6. Discussion

6.1. General aspects

The aim of the present work is to study the activity of the enzyme MPO in synovia from diseased joints and tendon sheaths in the horse and its relationship with synovial parameters and clinical signs of orthopedic maladies. Synovia was obtained from diarthrodial joints and tendon sheaths of animals suffering of natural occurring orthopedic disease presented at the Clinic for Horses, General Surgery and Radiology of the Freie Universität Berlin between 2003 and 2005. Synovia samples were classified according to criteria based on the anamnesic history, clinical, radiological and ultrasound examination and analysis of synovia into 7 groups, namely: healthy controls (C), chronic arthritis (CA) (n=28), osteoarthritis (OA) (n=12), septic arthritis (SA) (n=3), osteochondrosis dissecans (OCD) (n=15), osteochondrosis dissecans with secondary osteoarthritis (OCD + osteoarthritis) (n=4) and tenosynovitis (TS) (n=8).

The different diagnostic groups showed uneven sizes with some groups showing small number of samples. This should be taken into account when evaluating the results reported herein, since based on this, no general conclusions can be drawn for the whole equine population. The majority of the horses sampled in the present study were warmbloods, probably because it is the most common horse breed in the Federal Republic of Germany. Regarding the age of horses in this study, all animals in the group of OCD were 6 years old or younger and showed a mean age of 3, 2 ± 1, 74 years. The group with septic arthritis consisted of very young animals, with ages ranging from 1 to 3 years. The rest of the groups presented a wide range of ages. The sexual distribution among the different diagnostic groups was uneven. Synovia was obtained from patients presented at the clinic within a limited time period and the size of the groups and the distribution of ages and sexes in each group was probably influenced by and dependent on the number of cases presented at the clinic and may not necessarily represent the prevalence of disease in animals of certain breeds, ages or sexes in the equine population.

Different types of joints were not evenly distributed within the different diagnostic groups. Hence, no comparison between groups of any of the parameters studied in this work could be

made without being confounded by joint. For the group of healthy controls, only carpal (n=10) and tarsocrural (n=8) joints were punctuated. These are large and relatively easy-to-punctuate joints, enabling a faster, safer and less complicated sampling procedure, thus, reducing the risks associated with arthrocentesis. Synovia from healthy joints was used as a comparative control for synovia from diseased diarthrodial joints and tendon sheaths as well. This methodology was based on the similarity of synovial parameter of clinically healthy tendon sheaths with synovia from healthy joints (MALARK et al. 1991).

The joints punctuated in this study present a broad spectrum of duration and severity of disease. Except for the groups of OCD and septic arthritis, all horses with diseased joints presented a history of chronic joint disease of at least 5 weeks. The chronic character of joint disease is important when discussing the analysis of synovial parameters, as explained later in the present work.

Radiographic examination was used as one of the parameters for the classification of synovia from diarthrodial joints in a determined disease group. No attempt was made to classify radiographic signs of osteoarthritis into different categories. Joints classified in the group of chronic arthritis came from joints with clinical signs but no radiographic findings of disease. However, it is important to note that early pathological changes in articular cartilage are not apparent on radiographs (LOHMANDER et al. 1992; RAY et al. 1996; KIDD et al. 2001) and this may have led to a misleading classification of some joints into this group. However, radiography is the most frequently used imaging diagnostic method for the assessment of joint disease in the horse (WIDMER and BLEVINS 1994). The overall clinical and radiological examination performed in this work account for the regular procedure carried out in the daily veterinary practice to assess the clinical status of joints in the horse.

