

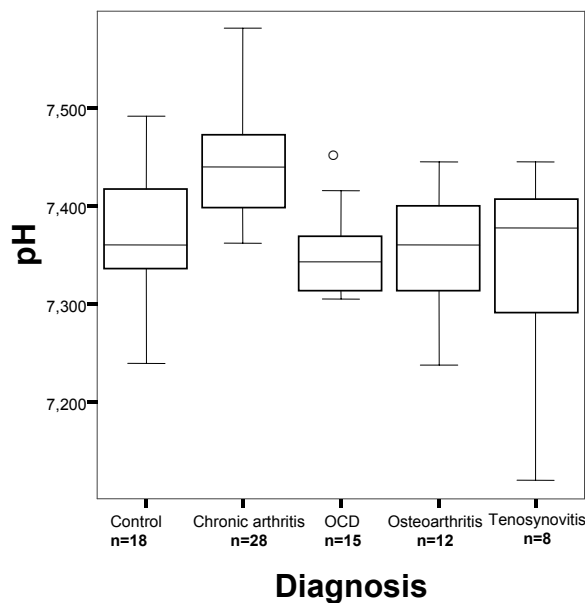
## 5. Results

### 5.1. pH

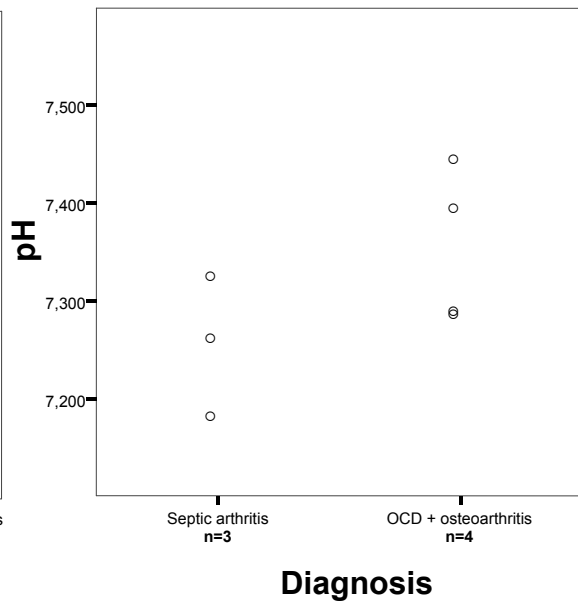
The pH mean value for the control group was  $7,377 \pm 0,062$  (range: 7, 239 – 7, 491). The group of samples with chronic arthritis had the highest mean value among the diseased groups ( $7, 446 \pm 0, 060$ , range: 7, 362 – 7, 580), while the septic arthritis the lowest ( $7, 274 \pm 0, 071$  range: 7, 200 – 7, 342) (Table 8). As shown on figures 4a and 4b, at least 75% of individual values of the groups with chronic arthritis, osteoarthritis, OCD, OCD + osteoarthritis and tenosynovitis fell within the range observed in the controls. This was also the case for 2 of the 3 samples diagnosed with septic arthritis.

**Table 8. pH values in synovia in the groups of controls and patients**

Diagnosis	n	Minimum	Maximum	Mean $\pm$ S.D.	Median	Percentiles	
						25%	75%
Control	18	7,239	7,491	$7,377 \pm 0,062$	7,360	7,336	7,425
Chronic arthritis	28	7,362	7,580	$7,446 \pm 0,060$	7,439	7,396	7,476
OCD	15	7,305	7,451	$7,351 \pm 0,044$	7,343	7,314	7,370
Osteoarthritis	12	7,237	7,445	$7,352 \pm 0,062$	7,361	7,306	7,400
Septic arthritis	3	7,200	7,342	$7,274 \pm 0,071$	7,280	7,200	7,342
OCD+osteoarthritis	4	7,300	7,456	$7,368 \pm 0,078$	7,357	7,301	7,445
Tenosynovitis	8	7,120	7,445	$7,339 \pm 0,105$	7,377	7,285	7,412



