-tubes)
Stoksolution (SL): 240 ng/ml

<table>
<thead>
<tr>
<th></th>
<th>500 µl SL</th>
<th>+</th>
<th>500 µl AP</th>
<th>→</th>
<th>120 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. 6:</td>
<td>500 µl St. 6</td>
<td>500 µl AP</td>
<td>→</td>
<td>60 ng/ml</td>
<td></td>
</tr>
<tr>
<td>St. 5:</td>
<td>500 µl St. 5</td>
<td>500 µl AP</td>
<td>→</td>
<td>30 ng/ml</td>
<td></td>
</tr>
<tr>
<td>St. 4:</td>
<td>500 µl St. 4</td>
<td>500 µl AP</td>
<td>→</td>
<td>15 ng/ml</td>
<td></td>
</tr>
<tr>
<td>St. 2:</td>
<td>500 µl St. 3</td>
<td>500 µl AP</td>
<td>→</td>
<td>7.5 ng/ml</td>
<td></td>
</tr>
<tr>
<td>St. 1:</td>
<td>500 µl St. 2</td>
<td>500 µl AP</td>
<td>→</td>
<td>3.75 ng/ml</td>
<td></td>
</tr>
</tbody>
</table>

St. 1-6 are used for the ELISA, they are supposed to be produced maximally one hour prior to Assay!

Plate pipetting (Doublesamples)

<table>
<thead>
<tr>
<th></th>
<th>100 µl AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>100 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

The plate is covered and incubated for one hour at 2-8°C. The plate is emptied, rinsed 4 times and beaten onto a tissue.
100 µl cold POD are pipetted into each well.

The plate is covered and incubated for one hour at 2-8°C. The plate is emptied, rinsed 4 times and beaten onto a tissue.

100 µl TMP-Substrate (RT!) are pipetted into each well.

The plate is covered and incubated for 30 min at RT (20-27°C).

The reaction is stopped with 100 µl (1M) sulphuric acid.

The measurement is performed at 450 nm OD (within 30 min).

6.2.6. Total Protein (TP) content

TP content was determined refractometrically.

6.3. Statistical analysis

The Spearman correlation analysis was performed to compare subjectively evaluated data such as chondral damage scores with subjectively evaluated data or with measured results.

The Pearson correlation analysis was performed to compare measured results such as individual synovial fluid markers with measured results.

A variance components model was used to analyse the data in order to obtain the variance between horses and within horses (interindividual and intraindividual variance). In a next step we looked at the correlation within each foot of each horse. Therefore a 2-level variance components model was performed.
7.0. Results

7.1. Cartilage analysis

7.1.1. Native lesion scoring following JOUGLIN et al. (2000)

First the cartilage surfaces were assessed following the semi-quantitative macroscopic scoring technique developed by JOUGLIN et al. (2000):

<table>
<thead>
<tr>
<th>Score</th>
<th>Score 0</th>
<th>Score 0.5</th>
<th>Score 1</th>
<th>Score 1.5</th>
<th>Score 2</th>
<th>Score 2.5</th>
<th>Score 3</th>
<th>Score 3.5</th>
<th>Score 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>32</td>
<td>10</td>
<td>27</td>
<td>11</td>
<td>31</td>
<td>12</td>
<td>28</td>
<td>1</td>
<td>26</td>
</tr>
</tbody>
</table>

To be able to compare if there was a general difference regarding the degree of cartilage damage between fetlock and coffin joints the values for the different joint surfaces were evaluated separately:

<table>
<thead>
<tr>
<th>Score</th>
<th>Score 0</th>
<th>Score 0.5</th>
<th>Score 1</th>
<th>Score 1.5</th>
<th>Score 2</th>
<th>Score 2.5</th>
<th>Score 3</th>
<th>Score 3.5</th>
<th>Score 4</th>
<th>Score 4.5</th>
<th>Score 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>14</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>9</td>
<td>4</td>
<td>11</td>
<td>0</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td>13</td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CB: Distal surface of the cannon bone:
P1: Proximal surface of P1
P2: Distal surface of P2
P3: Proximal surface of P3 plus distal sesamoid bone

7.1.2. Lesion scoring after Indian ink staining following CHANTLEY et al. (1999)

Slight cartilage fibrillation can not be detected macroscopically without Indian ink staining of the cartilage surface. In order to assess the cartilage damage as accurately as possible it was additionally evaluated following a lesion score developed by CHANTLEY et al. (1999):

<table>
<thead>
<tr>
<th>Score</th>
<th>Score 0</th>
<th>Score 0.5</th>
<th>Score 1</th>
<th>Score 1.5</th>
<th>Score 2</th>
<th>Score 2.5</th>
<th>Score 3</th>
<th>Score 3.5</th>
<th>Score 4</th>
<th>Score 4.5</th>
<th>Score 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>18</td>
<td>9</td>
<td>19</td>
<td>4</td>
<td>27</td>
<td>4</td>
<td>35</td>
<td>12</td>
<td>40</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>
For the comparison of the cartilage damage in different joints the values investigated for different joint surfaces were presented separately again:

<table>
<thead>
<tr>
<th>Score 0</th>
<th>Score 0.5</th>
<th>Score 1</th>
<th>Score 1.5</th>
<th>Score 2</th>
<th>Score 2.5</th>
<th>Score 3</th>
<th>Score 3.5</th>
<th>Score 4</th>
<th>Score 4.5</th>
<th>Score 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>4 3 8 1 5</td>
<td>2 11</td>
<td>6 19</td>
<td>0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>7 3 1 0 12</td>
<td>1 12</td>
<td>3 13</td>
<td>1 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>4 1 5 2 7</td>
<td>2 5</td>
<td>1 2</td>
<td>0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>3 2 5 1 3</td>
<td>0 7</td>
<td>2 6</td>
<td>0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CB: Distal surface of the cannon bone  
P1: Proximal surface of P1  
P2: Distal surface of P2  
P3: Proximal surface of P3 plus distal sesamoid bone

7.1.3. Computer assisted Grey level analysis

7.1.3.1. Cartilage degeneration index:

CDI index developed by BROMMER et al. (2003b) served as a quantitative measure for the assessment of the severity of cartilage degeneration.

CDI values for the proximal surface of P3 and the distal sesamoid bone ranged between 23.1 and 86.3.  
CDI values for the distal surface of P2 ranged between 30.1 and 65.2.  
CDI values for the proximal surface of P1 ranged between 20.7 and 80.8.  
CDI values for the distal surface of the cannon bone ranged between 20.2 and 52.

7.1.3.2. Colour degeneration index:  
The colour degeneration index was developed to give an estimation of the ratio of the mean grey value of healthy cartilage areas and the mean grey value of damaged areas.

CODI values for the proximal surface of P3 and the distal sesamoid bone ranged between 23.62 and 48.01.  
CODI values for the distal surface of P2 ranged between 21.73 and 33.39.  
CODI values for the proximal surface of P1 ranged between 11.85 and 30.56.  
CODI values for the distal surface of the cannon bone ranged between 16.41 and 33.45.
7.2. Synovia sample analysis

To rule out severe acute trauma or septic arthritis the synovia was first macroscopically assessed for normal amount, colour, transparency, viscosity and consistency as well as presence of foreign particles or if it showed signs of coagulation. No evidence indicating severe septic arthritis or acute trauma was found.

7.2.1. Evaluation of rheologic behaviour

\[ \eta_0 (Pa*s): \text{ Ranged between 0.0131 and 10.775 Pa*s.} \]

The viscosity curves (shear rate/viscosity) showed a large variety of \( \eta_0 \) indicating that the viscosity of the synovial fluid in joints under rest and at low speed varies dramatically.

\[ \text{Inflection Point (s'}): \text{ Ranged between 0.2 and 6.5 s'.} \]

With increasing speed the viscosity decreases due to the disruption of the entanglement network and orientation of the macromolecules in flow direction. This phenomenon occurs at different shear rates depending on \( \eta_0 \) (the smaller \( \eta_0 \) the less entanglements exist and the later occurs the onset of synovial fluid viscosity decrease).

\[ \text{Inclination: Ranged between 0.053918 and 0.83899.} \]

The higher the number of entanglements (therefore the higher \( \eta_0 \)) the more entanglements are disrupted under shear and the steeper is the inclination at medium shear rates.
Fig. 23: Viscosity curves


(Explanation of sample names:
1FG1  Horse 1 left front fetlock joint
2FG2  Horse 2 right front fetlock joint
1HG3  Horse 1 left hind coffin joint
2HG4  Horse 2 right hind coffin joint)

Viscoelasticity curves depicting G' and G'' (the first the storage modulus and the latter the loss modulus) describe the materials deformation behaviour in the form of an elastic and viscous part. The elasticity represents the stored energy as reversible extension of the polymere network whereas the viscosity represents the irreversibly dissipated energy in order to gain fluid displacement (flow). At low frequencies one observes that the viscosity is the dominant property. With increasing frequency elasticity develops and finally becomes dominant.

Point of intersection: Ranged between 0.045768 and 7.9367 1/s.
The intersection point is the characteristic frequency at which elasticity becomes starts to dominate over the viscosity, if an entanglement network is present.
$G_0$: Ranged between 0.0717 and 23.5 Pa. 

$G_0$, the almost constant $G'$ value at the highest frequencies, represents the behaviour of a viscoelastic body with mainly solid material's properties (elasticity) and less effective fluid properties ($G''$).