6.2. Discussion of the results of the analysis of synovial fluid

6.2.1. pH

Relatively few publications account for the pH values of equine synovial fluid. The mean value for synovial pH in the control group of the current study (7, 377 ± 0, 062) was more acidic than values reported in the equine literature for healthy joints (PERSSON 1971; BOLBOL 1983). A difference in the equipment used for the assessment of pH probably accounts for this apparent variation. The great majority of values of pH measured in synovia from joint suffering of osteoarthritis (7, 352 ± 0, 062), OCD (7, 351 ± 0, 044), OCD + osteoarthritis (7, 368 ± 0, 078) and tenosynovitis (7, 339 ± 0, 105) were within the range of values detected in the group of controls. This is in agreement with research demonstrating similar values of pH in synovia of healthy joints and of joints diagnosed with non-septic diseases (PERSSON 1971; AKENS 1994). This was also the case for the group of chronic arthritis, which interestingly showed a mean pH (7, 446 ± 0,060) that was above than the mean detected in the controls and other non-septic synovia. PERSSON (1971) reported a joint-type-dependent significative difference in pH values between healthy tarsocrural and the distal interphalangeal joints, the former presenting the lowest and the latter the highest pH value. This may have contributed to this higher mean pH in the group of chronic arthritis, since this group had the highest fraction of distal interphalangeal joints of this study (19 interphalangeal joints out of a total of 28 joints) while the control group presented 8 tarsocrural and 10 carpal joints. A time-dependent increase of pH after arthrocentesis (PERSSON 1971) may not explain this higher mean pH since all samples in the present study were treated in the same way and were immediately analyzed after arthrocentesis, regardless of their diagnosis.

As expected, the group of septic synovia (n=3) presented the lowest mean value (7, 274 ± 0, 071). The pH value of synovia decreases in joint infection (TULAMO et al. 1989a; LLOYD et al. 1990). Increased concentrations of lactate have been detected in synovia of septic joints (RIORDAN et al. 1982; HERTSCH 1983; TULAMO et al. 1989a), which may account for the decrease of synovial pH (GOBELET and GERSTER 1984). In infection, the concentration of lactate in the synovia increases because of the anaerobic glycolysis of the synovial membrane (TREUHAFT and McCARTY 1971; BROOK and CONTRONI 1978). Septic arthritis presents a very high total white blood cell count in synovia (McILWRAITH 1987)

and a close correlation between a high total number of leukocytes in the synovia and a low synovial pH value has been reported (WARD and STEIGBIGEL 1978).

6.2.2. Total protein concentration

Total protein concentration in synovial fluid of clinically normal horses has been described to be under 2, 5 g/dl (KAWCAK et al. 1997; FRISBIE et al. 1999; McILWRAITH 2002).

The content of total protein in the group of control was within the normal range, with a mean concentration of 0, 867 ± 0, 491 g/dl (range: 0, 1 – 1, 8 g/dl). During synovitis, an increased content of total protein in synovia is a consequence of the increased vascular permeability of the synovium and the widening of intercellular spaces, allowing a leakage of plasma protein molecules into the joint (SIMKIN 1991). The total protein concentration of synovia augments with increasing inflammation, approaching the protein level of plasma (McILWRAITH 1987). However, in the present work, the total protein concentrations of at least 75% of all samples from the groups suffering of chronic arthritis and osteoarthritis were within the range considered to be clinically normal (< 2, 5 g/dl). Moreover, the content of total protein detected in all samples in the groups of OCD and OCD + osteoarthritis fell within the physiologically normal range. This confirms data of the equine literature, suggesting that synovial total protein concentration from horses afflicted with non-septic chronic conditions may vary, showing increased values or remaining unchanged when compared with synovia from healthy joints (TEW and HOTCHKISS 1981; SANDER 1990; DIMOCK et al. 2000; McILWRAITH et al. 2001; MAHAFFEY 2002). These results suggest a low degree of synovitis, if any, in the great majority of samples from these groups. The chronic character of the disease may have also influenced the total protein content, since synovitis tends to be greater in the acute phases of osteoarthritis than in the chronic stages of the disease (BENITO et al. 2005). The group with tenosynovitis showed higher concentration of total protein, with a mean value of 2, 025 ± 1, 358 g/dl (range: 0, 4 – 4, 2 g/dl), possibly due to a higher degree of synovitis.

As expected, the highest mean value of total protein concentration was detected in the group of horses with septic arthritis (Mean: 3, 867 ± 1, 629 g/dl; range: 2, 0 – 5, 0 g/dl). This suggests a high degree of synovitis, since values above 2, 5 g/dl suggests the presence of inflammation while concentrations above 4 g/dl point to a severe inflammation (McILWRAITH et al. 2001; McILWRAITH 1987, 2002). Septic arthritis accounts for the

most aggressive kind of synovitis and shows a rather high concentration of protein because of the increased synovial permeability and the presence of products of cellular constituents (MAHAFFEY 2002).