**Figure 4a. pH values in the groups with 6 or more samples**



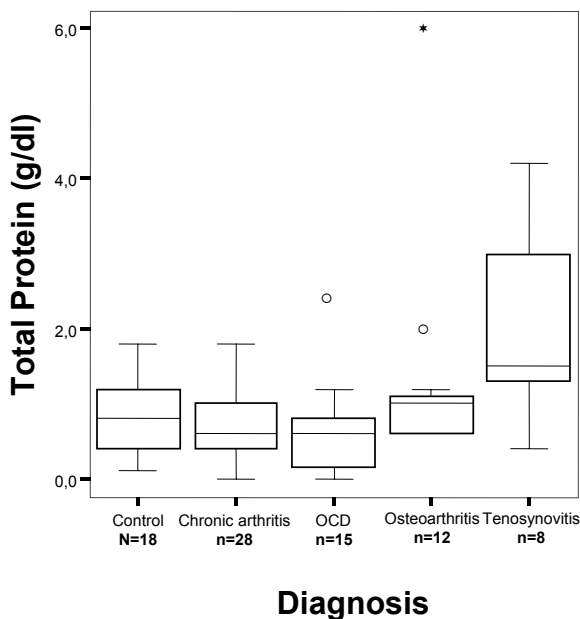
**Figure 4b. pH values in the groups with 5 or less samples**

## 5.2. Total protein concentration

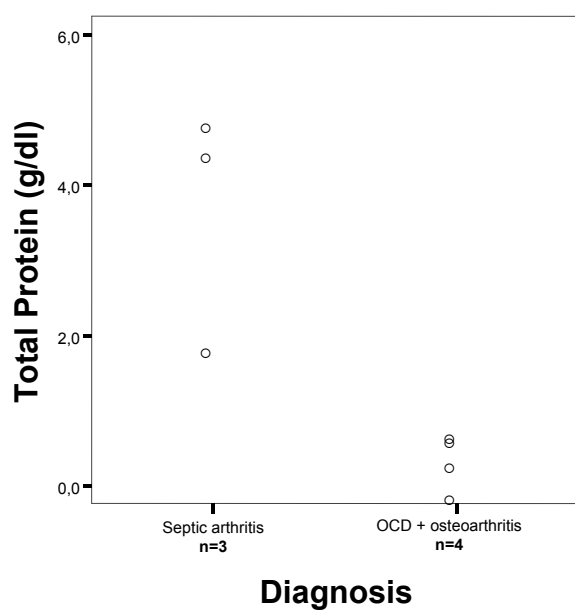
Synovial fluid total protein mean concentration in the control group was  $0,867 \pm 0,491$  g/dl (range: 0,1 – 1,8 g/dl). The group of septic arthritis had the highest mean total protein concentration (Mean:  $3,867 \pm 1,629$  g/dl; range: 2,0 – 5,0 g/dl), followed by the groups of tenosynovitis (Mean:  $2,025 \pm 1,358$  g/dl; range: 0,4 – 4,2 g/dl) and osteoarthritis (Mean:  $1,367 \pm 1,511$  g/dl; range: 0,6 – 6,0 g/dl) (Table 9). As shown in figures 5a and 5b, the majority of values of all groups, except for the septic arthritis and tenosynovitis overlapped with the range of total protein observed within the controls. Moreover, at least 75% of values from chronic arthritis, osteoarthritis, OCD and OCD + osteoarthritis were below 1,2 g/dl.