The viscoelastic properties might represent the synovial fluid's quality.
Tab. 5: Synovial fluid viscosity data

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\eta_0$ (Pa*s)</th>
<th>Inflection point</th>
<th>Inclination</th>
<th>Point of intersection (1/s)</th>
<th>$G_0$ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1HG2</td>
<td>1.5736</td>
<td>0.4</td>
<td>0.32898</td>
<td>0.1687</td>
<td>1.41</td>
</tr>
<tr>
<td>1HG1</td>
<td>1.2558</td>
<td>0.7</td>
<td>0.35534</td>
<td>0.32623</td>
<td>2.35</td>
</tr>
<tr>
<td>1FG1</td>
<td>0.1149</td>
<td>1.2</td>
<td>0.52602</td>
<td>0.51811</td>
<td>3</td>
</tr>
<tr>
<td>2FG2</td>
<td>0.0997</td>
<td>1.1</td>
<td>0.55181</td>
<td>0.32898</td>
<td>1.53</td>
</tr>
<tr>
<td>2FG1</td>
<td>0.3327</td>
<td>1</td>
<td>0.46758</td>
<td>0.32623</td>
<td>1.56</td>
</tr>
<tr>
<td>2HG2</td>
<td>0.0809</td>
<td>1.4</td>
<td>0.58296</td>
<td>0.51811</td>
<td>0.451</td>
</tr>
<tr>
<td>2HG1</td>
<td>0.4355</td>
<td>0.6</td>
<td>0.44613</td>
<td>0.4263</td>
<td>1.1</td>
</tr>
<tr>
<td>3FG1</td>
<td>0.0778</td>
<td>2.1</td>
<td>0.59573</td>
<td>0.704</td>
<td>0.704</td>
</tr>
<tr>
<td>3FG2</td>
<td>0.4544</td>
<td>0.55</td>
<td>0.43907</td>
<td>0.56617</td>
<td>1.18</td>
</tr>
<tr>
<td>3HG1</td>
<td>0.0362</td>
<td>1.95</td>
<td>0.69955</td>
<td>0.196</td>
<td>0.196</td>
</tr>
<tr>
<td>4HG4</td>
<td>1.0378</td>
<td>1.1</td>
<td>0.38656</td>
<td>4.45</td>
<td>2.6229</td>
</tr>
<tr>
<td>4FG1</td>
<td>3.0331</td>
<td>0.4</td>
<td>0.30404</td>
<td>5.2</td>
<td>0.29098</td>
</tr>
<tr>
<td>4FG4</td>
<td>5.275</td>
<td>0.4</td>
<td>0.27049</td>
<td>8.02</td>
<td>0.21166</td>
</tr>
<tr>
<td>4FG3</td>
<td>8.6589</td>
<td>0.9</td>
<td>0.24082</td>
<td>10.9</td>
<td>0.11575</td>
</tr>
<tr>
<td>4HG3</td>
<td>0.7451</td>
<td>1.1</td>
<td>0.41787</td>
<td>6</td>
<td>3.9452</td>
</tr>
<tr>
<td>4FG2</td>
<td>3.6237</td>
<td>0.25</td>
<td>0.30067</td>
<td>6.3</td>
<td>0.30313</td>
</tr>
<tr>
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<td>6.1753</td>
<td>0.35</td>
<td>0.22167</td>
<td>8.12</td>
<td>0.24951</td>
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7.2.2. Hyaluronic acid quantity

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<tr>
<td>11HG1</td>
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</table>
7.2.3. Hyaluronic acid molecular weight distribution

Explanation of the data in order to enable comprehension of the following results:

The detected HA-molecules were separated into 6 fractions depending on excluded volumes, whereas 1 comprised the smallest and 6 the largest excluded volumes. The percentage rate of each fraction, in relation to the overall amount of molecules and the minimal and maximal weight (in lg g/mol) of each fraction were determined as well.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Minimum Weight (lg g/mol)</th>
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<tr>
<td>16FG1</td>
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</tr>
</tbody>
</table>

Polydispersity is a value describing the degree of molecular weight variation in one sample.

The mean molecular weight of the overall smallest and largest 10% fraction was determined. Further the minimal and maximal molecular weight of the in total smallest and largest 10% was assessed. Each of these values was reported in logarithmic and non logarithmic version.

The mean HA-molecular weight (mass fraction Mw), number fraction (Mn) and centrifugation fraction (Mz) for each sample were calculated and again reported in logarithmic (lg) and non logarithmic version.
Mass fraction molecular weight distribution explained on basis of sample 4FG1:
The smallest fraction (1) of sample 4FG1 comprised 8.62% of the total mass of molecules in this sample.

Fraction 2 of sample 4FG1 comprised 9.5% of the total mass of molecules in this sample.

Fraction 3 of sample 4FG1 comprised 54.57% of the total mass of molecules in this sample.

Fraction 4 of sample 4FG1 comprised 20.85% of the total mass of molecules in this sample.

Fraction 5 of sample 4FG1 comprised 6.43% of the total mass of molecules in this sample.

The largest fraction (6) of sample 4FG1 comprised 0% of the total mass of molecules in this sample.

The polydispersity of sample 4FG1 was 9.15 (Mw/Mn) and 5.13 (Mz/Mw).

The mean molecular weight of the overall smallest 10% fraction was 2.76 for the lg version and 580 for the non lg version.
Further the minimal molecular weight of the in total smallest 10% was assessed to be 2,58 for the lg and 380 for the non lg version.

The maximal molecular weight of the in total smallest 10% was assessed to be 3.23 for the lg and 1700 for the non lg version.

The mean molecular weight of the overall largest 10% fraction was 5.35 for the lg version and 228990 for the non lg version.
Further the minimal molecular weight of the in total largest 10% was assessed to be 4.54 for the lg and 35420 for the non lg version.

The maximal molecular weight of the in total largest 10% was assessed to be 5.62 for the lg and 419410 for the non lg version.

Number average - mean Molecular weight Mn (lg): 3.55
Mean Molecular weight amount of molecules: 3600

Weight average mean molecular weight (lg): 4.51
Mean Molecular weight Mw: 32940
Z-average mean molecular weight (lg): 5.22
Mean Centrifuge average: 169150

Number fraction molecular weight distribution explained on basis of sample 4FG1:

The smallest fraction (1) of sample 4FG1 comprised 0% of the overall number of molecules in this sample.

Fraction 2 of sample 4FG1 comprised 40.35% of the overall number of molecules in this sample.

Fraction 3 of sample 4FG1 comprised 51.16% of the overall number of molecules in this sample.

Fraction 4 of sample 4FG1 comprised 8.48% of the overall number of molecules in this sample.

Fraction 5 of sample 4FG1 comprised 0% of the overall number of molecules in this sample.

The largest fraction (6) of sample 4FG1 comprised 0% of the overall number of molecules in this sample.

The mean molecular weight of the overall smallest 10% fraction was 3.18 for the lg version and 1520 for the non lg version.

Further the minimal molecular weight of the in total smallest 10% was assessed to be 3.01 for the lg and 1030 for the non lg version.

The maximal molecular weight of the in total smallest 10% was assessed to be 3.25 for the lg and 1780 for the non lg version.

The mean molecular weight of the overall largest 10% fraction was 4.40 for the lg version and 25530 for the non lg version.

Further the minimal molecular weight of the in total largest 10% was assessed to be 4.28 for the lg and 19120 for the non lg version.

The maximal molecular weight of the in total largest 10% was assessed to be 4.71 for the lg and 52080 for the non lg version.
Tab. 7: Comparison of weight fraction molecular weight

<table>
<thead>
<tr>
<th>Sample</th>
<th>4FG1</th>
<th>9FG2</th>
<th>2HG2</th>
<th>5FG4</th>
<th>7FG3</th>
<th>14FG3</th>
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<td>27.82%</td>
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<td>6.12%</td>
<td>12.19%</td>
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<td>470</td>
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Tab. 8: Comparison of number fraction molecular weight

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<tr>
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<th>2HG2</th>
<th>5FG4</th>
<th>7FG3</th>
<th>14FG3</th>
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<td>26.57%</td>
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<td>0.67%</td>
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<td>MG (largest 10%)</td>
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<td>11760</td>
<td>13370</td>
<td>9850</td>
<td>13190</td>
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</table>

F1: Fraction 1  
F2: Fraction 2  
F3: Fraction 3  
F4: Fraction 4  
F5: Fraction 5  
F6: Fraction 6  
PD: Polydispersity  
MG: Molecular weight  
Mn: Number average molecular weight  
Mw: Weight average molecular weight  
Mz: Z-average molecular weight (Centrifuge average)
Results:

**Sample 4FG1:**

Mass fraction curve of sample 4FG1:

The molecular weight of the HA molecules of sample 4FG1 was distributed between lg 2.6 g/mol (398.12 g/mol) and lg 5.5 g/mol (316227.77 g/mol).

The curve showed 3 peaks.

The first peak had a maximum at lg 2.7 g/mol (501.19 g/mol).

The second peak was the most prominent one with a maximum at lg 4.3 g/mol (19952.62 g/mol).

The third peak had a maximum at lg 5.4 g/mol (251188.64 g/mol).

![Fig. 25: Sample 4FG1, mass fraction molecular weight distribution](image)

Number fraction curve of sample 4FG1:

A measurable number of molecules was found at a molecular weight between lg 3 g/mol (1000 g/mol) and lg 4.7 g/mol (50118.72 g/mol).