6.2.3. Total white blood cell count

Total white blood cell count in healthy synovia measured with an automated cell coulter in the horse has been documented to be 500 cells/µl or less (DIRKS 2000). In the present study, the groups of samples of controls (range: 100 – 500 cells/µl) and the majority of samples from the group of chronic arthritis (range: 100 – 1800 cell/µl), osteoarthritis (range: 100 – 400 cells/µl), presented low values, as 75% of all observations of these groups were equal or below 200 cells/µl. The groups of OCD and OCD + osteoarthritis showed low counts of total white blood cells too, which were below the background threshold of the automated cell coulter (200 cells/µl). TWBC in synovia of joints diagnosed with non-septic chronic diseases in the horse may widely vary and at times may be to some extent increased or within the range established as physiologically normal (PERSSON 1971; GÄNGEL 1980; TEW and HOTCHKISS 1981; SANDER 1990; McILWRAITH et al. 2001; MAHAFFEY 2002). Only a small number of samples from the group of chronic arthritis showed TWBC above 1000 cells/µl, probable due to a flare-up of the disease. The group of tenosynovitis showed a TWBC ranging from 400 to 3200 cells/µl, probably reflecting a greater degree of synovitis (McILWRAITH 1987; McILWRAITH et al. 2001).

In the horse, septic arthritis is the joint disease with the most dramatic degree of synovitis and presenting the highest counts of white blood cells in synovia (HERTSCH 1983; McILWRAITH 1980, 1987; MAHAFFEY 2002). This was confirmed in the present study, as synovia from septic joints had extraordinarily high white blood cells counts ranging from 13100 to 29 800 cells/µl.

6.2.4. MPO-Activity

The group of controls showed a mean value of MPO-activity of 0, 150 ± 0, 313 mU/µl (range: 0 – 1, 2 mU/µl), while 75% of all observations in this group showed an activity below 0, 3 mU/µl. Mean values of MPO-activity were higher in all groups of diseased joints and tendon sheaths when compared with controls. However, a closer look at the results shows that at least

50% of all samples from chronic arthritis, osteoarthritis and OCD + osteoarthritis and 75% of all samples with OCD had values of MPO-activity that fell within the range of activities measured in the controls (range: 0 – 1, 2 mU/µl). This means that solely based on the activity of MPO, it would have been impossible to differentiate a great part of diseased joints in the groups mentioned above from healthy controls. All samples from the groups with septic arthritis and at least 75% of the samples from the group of tenosynovitis reported MPO-activity above the range observed within healthy controls, but these values overlapped with activities of the enzyme detected in a few samples of the groups mentioned previously in this paragraph.

The results showed herein contrast with the data published by LAMMER (2001) and SPELLMEYER (2003) in synovia of diseased joints in dogs, which reported a clearly higher activity of MPO in diseased joints when compared with healthy controls. Possible explanations for this fact are presented below.

Firstly, the activity of MPO in the present work was assessed with a kinetic study performed with an enzyme activity assay based on the peroxidative activity of MPO on o-diananisidine and utilizing the inhibitor 4 - Aminobenzoic acid hydrazide (ABAH) in the cell-free supernatant of synovia (FIETZ et al. 2004). This inhibitor would specifically inhibit the activity of MPO (KETTLE et al. 1995) and the values presented here would therefore account exclusively for peroxidative activity from MPO and not for the peroxidative activity of other enzymes.

MPO is an enzyme synthesized in the azurophilic granules of neutrophils (KLEBANOFF 1999) and liberated once these cells have been primed and activated (WEISS 1989). The presence of MPO in the extracellular milieu (e.g. synovial fluid) would account for massive liberation of the enzyme from activated or dead neutrophils during inflammation (SERTEYN et al. 2003). Mechanical lysis of neutrophils (e.g. during arthrocentesis or centrifugation) would not lead to the liberation of active enzyme into the extracellular milieu. Mechanically lysed neutrophils liberate the granules containing the MPO but this may not necessarily lead to the release of the active enzyme into the synovia (KJELDSEN et al. 1999).