**Table 9. Total protein concentration (g/dl) in the groups of control and patients**

Diagnosis	n	Minimum	Maximum	Mean $\pm$ S.D.	Median	Percentiles	
						25%	75%
Control	18	0,1	1,8	$0,867 \pm 0,491$	0,800	0,400	1,200
Chronic arthritis	28	0,0	1,8	$0,671 \pm 0,443$	0,600	0,400	1,000
OCD	15	0,0	2,4	$0,660 \pm 0,621$	0,600	0,000	0,800
Osteoarthritis	12	0,6	6,0	$1,367 \pm 1,511$	1,000	0,600	1,150
Septic arthritis	3	2,0	5,0	$3,867 \pm 1,629$	4,600	2,000	5,000
OCD+osteoarthritis	4	0,0	0,8	$0,500 \pm 0,383$	0,600	0,100	0,800
Tenosynovitis	8	0,4	4,2	$2,025 \pm 1,358$	1,500	1,250	3,500



**Figure 5a. Total protein values in the groups with 6 or more samples**



**Figure 5b. Total protein values in the groups with 5 or less samples**

### 5.3. Total white blood cell count

The group of controls showed a maximum total white blood cell count (TWBC) of 500 cells/ $\mu$ l. The groups of controls, chronic arthritis, osteoarthritis, OCD and OCD + osteoarthritis show the same minimal value (100 cells / $\mu$ l). Moreover, 75% of all observation of these groups remained below 200 cells/ $\mu$ l (Figures 6a/b/c). The group of samples with tenosynovitis showed a TWBC with a range of 400 – 3200 cells/ $\mu$ l. Septic arthritis showed the highest values of this study, with TWBC ranging from 13 100 cells/ $\mu$ l to 29 800 cells/ $\mu$ l (Table 10; Figure 6b).

**Table 10. Total white blood cell count (cells/ $\mu$ l) in the groups of controls and patients**

Diagnosis	n	Minimum	Maximum	Median	Percentiles	
					25%	75%
Control	18	100	500	100	100	100
Chronic arthritis	28	100	1800	100	100	200
OCD	15	100	100	100	100	100
Osteoarthritis	12	100	400	100	100	100
Septic arthritis	3	13100	29800	22200	13100	29800
OCD+osteoarthritis	4	100	100	100	100	100
Tenosynovitis	8	400	3200	550	500	2000

**Note:** The value of 100 cells/ $\mu$ l on table 10 was assigned to samples with total white blood cell counts below the background threshold of the automated cell couler (200 cells/ $\mu$ l).