However the highest number of molecules had a weight of lg 3.3 g/mol- 1995.26 g/mol (first peak) and even more had a weight of lg 4.2 g/mol- 15848.93 g/mol (second peak).
Sample 9FG2:

Mass fraction curve of sample 9FG2:

The molecular weight of the HA molecules of sample 9FG2 was distributed between $\lg 3 \text{ g/mol}$ (1000 g/mol) and $\lg 5.7 \text{ g/mol}$ (501187.23 g/mol). The sample showed 1 peak with a maximum at $\lg 4.3 \text{ g/mol}$ (19952.62 g/mol) and a long plateau between $\lg 4.7 \text{ g/mol}$ (50118.72 g/mol) and $\lg 5.6 \text{ g/mol}$ (398107.17 g/mol).
Number fraction curve of sample 9FG2:

A measurable number of molecules was found at a molecular weight between $\lg 3 \text{ g/mol}$ (1000 g/mol) and $\lg 4.9 \text{ g/mol}$ (79432.82 g/mol). However the highest number of molecules had a weight of $\lg 3.5 \text{ g/mol} = 3162.28 \text{ g/mol}$ (first peak) and even more had a weight of $\lg 4.3 \text{ g/mol} = 19952.62 \text{ g/mol}$ (second peak).
Sample 2HG2:

Mass fraction curve of sample 2HG2:

The molecular weight of the HA molecules of sample 2HG2 was distributed between \( \lg 2.7 \) g/mol (501.19 g/mol) and \( \lg 5.7 \) g/mol (501187.23 g/mol). The sample showed 4 peaks. The first peak had a maximum at \( \lg 2.6 \) g/mol (398.11 g/mol). The second peak had a maximum between \( \lg 3.3 \) (1995.26 g/mol) and \( \lg 3.6 \) g/mol (3981.07 g/mol). The third peak was the most prominent one with a maximum at \( \lg 4.2 \) g/mol (15848.93 g/mol). The fourth peak was a plateau between \( 4.9 \lg \) g/mol (79432.82 g/mol) and \( \lg 5.6 \) g/mol (398107.17 g/mol).

![mass_fraction](image)

Fig. 29: Sample 2HG2, mass fraction molecular weight distribution

Number fraction curve of sample 2HG2:

A measurable number of molecules was found at a molecular weight between \( \lg 2.8 \) g/mol (630.96 g/mol) and \( \lg 4.8 \) g/mol (63095.73 g/mol). However the highest number of molecules had a weight of \( \lg 2.8 \) g/mol = 630.96 g/mol (peak). After that it asymptotically approached \( \lg 0 \) g/mol (0 g/mol).
Sample 5FG4:

Mass fraction curve of sample 5FG4:

The molecular weight of the HA molecules of sample 5HG4 was distributed between $\log 2.6$ g/mol (398.11 g/mol) and $\log 5.7$ g/mol (501187.23 g/mol).
The sample showed 4 peaks. The first peak was the most prominent one with a maximum at $\log 2.5$ g/mol (316.23 g/mol). The second peak had a maximum at $\log 3.2$ g/mol (1584.89 g/mol). The third peak had a maximum at $\log 4.2$ g/mol (15848.93 g/mol). The small fourth peak had a maximum at $\log 5.5$ g/mol (316227.77 g/mol).
Number fraction curve of sample 5FG4:

A measurable number of molecules was found at a molecular weight between $\lg 2.7 \text{ g/mol}$ (501.19 g/mol) and $\lg 4.8 \text{ g/mol}$ (63095.73 g/mol). However the highest number of molecules had a weight of $\lg 2.7 \text{ g/mol} - 501.19 \text{ g/mol}$ (peak). After that it asymptotically approached $\lg 0 \text{ g/mol}$ (0 g/mol).
Sample 7FG3:

Mass fraction curve of sample 7FG3:

The molecular weight of the HA molecules of sample 7FG3 was distributed between $\lg 2.6 \text{ g/mol}$ (398.11 g/mol) and $\lg 5.6 \text{ g/mol}$ (398107.17 g/mol).

The sample showed 4 peaks.
The first peak had a maximum at $\lg 2.5 \text{ g/mol}$ (316.23 g/mol).
The second peak was the most prominent one with a maximum at $\lg 4.2 \text{ g/mol}$ (15848.93 g/mol).
The very small third peak had a maximum at $\lg 5 \text{ g/mol}$ (100000 g/mol).
The fourth peak was slightly bigger and had a maximum at $\lg 5.5 \text{ g/mol}$ (316227.77 g/mol).

![mass_fraction](image)

Fig. 33: Sample 7FG3, mass fraction molecular weight distribution

Number fraction curve of sample 7FG3:

A measurable number of molecules was found at a molecular weight between $\lg 2.7 \text{ g/mol}$ (501.19 g/mol) and $\lg 4.7 \text{ g/mol}$ (50118.72 g/mol).
However the highest number of molecules had a weight of $\lg 2.7 \text{ g/mol} - 501.19 \text{ g/mol}$ (peak).
After that it almost asymptotically approached $\lg 0 \text{ g/mol}$ (0 g/mol).
Sample 14FG3:

Mass fraction curve of sample 14FG3:

The molecular weight of the HA molecules of sample 14FG3 was distributed between $\lg 2.5$ g/mol ($316.23$ g/mol) and $\lg 5.7$ g/mol ($501187.23$ g/mol).

The sample showed 5 peaks.
The first peak was the most prominent one with a maximum at $\lg 2.5$ g/mol ($316.23$ g/mol).
The second peak had a maximum between $\lg 2.8$ g/mol ($630.96$ g/mol) and $\lg 3.2$ g/mol ($1584.89$ g/mol).
The third peak had a maximum at $\lg 4.2$ g/mol ($15848.93$ g/mol).
The very small fourth peak had a maximum between $\lg 4.8$ g/mol ($63095.73$ g/mol) and $\lg 5$ g/mol ($100000$ g/mol).
The even smaller fifth peak had a maximum between $\lg 5.2$ g/mol ($158489.32$ g/mol) and $\lg 5.5$ g/mol ($316227.77$ g/mol).
Number fraction curve of sample 14FG3:

A measurable number of molecules was found at a molecular weight between $\log 2.7$ g/mol (501.19 g/mol) and $\log 4.7$ g/mol (50118.72 g/mol). However the highest number of molecules had a weight of $\log 2.7$ g/mol $- 501.19$ g/mol (peak). After that it almost asymptotically approached $\log 0$ g/mol (0 g/mol).

Fig. 35: Sample 14FG3, mass fraction molecular weight distribution

Fig. 36: Sample 14FG3, number fraction molecular weight distribution
Sample 17HG3:

Mass fraction curve of sample 17HG3:

The molecular weight of the HA molecules of sample 17HG3 was distributed between $lg \ 2.5 \ g/mol \ (316.23 \ g/mol)$ and $lg \ 5.8 \ g/mol \ (630957.34 \ g/mol)$.

The sample showed 3 peaks.
The first peak was the most prominent one with a maximum at $lg \ 2.5 \ g/mol \ (316.23 \ g/mol)$.
The second peak had a maximum between $lg \ 3.4 \ g /mol \ (2511.89 \ g/mol)$ and $lg \ 3.6 \ g/mol \ (3981.07 \ g/mol)$.
The third peak had a maximum at $lg \ 4.2 \ g/mol \ (15848.93 \ g/mol)$.
The very small fourth peak had a maximum between $lg \ 5 \ g/mol \ (100000 \ g/mol)$ and $lg \ 5.6 \ g/mol \ (398107.17g/mol)$.

A measurable number of molecules was found at a molecular weight between $lg \ 2.7 \ g/mol \ (501.19 \ g/mol)$ and $lg \ 5.95 \ g/mol \ (891250.94 \ g/mol)$.
However the highest number of molecules had a weight of $lg \ 2.7 \ g/mol - 501.19 \ g/mol$ (peak).
After that it asymptotically approached $lg \ 0 \ g/mol \ (0 \ g/mol)$.
Sample 18FG4:

Mass fraction curve of sample 18FG4:

The molecular weight of the HA molecules of sample 18FG4 was distributed between $\lg 2.5$ g/mol (316.23 g/mol) and $\lg 5.8$ g/mol (630957.34 g/mol).

The sample showed 4 peaks.
The first peak was the most prominent one with a maximum at $\lg 2.5$ g/mol (316.23 g/mol). The second peak had a maximum between $\lg 3$ g/mol (1000 g/mol) and $\lg 3.3$ g/mol (1995.26 g/mol). The third peak had a maximum at $\lg 4.3$ g/mol (19952.62 g/mol). The very small fourth peak had a maximum at $\lg 5.5$ g/mol (316227.77 g/mol).
Number fraction curve of sample 18FG4:

A measurable number of molecules was found at a molecular weight between \( \lg 2.6 \text{ g/mol} \) (398.11 g/mol) and \( \lg 4.7 \text{ g/mol} \) (50118.72 g/mol). However the highest number of molecules had a weight of \( \lg 2.6 \text{ g/mol} \) - 398.11 g/mol (peak). After that it asymptotically approached \( \lg 0 \text{ g/mol} \) (0 g/mol).
Comparison of weight fraction molecular weight:

F1 % (lg 0-3 g/mol; 1-1000 g/mol) ranged between 0.34% and 25.98%
F2 % (lg 3-3.699 g/mol; 1000-5000 g/mol) ranged between 9.5% and 40.24%
F3% (lg 3.699-4.301 g/mol; 5000-20000 g/mol) ranged between 25.73% and 54.57%
F4% (lg 4.301-5.301 g/mol; 20000-200000 g/mol) ranged between 6.12% and 38.0%
F5 % (lg 5.301-6 g/mol; 200000-1000000 g/mol) ranged between 0.78% and 6.43%
F6 % (lg 6-9 g/mol; 1000000-1000000000 g/mol) 0%

PD
(Mw/Mn) 4.39-10.43

PD
(Mz/Mw) 4.04-12.79

MG
(smallest 10%) 450 -1960 g/mol

MG
(largest 10%) 45990-228990 g/mol

Mn 1480-8310 g/mol

Mw 8000- 36520 g/mol

Mz 71080- 169150 g/mol

Comparison of number fraction molecular weight

F1 % (lg 0-3 g/mol; 1-1000 g/mol) ranged between 0% and 67.14%
F2 % (lg 3-3.699 g/mol; 1000-5000g/mol) ranged between 26.57% and 40.35%
F3 % (lg 3.699-4,301 g/mol; 5000-20000 g/mol) ranged between 4.64% and 51.16%
F4 % (lg 4.301-5.301 g/mol; 20000-200000 g/mol) ranged between 0.21% and 15.27%
F5 % (lg 5.301-6 g/mol; 200000-1000000 g/mol) 0%
F6 % (lg 6-9 g/mol; 1000000-1000000000 g/mol) 0%
MG
(smallest 10%) 400-1520 g/mol

MG
(largest 10%) 9850-30420 g/mol

7.2.4. Matrix Metalloproteinases (MMP1 and MMP3)

MMP1 concentration ranged between 9.82 ng/ml and 12.42 ng/ml

MMP3 concentration ranged between 1.75 ng/ml and 2.19 ng/ml

<table>
<thead>
<tr>
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### 7.2.5. Total Protein content

TP content was determined refractometrically but unfortunately the results were not interpretable due to cryo-praecipitation caused by accidental storage of the samples in the freezer. For the same reason white blood cell count and cytology were also not analyzable.