The type of disease may determine the presence of MPO in the synovia. In the present study, it would have been impossible to differentiate a great part of diseased joints from the groups

of chronic arthritis, osteoarthritis, OCD and OCD + osteoarthritis from healthy controls solely based on the activity of MPO. In humans, the presence and the deleterious effect of the products of the MPO/H_20_2/Cl^- system in synovia of joints with rheumatoid arthritis has been demonstrated (HADLER et al. 1979; EDWARDS et al. 1988; SCHILLER et al. 1996). Yet, due to its highly inflammatory nature, rheumatoid arthritis is characterized by high numbers of neutrophils (EDWARDS and HALLETT 1997), with total white blood cell counts ranging from 5 000 to 75 000 cells/µl (CANOSO 1998). Conversely, as discussed before in the current work, non-inflammatory joint conditions in the horse, like osteoarthritis and OCD, usually present much lower cellularity. Since MPO is an enzyme synthesized in the azurophilic granules of neutrophils (KLEBANOFF 1999) and liberated once these cells are activated (WEISS 1989), a higher activity of MPO in synovia with high TWBC is to be expected, as the source of MPO is more abundant there. Besides, not only the number but the degree of activation of neutrophils may determine the amount of MPO secreted from neutrophils (NURCOMBE et al. 1991; KING et al. 1997). Factors like interleukins, immune complexes, leukotrienes and complement components leading to priming and activation of neutrophils are quite abundant in inflammatory joint diseases (e.g. rheumatoid arthritis) (EDWARDS and HALLETT 1997). As shown in the results of the current study, the great majority of samples coming from the groups chronic arthritis, osteoarthritis, OCD, OCD + osteoarthritis showed low TWBC (equal or below 200 cells/µl), coinciding with an overlapping of the great majority of MPO-activity with the controls. Conversely, all samples from the group of septic arthritis and the 75% of samples from the group of tenosynovitis showed values of MPO-activity above the range reported for controls, while showing relatively higher TWBC. Septic arthritis had the highest TWBC (range: 13 100 - 29 800 cells/µl) and MPO mean activity (mean: 6, 133 ± 3, 721) of the present work, followed by the group of tenosynovitis with TWBC ranging from 400 – 3200 cells/µl and a mean MPO activity of 3, 3 ± 2, 330 mU/ µl.

An interesting fact in the present study is the overlapping of MPO-activity values detected in the majority of samples from the groups of chronic arthritis (no radiographic findings) and osteoarthritis (positive osteoarthritic radiographic findings) (Figure 7a). The role of the products of the MPO/H_20_2/Cl^- system on the destruction of joint cartilage and synovial molecules has been widely demonstrated (BAKER et al. 1989; KOWANKO et al. 1989; GREEN et al. 1990; DAVIES et al. 1993; SCHILLER et al. 1996; OLSZOWSKI et al. 2003). Therefore, the overlapping of values of the groups with (osteoarthritis) and without (chronic

arthritis) radiographic findings might be seen at first as an inconsistency, since a higher activity of MPO would be expected in the group showing radiographic evidence of osteoarthritis. However, our results are not surprising considering the report of a negative correlation between concentration of MPO in human rheumatoid synovia and radiographic findings of cartilage degradation (HADLER et al. 1979). The development of radiographic findings is the results of damage to joint structures inflicted by disease over time. Conversely, data from synovial fluid analysis depends on the stadium and severity of disease at the time of arthrocentesis and provides information about the joint status at a single point of time. While comparing MPO-activity in these two groups it should also be borne in mind that low counts of total white blood cells were detected in both groups and that activated neutrophils are the only source of MPO (WEISS 1989; KLEBANOFF 1999).