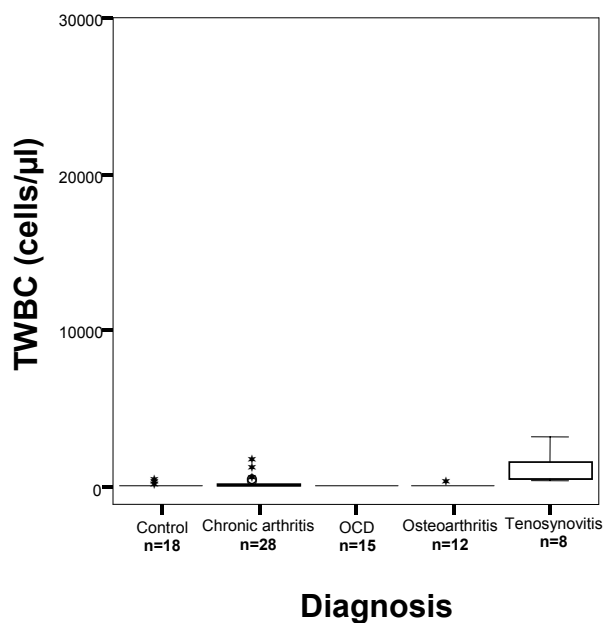


Figure 6a. TWBC in the groups with 6 or more samples

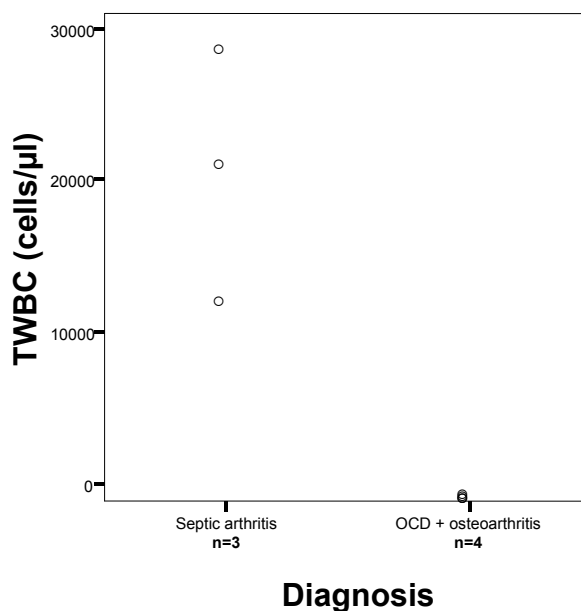


Figure 6b. TWBC in the groups with 5 or less samples

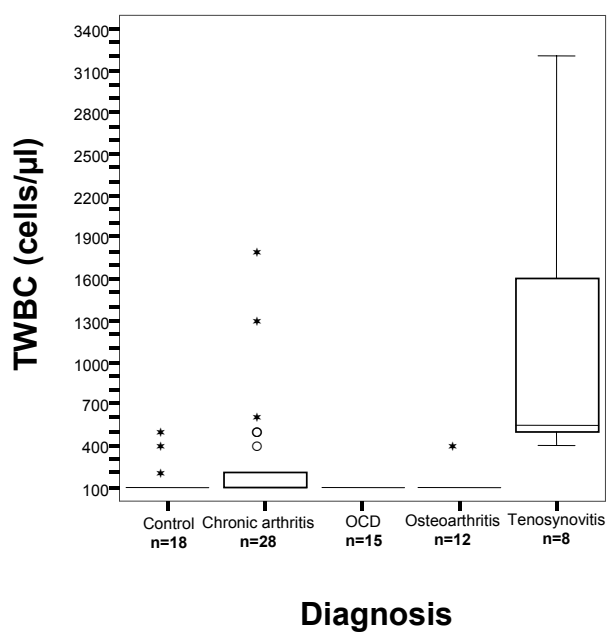


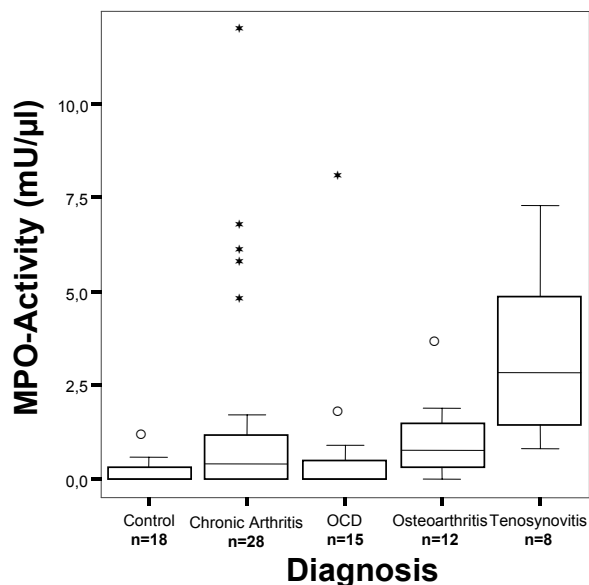
Figure 6c. TWBC in the groups with 6 or more samples using a different scale for the Y-axis than the one used in figure 6a.