### 7.3. Statistical analysis

#### 7.3.1. Correlation

The Spearman correlation analysis was performed to compare subjectively evaluated data with subjectively evaluated data or with measured results.

The Pearson correlation analysis was performed to compare measured results with measured results.
7.3.1.1. Correlation coefficients:

Overall correlation overview
Pearson:

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Proximal surface of P1 and distal surface of the cannon bone

Pearson:

Tab. 12: Proximal surface of P1 and distal surface of the cannon bone/ Pearson

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Spearman:

Tab. 13: Proximal surface of P1 and distal surface of the cannon bone/ Spearman

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Proximal surface of P3 plus distal sesamoid bone and distal surface of P2

Pearson:

**Tab. 18:** Proximal surface of P3 plus distal sesamoid bone and distal surface of P2/ Pearson

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**Tab. 19:** Proximal surface of P3 plus distal sesamoid bone and distal surface of P2/ Spearman

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Proximal surface of P3 plus distal sesamoid bone

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Distal surface of P2

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Jou: Cartilage degeneration by JOUGLIN et al. (1999)
Chan: Cartilage degeneration by CHANTLEY et al. (2000)
CDI: Cartilage degeneration Index
CODI: Colour degeneration Index
7.3.1.2. Significant correlation:

**Fig. 41:** $\eta_0$ – Inclination/correlation $-0.69$

**Fig. 42:** $\eta_0$ – $G_0$/ correlation $0.90$

**Fig. 43:** Inflection point
- Inclination/correlation $0.65$

**Fig. 44:** Point of intersection
- Inclination/correlation $0.70$
7.3.2 Data variance

The interindividual (between different individuals) and intraindividual (within the same individual) data variance of the correlation of the cartilage degeneration degree evaluated by CHANTLEY et al. (2000) and JOUGLIN et al. (1999) with different synovial fluid markers was analysed.

A variance components model was used to analyse the data in order to obtain the variance between horses and within horses (interindividual and intraindividual variance). Using the CHANTLEY score the variance between the horses was 1.587 and the variance within the
horses was 0.619. This results in an intraclass correlation of 0.72, which indicates that the measurements within the animals are correlated. Intraclass correlation is defined as the variance between subjects divided by the overall variance. The overall variance in turn is defined as the sum of the variances between and within animals. Using the JOUGLIN score the interindividual variance was 1.243 and the intraindividual variance was 0.621. The intraclass correlation for these results was 0.67.

In a next step we looked at the correlation within each foot of each horse. Therefore a 2-level variance components model was performed, where the feet of a horse were nested within each horse. The variance components of this model were 0.178 for a foot within a horse, 0.580 for the variance within horses, and 1.448 for the variance between horses. Thus, the correlation between the feet within an animal was \((0.58 + 1.448)/(0.178 + 0.58 + 1.448) = 0.92\). and the intraindividual correlation was \((1.448)/(0.178 + 0.58 + 1.448) = 0.66\). Using the JOUGLIN score the variance components were 0.103 for a foot within a horse, 0.6 for the variance within horses, and 1.163 for the variance between horses. Thus, the correlation between the feet within an animal was 0.94 and the intraindividual correlation was 0.62.

Both approaches showed a strong correlation within the feet and within animals.
8.0. Discussion

The standard clinical and radiographic methods used to assess OA are only able to provide crude and insensitive measures of pain and functional impairment going along with OA and of the degenerative changes that are severe enough to be appreciated on radiographs (SHARIF et al. 1995). Radiography and even magnetic resonance imaging are not sufficient for obtaining an early diagnosis (KATO et al. 2005). Therefore one can easily conclude that OA is usually at an advanced stage when it is detectable upon clinical and radiographic examination (ISRAEL et al. 1991) and that early diagnosis of OA is a major problem in veterinary medicine (JOUGLIN et al. 2000).

A possible solution for early enough diagnosis in order to be able to provide effective treatment could be the evaluation of SF biomarkers. Diagnostically valuable biomarkers are molecules that indicate an alteration in physiology and specifically and sensitively reflect a disease stage, providing information about the metabolic status of cartilage, synovium or other joint tissues (KATO et al. 2005).

However no biochemical marker has so far gained unrestricted acceptance for the diagnosis and monitoring of OA in clinical routine. Although there a wide variety of biochemical markers is acknowledged little is known about the value of these markers and hence no truly OA specific molecule or marker has yet been identified (SCHMIDT-ROHLFING et al. 2002). In order to find a distinct biomarker that would correlate with the degree of cartilage degeneration and allow an early OA diagnosis we carried out an extensive study using new and very accurate devices.

Within the scope of the study 60 legs from 17 polish draught-horses (16 mares and one gelding) were used for synovial fluid and cartilage sample collection. The ages of the horses included in the study ranged from three to twelve years (mean age 7.12 years). Synovial fluid samples were drawn through puncture of the dorsal pouch of the distal interphalangeal joints (of all four limbs), the palmar and respectively the plantar pouch of the metacarpo- and metatarsophalangeal joints, and the dorsal pouch of the intercarpal joints (for calibration purposes).

Previous research has indicated that exsanguination has no significant effect on viscoelastic properties if samples are collected within 3 hours after slaughter (LUMSDEN et al 1996). The synovia samples were stored first at -196°C in liquid Nitrogen and then at -80°C until further processing.

The influence of storage at -80°C and centrifugation on apparent viscosity and total protein content was determined before by LUMSDEN et al. (1996) for synovial fluid collected of a single horse without clinical signs of joint pain or lameness, gross enlargement of the joint and with normal range of motion. Storage at -80°C for up to 14 days had, according to Lumseden et al. (1996), no significant effect on apparent viscosity. The structural state of hyaluronate is essential for the rheologic characteristics of synovial fluid. A lack of significant changes in apparent viscosity after cold storage therefore allows the conclusion that the hyaluronate molecule is preserved under these conditions. For that reason synovial apparent viscosity measurement may be performed relatively unhesitatingly on previously frozen samples (LUMSDEN et al. 1996). According to REJNÖ (1976) storage at room temperature
for less than 1h may result in changes in the viscosity of synovial fluid whereas storage in a
frezer even for some days does not seem to influence viscosity. Centrifugation of the
synovial fluid prior to analysis had no significant effect on apparent viscosity either.
LUMSDEN et al. (1996) even consider it advisable to centrifuge samples prior to viscosity
measurement due to possible content of cellular and cartilage material which could influence
measurements especially in horses with joint disease.
The synovial fluid samples filled into sterile EDTA tubes without centrifugation for total
protein analysis, cytology and white blood cell count were accidentally stored in a freezer.
Therefore the originally planned conventional synovia analyses had to be left out.

Macroscopic semi-quantitative scoring of the cartilage surfaces revealed that the fetlock joints
generally showed more severe degeneration than the coffin joints, which is in accordance to
the well known fact that the fetlock joint is the joint generally exposed to the most strain
(BROMMER et al. 2003a; BROMMER et al. 2003b; CHANTLEY et al. 1999; JOUGLIN et
al. 2000). Following the scoring system by JOUGLIN et al. (2000), 26 specimens presented a
lesion score 4: three coffin joint specimens (2 proximal surfaces of P3-the distal phalanx- and
1 distal surface of P2- the intermediate phalanx) and 23 fetlock joint specimens (15 proximal
surfaces of P1- the proximal phalanx- and 8 distal surfaces of the cannon bone).
Of the 28 specimens presenting lesion score 3, 4 were coffin joint surfaces (2 proximal
surfaces of P3 and 2 distal surface of P2) whereas 24 were fetlock joint surfaces (13 proximal
surfaces of P1 and 11 distal surfaces of the cannon bone).
After Indian Ink staining and following the scoring system by CHANTLEY et al. (1999) this
finding was confirmed: 40 specimens presented a lesion score 4, thereof 8 were coffin joint
specimens (6 proximal surfaces of P3 and 2 distal surface of P2) versus 32 fetlock joint
specimens (13 proximal surfaces of P1 and 19 distal surfaces of the cannon bone).
9 specimens presented a lesion score 5, thereof 2 were coffin joint surfaces (1 proximal
surface of P3 and 1 distal surface of P2) compared to 7 fetlock joint surfaces (6 proximal
surfaces of P1 and 1 distal surface of the cannon bone).