However, factors other than the total white blood cell count in synovia and the degree of activation of neutrophils may also have had an effect on the results of the present work. EDWARDS et al. (1988) determined the **concentration** and **activity** of MPO in the cell-free supernatant in synovia samples obtained from rheumatoid knees of human patients. These authors measured the activity of MPO with an **enzymatic assay,** while the concentration was measured by an **immunological technique** (rocket immunoloelectrophoresis). These authors could detect the presence of the enzyme MPO in all 8 synovia samples by using the immunological technique, but the enzymatic assay could only detect positive MPO **activity** in 1 of the samples. In other words, the abnormal presence of the enzyme could indeed be identified by immunological methods but enzymatic assays failed to detect enzyme activity. This was due to the oxidative inactivation of the MPO once liberated into the extracellular space, in this case, the synovial fluid (EDWARDS et al. 1987, 1988). This inactivation of MPO outside the neutrophils has also been reported by other authors (VISSERS and WINTERBOURN 1987; KING et al. 1997) and it is believed to be a mechanism of control of the degradative capacity of neutrophils to host tissues. In the present work, activity of MPO was assessed with an **enzymatic assay** in the cell-free supernatant of synovia after centrifugation and it was based on the peroxidation of o-dianisidine by MPO (FIETZ et al. 2004), so that only **activity** of MPO which had been already liberated from activated neutrophils into the extracellular space was measured. Detection of activity of MPO based in the peroxidative activity of the enzyme (e.g. o-dianisidine) grossly underestimates the actual presence of MPO in the extracellular milieu (EDWARDS et al. 1987). It is probable that, at

least in some cases, the activity of MPO as detected in the current work did not accurately reflect the actual presence of the enzyme in the synovia of patients of the present study.
Finally, the influence of other factors on the detection of activity of MPO should also be mentioned. Trials have shown that the concentration of biomolecules in the synovia may be influenced by changes in the volume flux through the joint (effusion) (LEVICK 1992) and by changes in the clearance rate of proteins provoked by synovitis (MYERS et al. 1996). It is possible that these factors may have influenced to some extent the presence of MPO in synovia and thus the activity of the enzyme detected. Unfortunately, to the best of our knowledge, no study regarding the metabolism or half life of MPO within the joint has been performed.

In light of the information presented above, it seems that several factors may have influenced the activity of MPO in synovia. With the MPO-activity as the sole criterion, it would have been impossible to differentiate at least 50% of samples from the groups of chronic arthritis, osteoarthritis, OCD + osteoarthritis and 75% of samples with OCD from normal controls in the present study. MPO-activity in synovia of septic arthritis (n=3) and in the majority of samples with tenosynovitis was higher than in healthy controls but overlapped with activities detected in a few samples from joints diagnosed with non-septic joint diseases.

6.2.5. Viscosity of synovia

Viscosity of synovia is determined by the concentration of hyaluronate and its degree of polymerization (PERSSON 1971; van PELT 1974; GÄNGEL 1980). In disease, the viscosity of synovia may decrease as a result of a dilution effect in joint effusion, synthesis of a low-quality hyaluronate by inflamed synoviocytes (EISENMENGER 1974) and the destruction of the hyaluronate molecule (McILWRAITH 1987; GREEN et al. 1990; PALMER and BERTONE 1994; MAHAFFEY 2002).

In the horse, the viscosity of synovia in joints affected with non-septic joint diseases varies from normal to decreased or severely decreased (GÄNGEL 1980; TEW and HOTCHKISS 1981; SANDER 1990; MAHAFFEY 2002). This was confirmed in the present study, as the great majority of synovia from the group of osteoarthritis (n=10/12; 83, 3%), OCD (n= 14/15; 93, 3%), OCD + osteoarthritis (n=3/4; 75%) and all control samples showed a normal viscosity. The group of chronic arthritis had 57, 1% (n= 16/28) of its samples with a severely

decreased viscosity (non-stringing synovia), whereas the other 35, 7 % (n= 10/28) showed normal viscosity. The group of tenosynovitis had 50% of their observations (n= 4/8) below 3 cm and 37, 5% (n=3/8) of samples had a severely decreased viscosity. These results may probably reflect a greater degree of synovitis in these two groups.

All of the synovial samples taken from septic joints (n=3) showed severely decreased viscosity. This is in accordance with the literature reporting severely decreased viscosity in septic joints (REJNÖ 1976; HERTSCH 1983; McILWRAITH et al. 2001).

It is worth mentioning that synovial viscosity as assessed in this study is a subjective parameter and a decreased viscosity only suggests the presence but not necessarily the degree of inflammation (TROTTER and McILWRAITH 1996). Moreover, experimental data have revealed that viscosity does not always correlate to the rheologic properties of synovia (REJNÖ 1976).