## 5.4. MPO-Activity

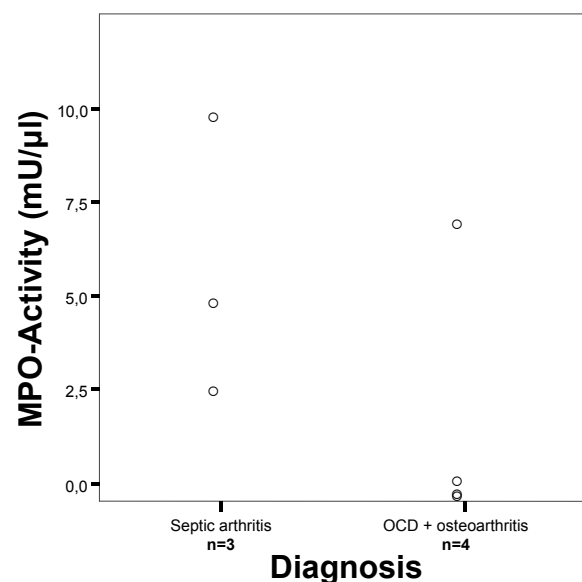
The lowest mean activity of MPO was detected in the control group (mean  $0,150 \pm 0,313$  mU/ $\mu$ l, range: 0,0 – 1,2 mU/ $\mu$ l), while 75% of the samples of controls showed activity of the enzyme below 0,3 mU/ $\mu$ l. All categories of diseased joints and tendon sheaths had mean values of MPO activity that were greater than that of the control group. At least 50% of the samples with chronic arthritis, osteoarthritis and OCD + osteoarthritis and 75% of samples with OCD presented MPO-activities that were within the range observed in the group of controls (Figures 7a and 7b). All samples from the groups with septic arthritis and at least 75% of the samples from the group of tenosynovitis reported an MPO-activity above the range observed within healthy controls, but these values overlapped with activities of the enzyme detected in a few samples in the groups with chronic arthritis, OCD, osteoarthritis and OCD+ osteoarthritis.

**Table 11. MPO-Activity (mU/ $\mu$ l) in the groups of controls and patients\***

Diagnosis	n	Minimum	Maximum	Mean $\pm$ S.D.	Median	Percentiles	
						25%	75%
Control	18	0,0	1,2	$0,150 \pm 0,313$	0,000	0,000	0,300
Chronic arthritis	28	0,0	12,0	$1,571 \pm 2,875$	0,400	0,000	1,275
OCD	15	0,0	8,1	$0,787 \pm 2,087$	0,000	0,000	0,700
Osteoarthritis	12	0,0	3,7	$1,025 \pm 1,047$	0,750	0,250	1,600
Septic arthritis	3	2,9	10,2	$6,133 \pm 3,721$	5,300	2,900	10,200
OCD+osteoarthritis	4	0,0	7,3	$1,950 \pm 3,574$	0,250	0,000	5,600
Tenosynovitis	8	0,8	7,3	$3,300 \pm 2,330$	2,850	1,425	5,525



**Figure 7a. MPO-Activity values in the groups with 6 or more samples\***



**Figure 7b. MPO-Activity values in the groups with 5 or less samples\***

\*All measurements of MPO presented here were performed by Simone Fietz or coworkers of the Institute of Veterinary Biochemistry of the Faculty of Veterinary Medicine of the Freie Universität Berlin and kindly provided based on a current scientific collaboration.

### 5.5. Viscosity of synovia

As shown on table 12, all synovia samples from the group of controls presented a normal viscosity (> 3cm). This was also the case for samples from the majority of joints afflicted with OCD (n=14/15; 93, 3%), osteoarthritis (n=10/12; 83, 3%), OCD + osteoarthritis (n=3/4; 75%). The majority of samples from the group of chronic arthritis (n= 18/28; 62, 2%) and tenosynovitis (n= 7/8; 87, 5%) had pathologically reduced viscosity. All samples obtained from septic joints (n=3) presented severely decreased viscosity.

**Table 12. Distribution of the degrees of synovial viscosity in the groups of controls and patients**

Diagnosis	n	Synovial Viscosity		
		*Normal	*Decreased	*Severely decreased
Control	18	18 (100%)	-	-
Chronic arthritis	28	10 (35,7%)	2 (7,1%)	16 (57,1%)
OCD	15	14 (93,3%)	1 (6,7%)	-
Osteoarthritis	12	10 (83,3%)	2 (16,7%)	-
Septic arthritis	3	-	-	3 (100%)
OCD + osteoarthritis	4	3 (75%)	-	1 (25%)
Tenosynovitis	8	1 (12,5%)	4 (50%)	3 (37,5%)

\*Normal: Synovial strand > 3 cm

Decreased: Synovial strand < 3 cm

Severely decreased: Non-stringing fluid

## 5.6. Degree of lameness

The table below presents the prevalence of the different degrees of lameness in each diagnostic group. All samples from the control group as well as the majority of samples of the OCD group (n = 10/15; 66, 7%) came from joints of sound limbs. This was also the case for 3 samples (25%) from the group diagnosed with osteoarthritis. Most of the samples from joints with chronic arthritis (n = 26/28; 92, 2%) were equally distributed in doubtful and mild degrees of lameness, whereas the great part of samples coming from joints diagnosed with osteoarthritis, OCD + osteoarthritis and tenosynovitis showed mild to moderate lameness. Severe degree of lameness was only observed in samples taken from joints affected with septic arthritis. Non-weight-bearing type of lameness was not observed in the present study.