The “Cartilage Degeneration Index” a reliable tool for the quantification of articular cartilage
damage across the entire joint surface based on computer assisted grey level analysis and
developed by BROMMER et al. (2003b) was assessed as well. Unfortunately in this study the
quantitative CDI did neither correlate with the semi-quantitative macroscopic scoring
technique by CHANTLEY et al. (1999), as reported by BROMMER et al (2003a), nor with
the cartilage degeneration score developed by JOUGLIN et al. (2000). The overall correlation
in our study was 0.24 between the lesion score by CHANTLEY et al. (1999) and CDI and
only 0.10 between the lesion score by JOUGLIN et al. (2000) and CDI. This might be due to
the fact that BROMMER et al. (2003b) restricted the determination of articular cartilage
degeneration to the surface of P1. Translation of the 3-dimensional architecture of the joint
surface into a 2-dimensional digital image has consequences for the assessment of articular
cartilage degeneration. The reflected light intensity is significantly influenced by articular
cartilage geometry. This problem is of minor importance for the proximal articular cartilage-
surface of P1 but becomes more important with the increasing joint curvature. In an earlier
experiment the reflected light intensity was within 5% of the maximum in a central region of
a 6.4 mm diameter spherical target, defined by surface angles of 36° or less with the
horizontal. Near the edges of the spherical target the intensity of the reflected light fell but
remained within 10% of the maximum at surface angles of 50° with the horizontal (BROMMER et al. 2003b). In the presented study however CDI was used for the determination of cartilage degeneration of all articular surfaces including the surfaces of the distal part of the cannon bone and the distal joint surface of P2, which have a very large curvature. This might be the reason for the described lack of correlation with the semi-quantitative macroscopic scoring technique. Another explanation might be that if cartilage ulceration extends to the level of the underlying subchondral bone, these two methods for the quantification of cartilage degeneration will be inadequate, as Indian ink does not stain subchondral bone. This makes the methods particularly useful for the initial stages of cartilage degeneration (BROMMER et al. 2003b) but causes them to fail in cases of very severe cartilage damage. Due to the lacking correlation the CDI was not taken into account for the statistical analysis of the inter- and intraindividual variation.

Prior to the discussion of the results of synovial fluid analysis it needs to be said that because the volume of synovial fluid collected per joint was variable, there was not always enough for all assays to be performed.

Viscosity is defined as the ratio of shear stress to shear rate of a fluid (KORENEK et al. 1992; REJNÖ 1976). Shear stress (Pa, Newton per square meter) is defined as the force per unit area required to move adjacent layers of fluid. Shear rate (1/s) on the other hand is the velocity gradient at which adjacent layers of fluid move. High viscosity is attributable to an increase in shear stress or a decrease in shear rate. Viscosity is defined as shear stress/shear rate (KORENEK et al. 1992). So called Newtonian fluids show a constant viscosity at different shear rates. Examples are water, alcohol or all but the heaviest oils (REJNÖ 1976). Non Newtonian fluids (e.g. blood) are complex fluids which show a shear rate dependent change in viscosity. This phenomenon is defined as shear thinning (KORENEK et al. 1992): When the structure broken down by shearing is recovered fast enough that all that is observed is a fall in viscosity with increasing rate to shear it is referred to as shear thinning. The reverse effect is therefore called shear thickening. Synovial fluids are non Newtonian, therefore showing shear thinning in both normal and pathological stages. Only at high shear rates they start showing Newtonian behaviour. Elastic materials exhibit a special, complex relation between stress and strain. They either show a reduction in viscosity as the stress increases or show a rise. Sometimes the viscosity reduction is slowly recovered on standing (REJNÖ 1976).

Synovial fluid from diseased joints loses its functional properties and behaves as Newtonian fluid (KORENEK et al. 1992). A decrease in SF viscosity is often, but not always associated with arthritis (TULAMO et al. 1994).

Hyaluronate is a high molecular weight constituent of SF. It decisively contributes to viscosity (KORENEK et al. 1992). Hence destruction, depolymerisation and reduced concentration are supposedly responsible for the changed flow behaviours (KORENEK et al. 1992; REJNÖ 1976; TULAMO et al. 1994). The reason why more concentrated HA solutions show a greater linear deformation can be explained as follows:

Hyaluronic acid is a GAG bipolymer composed of unbranched repeating disaccharide units. HA molecules typically form temporary network structures. Molecules join with each other temporarily and disentangle after over time. Viscoelasticity is dependent on the phenomenon of entanglement coupling and network properties of HA solutions. When the total volume
occupied by hydrated polymer molecules equals the total volume of the solution, the polymer coils will overlap and entangle with each other. They form an intermolecular network stabilized by non covalent intermolecular associations. The higher the number and density of entanglements in the polymer solution the lower is the relative effect of some broken junctions on the sample's $G'$ value (elasticity value). In concentrated solutions the time necessary to create a new entanglement point immediately after the destruction of the old one is much shorter. Increasing $G'$ values due to increasing concentration also indicate a change of elastic properties caused by increasing network properties (DE SMEDT et al. 1993). Therefore it is suggested that measurement of viscosity provides valuable information about the structural state of the hyaluronate and the functional properties of SF (KORENEK et al. 1992; REJNÖ 1976). A prerequisite for an accurate measurement of synovial fluid apparent viscosity is elimination of time-dependent thixothropic behaviour (thinning and subsequent recovery of viscosity when shearing is removed) as well as multiple measurements of shear stress over a range of known shear rates (LUMSDEN et al. 1996). Rotational cone and plate microviscosimeter is a suitable instrument for the determination of SF viscoelasticity at multiple rpm (rotations per minute) or shear rates (KORENEK et al. 1992).

To characterise and describe the viscoelastic properties of synovial fluid samples the typical parameters of the flow curves as mentioned above need to be explained:

$\eta_0$ (Pa*s): $\eta_0$ is the plateau value of the initial viscosity measured by means of a viscosity curve. It is directly influenced by the molecular weight and concentration of HA. $\eta_0$ is high at a high HA molecular weight together with a constant HA concentration and at a high HA concentration at low molecular weight. HA molecules form a temporary network structure: Molecules join with each other temporarily (entanglement) and disentangle after a certain time. This phenomenon decisively influences $\eta_0$.

Inclination: The higher the HA molecular weight the higher is the inclination. This phenomenon is due to the relatively fast viscosity decrease following frequency increase. It is based on the relatively high disengagement rate of mechanical connections in high molecular weight samples compared to low molecular weight samples.

Both parameters were calculated from a Carreaux fit of the viscosity curves.

Inflection Point (s$^{-1}$): The inflection point represents the force at which mechanical connections start being disengaged and is determined via a strain sweep.

Point of intersection: Based on the analysis of a frequency sweep one can obtain an intersection point between the viscous and elastic components" (DE SMEDT et al. 1993). Above that point of intersection the samples show more elastic than viscous properties. The frequency applied is higher than the reaction time of the fluid, causing the fluid to react mainly elastic like a solid body. The
point of intersection is proportional to the longest relaxation time (s) of the molecular network.

\( G_0 \):
“\( G_0 \) is the plateau value of the final elasticity”. It represents the frequency at which the fluid reacts exclusively like a solid body (DE SMEDT et al. 1993).

The rheologic data (\( \eta_{ao} \), inflection point, inclination, point of intersection and \( G_0 \)) were consistent and correlated well with each other. Therefore the results can serve as prove of principle regarding the method and the correctness and reliability of the measured viscosity and elasticity data. The higher the values representing the viscoelasticity (\( \eta_{ao} \) and \( G_0 \)) the lower is the point of intersection, the shorter the relaxation time (time that a system under load requires to return into its original state after load cessation) and the better is the joint lubrication and shock absorption. The overall correlation between \( \eta_{ao} \) and \( G_0 \) was 0.90. Since both \( \eta_{ao} \) and \( G_0 \), who are detected by two independent measurement techniques, are parameters dependent on the molecular weight and concentration of HA the good correlation may serve as prove of the data’s validity. \( \eta_{ao} \) and the inclination showed a relatively good correlation of -0.69. The correlation between the inflection point and the inclination was 0.65, between the point of intersection and the inclination 0.70 and between the inclination and \( G_0 \) - 0.67. Interestingly for the fetlock joints no significant correlation between the inclination and the inflection point was detected, only for the overall comparison and the coffin joints (0.65 and 0.76 respectively). However in the fetlock joints a significant correlation (0.72) between the point of intersection and the inflection point was detected. These two parameters determine the so called relaxation time. The lack of correlation between these two parameters in the coffin joints could probably indicate that these samples have a larger variety of HA concentrations rather influencing the inclination than the inflection point.

Unfortunately no correlation between the macroscopic appearance of the cartilage degeneration and the rheologic data could be found (\( \eta_{ao}-JOUGLIN: -0.11 \); inflection point- \( JOUGLIN: 0.07 \); inclination- \( JOUGLIN: 0.09 \); point of intersection- \( JOUGLIN: 0.12 \); \( G_0 -JOUGLIN: -0.07 \) and \( \eta_{ao}-CHANTLEY: -0.14 \); inflection point- \( CHANTLEY: 0.16 \); inclination- \( CHANTLEY: 0.15 \); point of intersection- \( CHANTLEY: 0.09 \); \( G_0 -CHANTLEY: -0.11 \)).

Interestingly LINDHOLM et al. (1996) and PRAEST et al. (1997) observed a decrease in viscosity of SF during inflammatory joint disease though. The degenerative process causes a release of lysosomal enzymes, cytokines and also a release of PG fragments into the synovial fluid. The activity of these inflammatory cells in turn causes a decrease of viscosity of the synovial fluid. The reduction of viscosity has also been associated with depolymerization of the synovial fluid due to the inflammatory effusion (LINDHOLM et al. 1996). McILWRAITH (2002) stated though that a clinician must not place too much significance on viscosity findings: This parameter does not give a complete picture of the rheologic behaviour of synovial fluid and should not be considered a direct quantitative or qualitative estimate of the hyaluronan content. Even some fluid samples from joints with only mild changes have markedly decreased viscosity.

Though our preliminary test showed that centrifugation and storage at -170° does not influence the results of the viscoelasticity measurements (6 different samples from 2 different horses were measured fresh, after storage at -170° for 3-5 days and again after 4 days of
storage in a fridge. The samples were centrifuged prior to each measurement.) - see figures below - opinions in other previous papers are controversial.