6.2.6. Degree of lameness

All samples from healthy controls and the majority of samples from joints with OCD (n=10/15; 66, 7 %) came from sound limbs. This has been reported in the equine literature, as OCD may be a silent disease showing no clinical signs of illness (SANDER 1990; HERTSCH 1991; McILWRAITH 2002). Samples of the group of OCD + osteoarthritis showed a mild (n=3/4; 75%) and moderate (n=1/4; 25%) lameness. On the other hand, the majority of samples from chronic arthritis (n= 26/28; 92, 8%) came from joints of limbs showing doubtful (n= 13/28; 46, 4%) or mild (n= 13/28; 46, 4%) lameness.

The majority of horses of the group with osteoarthritis showed a mild (n= 4/12; 33, 3%) to moderate (n= 3/12; 25 %) degree of lameness, whereas 25% of the observations (n= 3/12) showed no signs of lameness. These 3 sound horses from the group of osteoarthritis were animals presented for a follow-up lameness examination. Severe type of lameness was only observed in all 3 samples from the group of septic arthritis. Severe lameness is a consistent feature of septic arthritis (BERTONE 1996; HAGUE and CARTER 1999).

6.2.7. Relationship between pH and MPO-Activity

Samples with the same activity of MPO showed different pH values, independent of their diagnosis (Figure 8). Based on these results, there seems to be no relationship between pH and MPO-activity.

6.2.8. Relationship between total protein concentration and MPO-Activity

Independent of their diagnosis, samples with the same content of total protein showed different activities of MPO. There does not seem to be any relationship between total protein concentration and MPO-activity in synovia as detected in the present study (Figure 9).

6.2.9. Relationship between total white blood cell count and MPO-activity

The relationship between total white blood cell count and MPO-activity in synovia was analyzed independently of their diagnosis, as shown on figure 10. There seems to be no relationship between TWBC and activity of MPO. Factors probably related to the degree of activation of neutrophils and the deactivation of the enzyme in the extracellular milieu may possibly explain this fact (see discussion about MPO-activity above).

6.2.10. Relationship between the viscosity of synovia and MPO-activity

The relationship between activity of MPO and the viscosity of synovial fluid was analyzed irrespective of the clinical diagnosis. Samples with normal viscosity (n=56) had the lowest MPO-activity with a mean value of 0,668 ± 1, 520 mU/µl (range: 0 – 8, 1 mU/µl). The mean MPO-activity in the groups with reduced viscosity was higher, as the groups with decreased viscosity (<3 cm) (n=9) had a mean MPO-activity of 3, 211 ± 3, 737 mU/µl (range: 0 – 12 mU/µl) and the group with severely decreased viscosity (non-stringing synovia) showed a mean activity of 2, 483 ± 3, 091 mU/µl (range: 0 – 10, 2 mU/µl). However, as shown on figure 11, more than 50% of synovial samples with severely decreased viscosity overlapped with samples with decreased and physiologically normal viscosity. Therefore, it was impossible to differentiate at least 50% of samples with decreased or severely decreased synovia from samples with normal viscosity based on the activity of MPO.

As mentioned before, the destructive effect of the MPO/H_20_2/Cl^- system-products on the hyaluronate molecule of synovial fluid has been demonstrated (BAKER et al. 1989; GREEN et al. 1990). Despite this evidence, it is difficult to quantify the role of the MPO/H_20_2/Cl^- on the reduction of the synovial viscosity in the patients of the current study. The overlap of MPO-activity between groups with physiologically normal or pathologically decreased viscosity in the present work possibly suggests that factors other than the MPO/H_20_2/Cl^- system may have led also to a reduction of the viscosity. At the same time, it could also means that features that may have influenced the detection of the presence of MPO in this work should also be considered when comparing MPO activity and viscosity (see discussion about MPO-activity above).

According to our results, there does not seem to be any relationship between the viscosity of synovia and the activity of MPO.