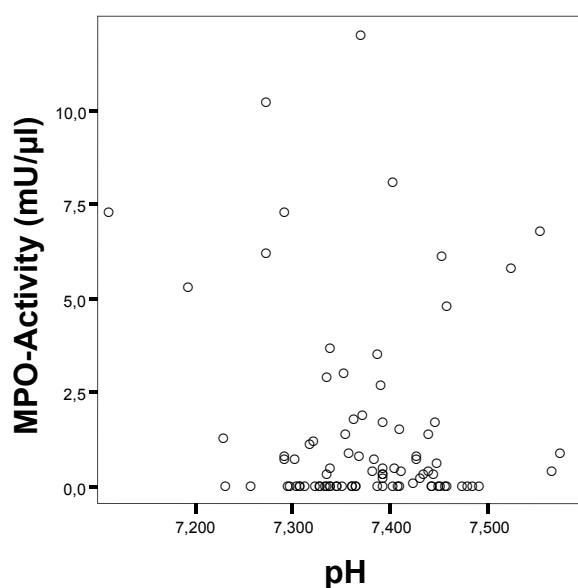
**Table 13. Distribution of the different degrees of lameness in the groups of controls and patients**

Diagnosis	n	Degree of Lameness				
		Sound	Doubtful	Mild	Moderate	Severe
Control	18	18 (100%)	-	-	-	-
Chronic arthritis	28	-	13 (46, 4%)	13 (46, 4%)	2 (7, 1%)	-
OCD	15	10 (66, 7%)	4 (26, 7%)	1 (6,7%)	-	-
Osteoarthritis	12	3 (25%)	2 (16,7%)	4 (33, 3%)	3 (25%)	-
Septic arthritis	3	-	-	-	-	3 (100%)
OCD + osteoarthritis	4	-	-	3 (75%)	1 (25%)	-
Tenosynovitis	8	1 (12, 5%)	2 (25%)	3 (37, 5%)	2 (25%)	-

### 5.7. Relationship between pH and MPO-activity

Figure 8 shows the values of pH of each sample and their correspondent activity of MPO, irrespective of their diagnostic group. As shown below, samples with the same pH value showed different activity of MPO.

pH	MPO (mU/ $\mu$ l) *
7,265	0
7,311	0
7,400	0
7,491	0
7,390	0,7
7,310	0,7
7,434	0,7



**Figure 8: Relationship between synovial pH and MPO-Activity \***

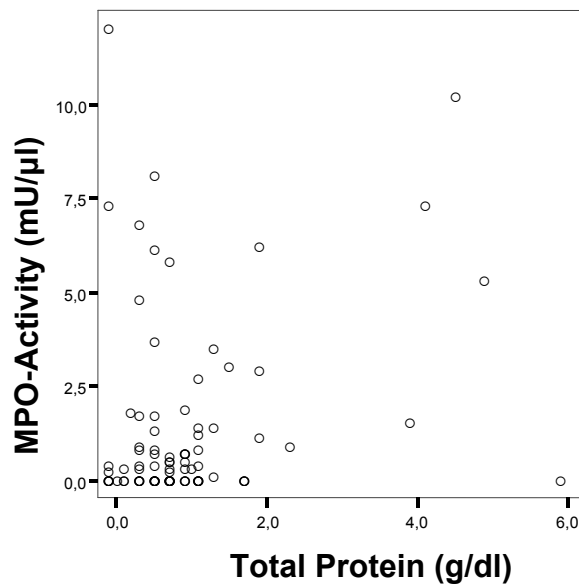
\*All measurements of MPO presented here were performed by Simone Fietz or coworkers of the Institute of Veterinary Biochemistry of the Faculty of Veterinary Medicine of the Freie Universität Berlin and kindly provided based on a current scientific collaboration.