Fig. 49: Storage related viscoelasticity changes - 1
Fig. 50: Storage related viscoelasticity changes - 2

Fig. 51: Storage related viscoelasticity changes – 3
LUMSDEN et al. (1996) reported that storage of synovial fluid at -80°C for up to 14 days after collection had no significant effect on apparent viscosity. Because the structural state of hyaluronate is largely responsible for the rheologic characteristics of synovial fluid a lack of significant change in apparent viscosity after cold storage would suggest that the hyaluronate molecule is preserved under these conditions. Therefore SF apparent viscosity determination may be performed on previously frozen samples. Centrifugation of the synovial fluid before analysis of the supernatant had no significant effect on apparent viscosity either. LUMSDEN et al. (1996) even consider it advisable to centrifuge samples prior to viscosity measurement because cellular and cartilage material could influence measurements in horses with joint disease. The effect of storage at -80°C and centrifugation on apparent viscosity and total protein concentration was determined for synovial fluid collected from the tibiotarsal joint of a single horse which had no clinical signs of joint pain or lameness, normal range of motion and no gross enlargement of the joint (LUMSDEN et al. 1996). However KORENEK et al. (1992) plead that storage of SF in a freezer for a few days does not affect viscosity, but long-term storage may cause alterations in the hyaluronate molecule and may lead to increased viscosity. These contrary opinions should be kept in mind upon interpretation of the data presented in this study.

In the past widely ranging HA concentrations have been reported for equine SF from normal and from diseased joints (TULAMO et al. 1994). A variety of pathophysiologic mechanisms seem to affect HA concentration. Different rates of synthesis and degradation of HA as well as mobilization to the lymph, influence SF HA concentration. In joints with acute inflammation the degradation may be increased by inflammatory mediators in the SF. On the other hand Interleukin 1 has been documented to stimulate HA synthesis of synovial fibroblasts. Concurrent events may thus result in a normal HA concentration range within the joint (TULAMO et al. 1996).

In this study a significant correlation of the synovial fluid HA concentration and the synovial fluid's viscoelastic properties could be shown. The overall correlation between \( E_{\eta 0} \) and HA was calculated to be 0.73. The inclination and HA concentration showed a negative correlation of -0.78 and \( G_0 \) and HA had a correlation coefficient of 0.72. A correlation between the HA concentration and the degree of cartilage degeneration could not be found. This may be, as already suggested by FULLER et al. (2001), because the scoring system used did not account for changes in the synovium which is the principle source of HA. Our results are in accordance with the results of SAARI et al. (1989) who did not find a difference between the fetlock SF HA concentration in control joints and in joints with positive response to local anaesthetic administration or TAYLOR et al. (2006) who showed that there was no correlation between values of HA and radiographic changes. FULLER et al. (2001) as well as VAN DEN BOOM et al. (2004b) did not find a significant correlation between HA concentration and cartilage damage within a joint either. ELSAID et al. (2003) compared synovial fluid HA concentration of knees with joint synovitis, OA and rheumatoid arthritis and showed that Hyaluronic acid concentration did not differ significantly between the three
patient groups. Therefore several authors have the opinion that levels of HA may not be a useful marker for joint disease (TAYLOR et al. 2006; TULAMO et al. 1994).

In contrast PRAEST et al. (1997) stated that measurement of the SF HA concentration may have prognostic value in the evaluation of different kinds of joint disease and TULAMO et al. (1996) (though 1994 she still suggested that HA may not be a useful marker for joint disease) showed that the degree of cartilage degeneration of the joints with chronic traumatic arthritis documented by arthroscopy correlates negatively with SF HA concentration. KATO et al. (2005) wrote that SF HA in osteoarthritic joints decreases in proportion to progression of radiographic stage.

Many authors on the other hand report a correlation between the inflammatory process in a joint and the HA concentration whereas others reported about the concentration and MW distributions of HA in SF from human beings without considerable significant correlation to clinical or inflammatory variables: Significant correlation was found between the conventional SF inflammatory variables (WBCC, and TP) and the concentration or retention time of HA in control joints and in joints with acute traumatic arthritis or between HA concentration of the diseased joint and lameness degree (TULAMO et al. 1994). That SF HA concentration can be used as a marker for differentiating between different degrees of inflammation was also confirmed by KATO et al. (2005) and PRAEST et al. (1997). Another study suggested that HA concentration in clinically active joints was significantly lower than in the contralateral joints (FULLER et al. 2001). One explanation for the correlation between the SF HA concentration and the degree of joint inflammation might be that the deteriorated blood SF barrier leads to an increased fluid influx reducing the HA concentration (PRAEST et al. 1997). The concentration of HA in SF has been shown to be lower in acutely injured effusive joints compared with acutely injured non effusive or normal joints (VAN DEN BOOM et al. 2004b).

The evaluations in this study revealed a significant correlation between HA concentration and certain viscosity respectively elasticity values. The over all correlation coefficients were as follows: 0.73 for the correlation between $\eta_0$ and HA concentration; -0.78 for the correlation between the inclination and HA concentration and 0.72 for the correlation between $G_0$ and HA concentration. The results comparing only the fetlock joints were slightly different, with a correlation of $\eta_0$ and HA concentration at 0.73; HA concentration and inclination at -0.83 and HA concentration and $G_0$ at 0.76.

Interestingly another rheologic value -the point of intersection- showed a relatively good correlation (-0.62) with the HA concentration when only the fetlock joints were taken into account. This finding can only be explained by the theory that in case of the fetlock joints the molecular weight was relatively constant. Further it indicates that a large variety of molecular weights existed in the coffin joints which in turn caused the lack of correlation between the point of intersection and the HA concentration in these joints. Despite the correlation of the point of intersection with the HA concentration the results for the coffin joints were similar to the over all results and the results of the fetlock joints (HA concentration– $\eta_0$: 0.74; HA concentration– inclination: -0.83; HA concentration – $G_0$: 0.79).

Our results are in agreement with PRAEST et al. (1997) who stated that the correlation between viscosity and HA concentration was much better than between viscosity and weight average molecular weight. They even suggested that HA concentration and the viscosity maybe of equal diagnostic value for differentiating between acute and chronic joint disease.
TULAMO et al. (1996) found an explanation for this phenomenon as reduced viscosity is strongly dependent on SF HA concentration. They found that joints with DJD, infectious arthritis and with intra articular fractures had significantly lower SF HA concentration than control joints. They explained that this finding is based on the fact that acute severe or persistent inflammation of synovial structures leads to joint effusion, with SF dilution effect or altered SF synthesis which in turn reduces HA concentration and viscosity. In an earlier study TULAMO et al. (1994) already concluded that because SF is a non Newtonian fluid the reduced viscosity is strongly dependent on HA concentration and that clinical observations verify that SF viscosity is reduced in horses with infectious and chronic arthritis and with radiographic evidence of DJD.

From the chemical point of view the correlation between the rheologic properties of SF and the HA concentration can be explained as follows:
The change of the viscoelastic properties is a function of the HA concentration and molecular weight. As soon as the total volume occupied by hydrated polymer molecules equals the total volume of the solution, the polymer coils will begin to overlap and entangle to form an intermolecular network stabilized by non covalent intermolecular associations. HA solutions with concentrations lower than 1 mg/ml form particle solutions. At concentrations higher than 1 mg/ml the HA molecules start to entangle with each other and form a network structure. This means that the synovial fluids with the lowest viscosities represent particle solutions (Eta0 values below 0.2 Pa*s). All other synovial fluids are network solutions with a concentration dependent entanglement density.

The correlation of the inclination with the HA concentration confirms the concentration-dependent entanglement density and the effect of a larger disentanglement caused by shear forces in case of a high HA concentration. This phenomenon is called a transient network. In addition to concentration and molecular weight the number of entanglement points depends on the applied shear rate. With increasing shear rate the number is reduced resulting in a decreased resistance against flow. With decreasing shear rate the number of entanglement points increases. This process is reversible and does not cause a reduction of molecular weight, except at extremely high shear rates.

The higher G' values with increasing concentration also indicate augmentation of the elastic properties due to the denser network, as explained earlier.

The point of intersection (frequency at which G' and G'' cross each other) changes from high to low frequencies with increasing HA concentration. As the appearance of the G' plateau is a consequence of the existence of a network, the G'' values give an estimation of the network structure changes (density, number of entanglement points per volume) as a function of the HA concentration. The profile of the G' curve tends to form a plateau at high frequencies whereas the decay of the modulus is temporarily inhibited by the entanglement network of the samples (DE SMEDT et al. 1993). McILWRAITH (2002) summarized that the viscosity of hyaluronan apparently depends on, the length of the polysaccharide chains, the conformation of the chains and the interaction between adjacent chains and other molecules. Therefore, the decrease in viscosity may also be due to a change in the overall relationship of hyaluronan and other molecules.