6.2.11. Relationship between the degree of lameness and MPO-activity

Recent scientific data may suggest that products of the MPO/H_20_2/Cl^- system could modify the transmission of stimulation to mechanosensory nerve channels of nociceptive nerve terminals within diseased joints. This evidence suggest that synovial hyaluronate surrounds all neural elements of joint tissue, acting as an elastoviscous filter to the transmission of mechanical forces and inflammation-mediated stimuli of nociceptors, while degraded hyaluronate may not fulfil this protective function as a result of its decreased rheologic (elastoviscous) characteristics (POZO et al. 1997; de la PEÑA et al. 2002). In view of the fact that the role of the MPO/H_20_2/Cl^- system-products in the degradation of the hyaluronate molecule of synovia has been demonstrated (BAKER et al. 1989; GREEN et al. 1990), it could be hypothesized that the depletion of hyaluronate by the MPO/H_20_2/Cl^- system may be involved in the painful sensation of diseased joints and tendon sheaths in the horse.

In the current study, the relationship between the activity of MPO in synovia and the degree of lameness was analyzed irrespective of the clinical diagnosis. The group of sound joints (n=32) presented the lowest mean MPO-activity (0, 538 ± 1, 505 mU/µl; range: 0 – 8, 1 mU/µl). Mean activity of MPO was higher in all 4 groups of samples with different degrees of lameness, while the highest mean values were reported in the group of moderate (n=8) (Mean: 2, 750 ± 2, 755 mU/µl; range: 0, 0 – 7, 3 mU/µl) and severe lameness (n=3) (Mean: 6, 133 ±

3, 721 mU/ µl; range: 2, 9 – 10, 2 mU/ µl). The group with severe lameness corresponds to the three samples obtained from septic joints. Unfortunately, no animal diagnosed with non-septic joint disease presented a severe lameness in the present study. It would have been interesting to observe MPO-activity in a joint with non-septic disease and presenting a severe degree of lameness, as the number of leukocytes and degree of synovitis would be expected to be lower than in a septic joint. The number of samples was low in the groups of moderate (n=8) and severe (n=3) lameness when compared to the size of the other groups. This should be considered when evaluating these results.

In spite of these higher mean values of MPO-activity in synovia from lame limbs when compared to MPO-activity detected in synovia obtained from sound limbs, minimum values of MPO-activity observed in the groups of sound and doubtful, mild and moderate degrees of lameness were the same (0 mU/µl), while at least 50% of the observations of these groups overlapped with values detected in synovia obtained from sound joints.

Pain perception is the consequence of intricate and multiple interactions between local events in the different joint structures, pain sensitisation, cortical experience of pain and the influence of everyday activity (DIEPPE and LOHMANDER 2005). When considering the evidence of POZO et al. (1997) and de la PEÑA et al. (2002), it must be borne in mind that the MPO/H_20_2/Cl^- system may not be the only factor associated with the presence of a molecule of hyaluronate with decreased rheological characteristics. On the other hand, the results presented by POZO et al. (1997) and de la PEÑA et al. (2002) are based on experimental trials performed on acute cases and therefore may not be extrapolated to cases of chronic disease. Furthermore, this mechanism may only explain the pathway effect of the destruction of hyaluronate and painful stimuli from innervated joint structures that are in direct contact with the synovial fluid hyaluronate, i.e. the synovial membrane and intraarticular ligaments (articular cartilage is an aneural structure and consequently it cannot generate pain). Yet besides the synovial membrane and intraarticular ligaments, other joint structure that are not in contact with synovia, like the fibrous capsule, periostium and/or the subchondral bone, may also be the source of pain (LOZADA and ALTMAN 1997; DIEPPE and LOHMANDER 2005) whereas the contribution of each of these structures to the overall painful sensation varies among individuals and may depend on the stage of the disease and its particular anatomic situation (CARON 1996).

It is not possible to quantify precisely the relative potential contribution of the MPO/H_20_2/Cl^- system-dependent and independent sources of pain in joint structures in the patients of this study with the information at our disposal. In addition, factors possibly influencing the detection of the presence MPO in synovia (see discussion about MPO-activity above) should as well be considered when comparing the relation between MPO-activity and the different degrees of lameness.

According to our results, there does not seem to be any relationship between MPO-activity and the degree of lameness in the equines studied in the present work.