### 5.8. Relationship between total protein concentration and MPO-activity

The total protein content and MPO-Activity of each sample is shown on the dispersion diagram in figure 9, irrespective of their diagnostic group. As shown below, different activities of MPO were detected in samples with the same content of total protein.

Total Protein (g/dl)	MPO-Activity (mU/μl) *
0, 6	0
0, 6	1, 3
0, 6	3, 7
0, 6	8, 1



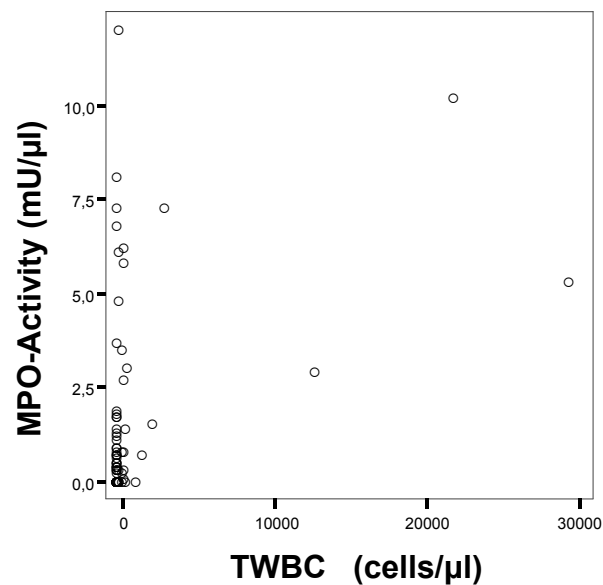
**Figure 9: Relationship between synovial total protein concentration and MPO-activity \***

\*All measurements of MPO presented here were performed by Simone Fietz or coworkers of the Institute of Veterinary Biochemistry of the Faculty of Veterinary Medicine of the Freie Universität Berlin and kindly provided based on a current scientific collaboration.

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

Total count of leukocytes and the MPO-Activity detected in each synovia sample is shown in figure 10, irrespective of the diagnosis. A different activity of MPO was detected in samples with the same total number of leukocytes.

TWBC (cells/ $\mu$ l)	MPO-Activity (mU/ $\mu$ l) *
200	0
200	4, 8
200	12



**Figure 10: Relationship between synovial total white blood cell count and MPO-activity \***

\*All measurements of MPO presented here were performed by Simone Fietz or coworkers of the Institute of Veterinary Biochemistry of the Faculty of Veterinary Medicine of the Freie Universität Berlin and kindly provided based on a current scientific collaboration.

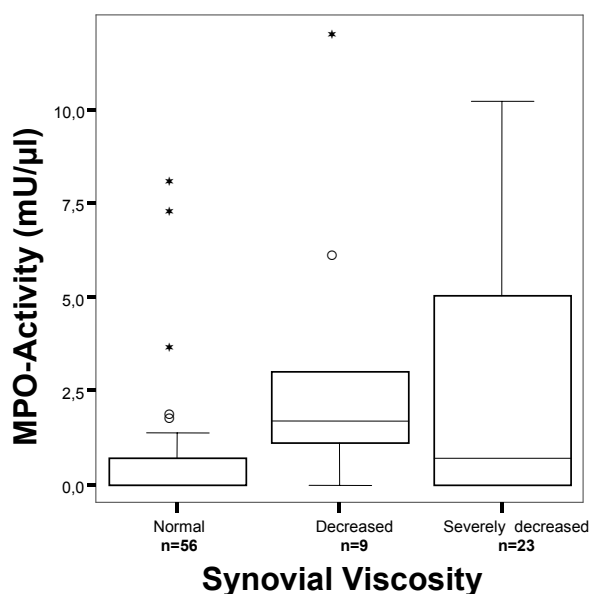
### 5.10. Relationship between viscosity of synovia and MPO-activity