Another factor assessed in this study was the molecular weight of HA. Separation techniques and in particular liquid chromatography (LC) techniques are major tools in the analysis/characterisation of carbohydrates and polysaccharides. Entropy controlled GPC
enables the determination of molecular dimensions of oligo- and polysaccharides in terms of molecular weight/degree of polymerization distribution and excluded volume distribution. The GPC separation criterion (separation of components according to differences in excluded volume and molecular dimension) matches perfectly with the major property of polysaccharides (filling volume in a more or less regular way) (HUBER et al. 2004). GPC was chosen for HA-molecular-weight-assessment because synovial fluid besides HA consists of a variety of proteins, glycosaminoglycans and other molecules (TULAMO et al. 1994). In most other studies HPLC (High Performance Liquid Chromatography), another method to determine HA molecular weight, was chosen. Retention times are inversely proportional to the molecular weight/size (TULAMO et al. 1994; TULAMO et al. 1996). In HPLC the separation of molecules is based exclusively on molecule sizes (TULAMO et al. 1994) - retention times are inversely proportional to the molecular weight/size (TULAMO et al. 1994; TULAMO et al. 1996) - and therefore, though HA is by far largest constituent of SF (TULAMO et al. 1994), might be less accurate when it comes to molecules of the same size. Assuming that in joints with OA HA molecules of smaller size occur due to either the production of smaller molecules by the synoviocytes or fragmentation of the molecules, these smaller HA molecules can not be distinguished from other proteins by HPLC. This would require another step of evaluation. The GPC procedure in contrast allows to determine MW of very small HA molecules. Unfortunately we were not able to evaluate the molecular weight distribution of more than 8 samples. Therefore an adequate statistical analysis of these results was not possible. Despite TULAMO’s et al. (1994) remark that this technique requires extensive sample purification, is complicated and that sample preparation and exposure to chemicals necessary for the assay could cause substantial variations in the HA molecular configuration, resulting in widely diverse MW of equivalent preparation we are convinced that this method is particularly suitable to prove the suggested theory that DJD and/or inflammation causes fractioning of Hyaluronic acid molecules (McILWRAITH 2002) or the production of Hyaluronic acid with shorter chain-length in the first place. In the course of the inflammatory process high molecular weight HA seems to be depolimerized by oxygen derived free radicals into very small fragments (PRAEST et al. 1997; TULAMO et al. 1994). However comparing the 8 samples assessed for their HA molecular weight distribution no correlation could be found. Neither with the degree of cartilage degeneration nor with any of the other evaluated synovial fluid quality parameters. The results of this study results are in agreement with PRAEST et al. (1997) who could not find a correlation between viscosity of SF and Mw. However the results are in contrast to TULAMO et al. (1994) who found a significant correlation of HA molecular weights and radiographic changes of joints. SAARI et al. (1989) reported that the correlation between SF HA concentration and its degree of polymerization was 0.760. TULAMO et al. (1996) also described a significant correlation between HA concentration and molecular weight and the degree of arthritis which PRAEST et al. (1997) partly confirmed as the research group showed that smaller amounts of high MW HA are detectable in the SF obtained from inflammatory joints compared to the SF obtained from DJD. There was more high molecular weight HA in non inflammatory than in inflammatory joint disease. Again these results indicate that the evaluated parameter might be rather influenced by the degree of joint inflammation that the cartilage degeneration.
Imbalance of MMP activities is crucial for the disease evolution of OA (MARINI et al. 2003). Thus the correlation of MMP 1 and 3 with the cartilage degeneration, the other synovial fluid markers and one another was evaluate in this study, again with no results that would allow a diagnosis of joint disease or an estimation of the degree of cartilage damage. SCHMIDT-ROHLFING et al. (2002) and YOSHIHARA et al. (2000) reported about a significant correlation between the levels of MMP1 and MMP3 in OA, which we could not confirm.

For Bioassays the assayed enzymes usually need to be present in an active and uninhibited form whereas Elisa using antibodies against various MMPs can provide information on potential enzyme activities. It is not possible to achieve any information whether the detected enzyme actually is in an active state or not (CLEGG et al. 1997).

In this study an Elisa test was chosen for the determination of the MMP1 and MMP3 concentration. Maybe if only the active forms of MMPs had been taken into consideration better results would have achieved.

The findings of this study are in accord with McLWRAITH et al. (2002) who wrote that MMPs, are considered of minimal value in assessing the amount of articular cartilage damage or defining the status of the joint. VAN DEN BOOM et al. (2004b) also reported about no significant correlation between MMP activity and CDI value for unaffected joints or joints with OA. SCHMIDT-ROHLFING (2002) reported that the highest levels of MMP3 and MMP1 were not seen in the most advanced stages of DJD. The highest levels of MMP3 were found in the intermediate stages. Similar results were obtained for MMP1 with the highest levels at the initial and intermediate stages.

In contrast MARINI et al. (2003) showed that the degree of cartilage degradation as seen by arthroscopy is strictly related to the activity of some synovial MMPs and therefore concluded that the severity of the disease must be associated to the production of MMPs with either enhanced activity or reduced inhibition or both. They could even describe a slight increase of MMP1 related to the severity of chondromalazia. JOUGLIN et al. (2000), MAIOTTI et al. (2000) and VAN DEN BOOM et al. (2005) also detected a good correlation between MMP concentration and cartilage degeneration. JOUGLIN et al. (2000) even concluded that to a certain extend SF MMPs may be used as a marker of changes that occur in joints with OA.

Other authors such as TRUMBLE et al. (2001) and VOLK et al. (2003) found that MMPs but not necessarily MMP1 and 3, as assessed in this study, were elevated in diseased joints compared to normal joints. In man increases in stromolysin and collagenase have been demonstrated in SF from OA patients and in post traumatic joint disease (CLEGG et al. 1997).

Again the correlation of MMP concentration and inflammatory state of the joint would probably provide important, additional information. TRUMBLE et al. (2001) identified an association between MMP9 and inflammatory arthritides in the form of a correlation between the WBC concentration and pro- as well as active MMP9. Interestingly although VAN DEN BOOM et al. (2005) found a good correlation between the condition of the articular cartilage and MMP activity they still conclude that it seems to be a better indicator for active processes than for the cartilage status in chronic cases.

Other than the correlation between HA concentration and viscosity values (initial viscosity, inclination, point of intersection and final elasticity) no statistically significant correlation could be found. This may be because the cartilage scoring systems used did not account for
changes in the synovium which is not only the principle source of HA (FULLER et.al. 2001) but also of joint inflammation. Many other features that go along with OA, for example changes in the subchondral bone or joint capsule were not taken into account. However despite the very different results for the degree of cartilage degeneration and the concentration of the assessed biomarkers in different joints within a horse the intraindividual variance was low (0.619 using the CHANTLEY score and 0.621 using the JOUGLIN score). The data variance within one foot was even lower (Interclass correlation of 0.92 and 0.94 respectively) indicating that the coffin and fetlock joint of the same foot seem to suffer a similar destruction. However since no significant correlation of the synovial fluid data and the cartilage degeneration could be found this might only indicate that usually several joints of one individual suffering from degenerative joint disease are affected. Therefore not even the contralateral joints can serve as a control or even give a reliable estimation of the healthy values because it has to be suspected that other joints are affected as well.

This conclusion is supported by the suggestion that HA concentration and degree of polymerization in equine SF have a wide range of biologic variation even in healthy joints (TULAMO et al. 1994). The amount and concentration of HA has been reported to vary between animals and also between joints within the same animal. SF of joints with smaller volumes generally comprises higher concentrations of HA (TAYLOR et al. 2006). SAARI et al. (1989) reported about variation not only in the HA concentration but also in the degree of polymerization.

Apparent viscosity values have a substantial range as well but measurement of apparent viscosity of equine synovial fluid is repeatable. Therefore the observed variation in apparent viscosity may represent true variation among horses. Clearly such large variations in normal values may limit usefulness in facilitating detection of arthrosis (LUMSDEN et al. 1996).

A reasonable answer to the question why there seems to be a general lack of correlation between cartilage degeneration and synovial fluid biomarkers might be synovial clearance. BELLO et al. (1997) and MYERS et al. (2000) reported that synovial clearance in inflamed joints was enhanced. Further TAYLOR et al. (2006) reported about increased clearance of protein from synovial fluid especially in inflammatory phases of OA and LINDHOLM et al. (1996) found that Hyaluronan disappears from the joints or is metabolized in approximately 3 days whereas different molecular weights do not seem to influence the rate of biological turnover (LINDHOLM et al. 1996; Meyers et al. 2000). POPOUT et al. (2004) even calculated the SF clearance to be much faster. According to them synovial clearance can be estimated to be about 4 m/l/h (a synovial fluid turnover rate of about 10% assuming a joint volume of 30 ml). According to VAN DEN BOOM et al. (2005) GAG released into SF is subsequently cleared from the joint very rapidly and levels therefore do not accumulate to values greater than in normal joints. Within a few hours the GAGs pass via lymph vessels into the bloodstream.

Therefore there is a reasonable suspicion that after the inflammatory phase has seized and the synovial membrane has recovered, new and healthy synovial fluid might be produced and synovial fluid might be completely cleared of any markers indicating DJD (in case of this study depolymerised HA, MMPs, low HA concentration and low viscosity). Synovial fluid samples drawn from such joints might therefore appear as if they were drawn from a healthy joint despite of the cartilage degeneration. The joint might have rapidly been cleared from
impaired synovial fluid and new healthy synovial fluid might have been produced by the recovered synovial membrane. Therefore the results of this study confirm the conclusion of SHARIF et al. (1995) and TAYLOR et al. (2006) who suggested that measures of molecular markers taken at a single point in time cannot reflect a dynamic disease process. This fact is even more important since in contrast to the situation described above an inflammation of the synovial membrane might be present (therefore an impaired synovial fluid) though the articular cartilage surface is intact.

This study was designed to evaluate and describe the correlation of cartilage degeneration with the changes in synovial fluid parameters due to DJD. Therefore all scoring techniques used were focused on the detection of cartilage damage. Non of the scoring techniques in this study took changes in other tissues of the joints that might also be impaired by DJD into account. In future studies focus should also be put on the changes of the synovial membrane especially since synovial fluid is produced by the synoviocytes situated within this tissue. This is even more important since some studies (see above) reported that several synovial fluid parameters mainly represent the degree of joint inflammation and respectively the condition of the synovial membrane. Besides the majority of markers are not only produced in articular cartilage but to varying extents also in the synovial membrane and other organs (SCHMIDT-Rohlfing et al. 2002).

In summary it seems that synovial fluid biomarkers at this point in time rather provide information about the degree of joint inflammation than cartilage degeneration. However also in this field further research needs to be done since SAARI et al. (1989) for example could not find a significant correlation between conventional SF inflammatory variables and concentration or degree of polymerization of HA. ELSAID et al. (2003) were only able to show that TP concentrations were significantly higher in patients with knee joint synovitis compared to OA and rheumatoid arthritis, but could not find a significant difference between synovial fluid samples from patients with OA and rheumatoid arthritis. In a study by BELLO et al. (1997) no significant effect of the presence of abnormal synovium on marker levels was found.