Synovial samples showing a normal viscosity (> 3 cm) (n=56) presented the lowest mean value of MPO-activity from the 3 groups of viscosity (mean: 0, 668 ± 1, 520 mU/μl; range: 0 – 8, 1 mU/μl) (Table 14). The mean MPO activity was higher in the two groups of samples with pathologically reduced viscosity, being maximal in the group presenting severely decreased viscosity (n=23) (mean: 2, 483 ± 3, 091 mU/μl; range: 0 – 10, 2 mU/μl) (Table 14). All three groups presented the same minimum of MPO-activity (0 mU/μl) and at least 50% of the MPO-activity detected in samples with normal, decreased and severely decreased viscosity overlapped with each other (Figure 11).

**Table 14. MPO-Activity (mU/μl) in samples with different degrees of synovial viscosity \*\***

Viscosity	n	Minimum	Maximum	Mean ± S.D.	Median	Percentiles	
						25 %	75 %
*Normal	56	0,0	8,1	0,668 ± 1,520	0,000	0,000	0,700
*Decreased	9	0,0	12,0	3,211 ± 3,737	1,700	0,950	4,550
*Severely decreased	23	0,0	10,2	2,483 ± 3,091	0,700	0,000	5,300

\* Normal: Synovial strand > 3 cm  
 Decreased: Synovial strand < 3 cm  
 Severely decreased: Non-stringing fluid



**Figure 11. MPO-Activity in samples showing different degrees of synovial viscosity \*\***

\*\*All measurements of MPO presented here were performed by Simone Fietz or coworkers of the Institute of Veterinary Biochemistry of the Faculty of Veterinary Medicine of the Freie Universität Berlin and kindly provided based on a current scientific collaboration.

### 5.11. Relationship between the degree of lameness and MPO-activity

From table 15, it can be seen that the size of the groups of different degrees of lameness was uneven. The group of samples obtained from joints of sound limbs (n=32) showed the lowest mean activity of MPO ( $0,538 \pm 1,505$  mU/ $\mu$ l; range: 0 – 8,1 mU/ $\mu$ l), whereas mean values were higher in all groups of samples from lame joints. The highest mean activity of MPO was detected in the group presenting a severe lameness (n=3) (mean:  $6,133 \pm 3,721$  mU/ $\mu$ l; range: 2,9 – 10,2 mU/ $\mu$ l). Minimum MPO-activity detected in the groups of sound limbs and in the groups presenting doubtful, mild and moderate lameness was the same (0 mU/ $\mu$ l). Moreover, the median of the groups with doubtful, mild and moderate lameness are smaller than the maximum value detected in the group of sound horses (8,1 mU/ $\mu$ l). Therefore, at least 50% of all observation from the groups of doubtful, mild and moderate lameness overlapped with the range of activity of MPO detected in the group of sound limbs.

**Table 15. MPO-Activity (mU/ $\mu$ l) detected in samples taken from limbs showing different degrees of lameness \***

Degree of Lameness	n	Minimum	Maximum	Mean $\pm$ S.D.	Median	Percentiles	
						25%	75%
<b>Sound</b>	32	0,0	8,1	$0,538 \pm 1,505$	0,000	0,000	0,450
<b>Doubtful</b>	21	0,0	7,3	$1,481 \pm 2,454$	0,400	0,000	1,300
<b>Mild</b>	24	0,0	12,0	$1,446 \pm 2,622$	0,600	0,000	1,475
<b>Moderate</b>	8	0,0	7,3	$2,750 \pm 2,755$	2,050	0,350	5,450
<b>Severe</b>	3	2,9	10,2	$6,133 \pm 3,721$	5,300	2,900	10,200

\*All measurements of MPO presented here were performed by Simone Fietz or coworkers of the Institute of Veterinary Biochemistry of the Faculty of Veterinary Medicine of the Freie Universität Berlin and kindly provided based on a current scientific collaboration.