The original plan of this study was to take the degree of inflammation into account as well but unfortunately the samples were accidentally frozen which made diagnosis of joint inflammation by means of cytology, white blood cell count or total protein concentration impossible. Total protein concentration assessments were carried out but due to cryo-precipitation no reasonable results could be obtained. However the degree of joint inflammation is certainly a very important information and should definitely be taken into account in future studies because whether inflammation was present in the joints at the moment of arthrocentesis will affect the results obtained and may (in part) explain contradictory results presented by various researchers in the past (VAN DEN BOOM et al. 2004b).

Some authors suggest bone markers to be a valuable tool in early diagnosis of DJD or the prediction of it's progression. Their theory is based on positive correlation between bone markers and cartilage damage which suggests that there might be a link between bone turnover and cartilage damage in OA. Recent studies have shown that subchondral bone
changes may precede articular cartilage changes and in that case bone may be the more important tissue in OA research (FULLER et al. 2001; SHARIF et al. 1995).

The conclusion of this study is therefore that neither diagnosis nor an estimation of the degree of cartilage degeneration can be made based on synovial fluid analysis so far. To make a legitimate diagnosis it will be necessary to establish generally valid limits for synovial fluid parameters in health and disease. However even the values that different authors refer to as "normal" vary considerably. Our results confirm the fact that synovial fluid parameters change due to DJD but can not be pulled up for diagnostic purposes, only to gain additional information. In future studies it will be necessary to determine the physiologic values of different parameters commonly used in synovial fluid quality analysis, in order to provide a well-founded baseline, using new and very accurate devices. Only then it will be possible to make a profound statement about the changes in synovial fluid parameters caused by degenerative and inflammatory joint disease. Based on that knowledge one can try to assess if and how these values are affected by different commonly used pharmaceuticals, how their activity can be improved or what criteria new pharmaceuticals would have to fulfil.
9.0. Summary

9.1. English summary

Currently therapies for osteoarthritis are only capable of alleviating the symptoms (BELLO et al. 1997; GUIDOLIN et al. 2001) but no preparation has so far definitely slowed down the progression of the contributing factors (GUIDOLIN et al. 2001). A main limiting factor is, that by the time that changes in the joint can be appreciated radiographically or by ultrasound the disease has progressed so far that it already caused severe and irreparable cartilage lesions (BELLO et al. 1997; FULLER et al. 2001; TULAMO et al. 1996) and/or has even lead to specific alterations in the underlying bone such as bone sclerosis or osteophyte formation (TULAMO et al. 1996). Therefore the presented study aimed at comparing osteoarthritis associated changes of specific synovial fluid biomarkers and properties with the degree of articular cartilage degeneration in horses, in order to find a possibility to diagnose osteoarthritis at an early stage.

The cartilage degeneration degree of 60 equine fetlock and coffin joints was determined using 3 different, well established methods. Synovial fluid viscoelastic properties, hyaluronic acid molecular weight distribution, hyaluronic acid concentration, total protein concentration and the biomarkers MMP1 and MMP3 were chosen to be correlated with the cartilage degeneration degree.

Significant correlation could be shown between the synovial fluid viscoelastic properties and the hyaluronic acid concentration as well as between the different viscoelasticity data. Unfortunately correlation between any of the synovial fluid analysis data and the degree of cartilage degeneration which could serve as basis for an early osteoarthritis diagnosis could not be found.

The conclusion of this study is therefore that neither diagnosis nor an estimation of the degree of cartilage degeneration can be made based on the analysis of the synovial fluid parameters assessed within the scope of this study. The results confirm the fact that synovial fluid parameters change due to DJD but can not be pulled up for diagnostic purposes, only to gain additional information. In future studies it will be necessary to determine the physiologic values of different parameters commonly used in synovial fluid quality analysis, in order to provide a well-founded baseline, using new and very accurate devices. Only then it will be possible to make a profound statement about the changes in synovial fluid parameters caused by degenerative and inflammatory joint disease.

Key words: Osteoarthritis, cartilage, synovial fluid, cartilage degeneration, hyaluronic acid, MMP
9.2. Zusammenfassung


Schlüsselwörter: Osteoarthritis, Knorpel, Synovialflüssigkeit, Knorpel Degeneration, Hyaluronsäure, MMP
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Annals of the Rheumatic Disease, 59, 455-461
11.0. Figures

**Figure 1:** Hyaluronic Acid (HA) - polymer based on a repeating disaccharide 10

**Figure 2:** Radiographic image of an osteoarthritic joint 40

**Figure 3:** Sonographic image of an osteoarthritic joint 40

**Figure 4:** 3-Dimensional reconstruction of CT images 41

**Figure 5:** Arthroscopic view of a cartilage defect 46

**Figure 6:** Cannon bone/ JOUGLIN lesion score 0 48

**Figure 7:** Proximal surface of proximal phalanx (P1)/ JOUGLIN lesion score 1 48

**Figure 8:** Cannon bone/ JOUGLIN lesion score 2 49

**Figure 9:** Proximal surface of P1/ JOUGLIN lesion score 3 49

**Figure 10:** Cannon bone/ JOUGLIN lesion score 4 49

**Figure 11:** Cannon bone/ CHANTLEY lesion score 0 50

**Figure 12:** Distal surface of middle phalanx (P2)/ CHANTLEY lesion score 1 51

**Figure 13:** Distal surface of P2/ CHANTLEY lesion score 2 51

**Figure 14:** Proximal surface of P1/ CHANTLEY lesion score 3 52

**Figure 15:** Cannon bone/ CHANTLEY lesion score 4 52

**Figure 16:** Proximal surface of P1/ CHANTLEY lesion score 5 53

**Figure 17:** unstained 54

**Figure 18:** stained 54

**Figure 19:** CDI: 48,8%; CODI: 18,5% 54

**Figure 20:** Synovial fluid colours 55

**Figure 21:** Degree of Polymerization Distribution (dpD) (HUBER 2008) 60
Figure 22: Methodical illustration of GPC assembling (HOLZER 2004; HUBER 2008) 61

Figure 23: Viscosity curves 68

Figure 24: Frequency sweep 69

Figure 25: Sample 4FG1, mass fraction molecular weight distribution 81

Figure 26: Sample 4FG1, number fraction molecular weight distribution 82

Figure 27: Sample 9FG2, mass fraction molecular weight distribution 83

Figure 28: Sample 9FG2, number fraction molecular weight distribution 83

Figure 29: Sample 2HG2, mass fraction molecular weight distribution 84

Figure 30: Sample 2HG2, number fraction molecular weight distribution 85

Figure 31: Sample 5FG4, mass fraction molecular weight distribution 86

Figure 32: Sample 5FG4, number fraction molecular weight distribution 86

Figure 33: Sample 7FG3, mass fraction molecular weight distribution 87

Figure 34: Sample 7FG3, number fraction molecular weight distribution 88

Figure 35: Sample 14FG3, mass fraction molecular weight distribution 89

Figure 36: Sample 14FG3, number fraction molecular weight distribution 89

Figure 37: Sample 17HG3, mass fraction molecular weight distribution 90

Figure 38: Sample 17HG3, number fraction molecular weight distribution 91

Figure 39: Sample 18FG4, mass fraction molecular weight distribution 92

Figure 40: Sample 18FG4, number fraction molecular weight distribution 92

Figure 41: \( \eta_0 - \text{Inclination/ correlation } 0.69 \)

Figure 42: \( \eta_0 - G_\beta \text{/ correlation } 0.90 \)

Figure 43: Inflection point – Inclination/ correlation 0.65

Figure 44: Point of intersection – Inclination/ correlation 0.70
Figure 45: Inclination – $G_0$/ correlation -0.67

Figure 46: Eta$_0$ – HA/ correlation 0.73

Figure 47: Inclination – HA/ correlation -0.78

Figure 48: G$_0$ – HA/ correlation 0.72

Figure 49: Storage related viscoelasticity changes – 1

Figure 50: Storage related viscoelasticity changes – 2

Figure 51: Storage related viscoelasticity changes – 3
12.0. Tables:

Table 1: Lesion score overview of all horses and all joints
Table 2: Lesion score differences between fetlock and coffin joints
Table 3: Lesion score overview of all horses and all joints
Table 4: Lesion score differences between fetlock and coffin joints
Table 5: Synovial fluid viscosity data
Table 6: HA- concentration data (ranged between 0.24 and 4.15 mg/ml)
Table 7: Comparison of weight fraction molecular weight
Table 8: Comparison of number fraction molecular weight
Table 9: MMP1 and MMP3 data/ VF5: Delution factor 5
Table 10: Overall correlation overview/ Pearson
Table 11: Overall correlation overview/ Spearman
Table 12: Proximal surface of P1 and distal surface of the cannon bone/ Pearson
Table 13: Proximal surface of P1 and distal surface of the cannon bone/ Spearman
Table 14: Proximal surface of P1/ Pearson
Table 15: Proximal surface of P1/ Spearman
Table 16: Distal surface of the cannon bone/ Pearson
Table 17: Distal surface of the cannon bone/ Spearman
Table 18: Proximal surface of P3 plus distal sesamoid bone and distal surface of P2/ Pearson
Table 19: Proximal surface of P3 plus distal sesamoid bone and distal surface of P2/ Spearman
Table 20: Proximal surface of P3 plus distal sesamoid bone/ Pearson
Table 21: Proximal surface of P3 plus distal sesamoid bone/ Spearman

Table 22: Distal surface of P2/ Pearson

Table 23: Distal surface of P2/Spearman
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