Pferdeheilkunde 26 (2010) 4 (Juli/August) 541-552

Histological evaluation of intraarticular osteochondral fragments

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Summary

Scientific studies, investigating the aetiology of osteochondrosis focus mainly on the articular-epiphyseal cartilage complex and the growth plate. In osteochondrosis dissecans cleft formation through the necrotic cartilage leads to the formation of intraarticular osteochondral fragments. In contrast to osteochondral fragments located at typical predilection sites, a causative allocation of fragments located at different sites may be more challenging. Only limited numbers of studies have focused on the histological appearance of osteochondral fragments. They are mainly limited to the identification of types of tissue present, but do not further investigate possible specific characteristics and differences of involved components.The aim of the present study was to histologically evaluate osteochondral fragments more in detail to identify possible characteristics which may allow a causative and topographical allocation. A total of 76 osteochondral fragments were examined by two blinded observers. Signs of bone remodeling, enchondral ossification and proteoglycan staining properties were graded semi-quantitatively. The appearance of fibrous tissue or enchondral ossification at the separation border as well as the presence of ligamentous attachments were graded qualitatively. The articular cartilage present was evaluated applying the Mankin score. Significant differences in enchondral ossification (within the fragment and at the separation border), proteoglycan staining properties, the appearance of fibrous tissue at the separation border as well as differences in bone remodeling and in the Mankin scores were detected in osteochondral fragments from different locations. It was concluded that histological evaluation of osteochondral fragments may help in their aetiological allocation and may contribute to future understanding of the disease.

Keywords: osteochondrosis, osteochondral fragment, bone remodelling, enchondral ossification, histology

Histologische Evaluierung von intraartikulären osteochondralen Fragmenten

Charakteristisches Merkmal der Osteochondrose ist eine Störung der enchondralen Ossifikation im artikulär-epiphysären Gelenkknorpel und der Wachstumsfuge. Die Osteochondrosis dissecans ist durch die Entstehung von osteochondralen Fragmenten charakterisiert. Die ätiologische Zuordnung dieser Fragmente ist an den Prädilektionsstellen der Osteochondrose in vielen Fällen zweifelsfrei möglich. Problematisch wird die kausale Zuordnung an, von der Literatur uneinheitlich beurteilten und an für die Osteochondrose untypischen Lokalisationen. Während sich die meisten wissenschaftlichen Studien auf den artikulären-epiphysären Knorpel und die Wachstumszone konzentrieren, existieren nur wenige histologische Untersuchungen der Fragmente. Diese konzentrieren sich überwiegend auf die Klassifizierung der vorhandenen Gewebetypen und nicht auf die innerhalb der einzelnen Gewebearten, möglicherweise für die zugrundeliegende Ätiologie charakteristischen Vorgänge. Ziel der vorliegenden Studie war, anhand einer detaillierten histologischen Analyse, der an der Bildung von osteochondralen Fragmenten beteiligten Gewebeanteilen, mögliche, für die zugrunde liegende Ätiologie charakteristische Veränderungen zu identifizieren. Die Studie basierte auf der Hypothese, dass histologisch identifizierbare Parameter eine topographische sowie eine kausale Zuordnung der intraartikulären osteochondralen Fragmente und damit eine Differenzierung zwischen der Osteochondrose oder einer Fraktur als Entstehungsweg erlauben. Insgesamt 76 osteochondrale Fragmente aus 51 Pferden wurden untersucht. Bei 49 Pferden wurden die Fragmente arthroskopisch entfernt. Vor der Operation wurden die Pferde einer klinischen und radiologischen Untersuchung unterzogen. Nicht dekalzifizierte, mit Toluidinblau oder van Kossa/Mc Neal gefärbte Dünnschnitte wurden von zwei verblindeten Untersuchern histologisch evaluiert. Hinweise für aktiven Knochenumbau im trabekulären und kortikalen Knochen, für enchondrale Ossifikation und die Proteoglykananfärbbarkeit innerhalb der osteochondralen Fragmente wurden semiquantitativ beurteilt. Das Auftreten von fibrösem Gewebe oder Hinweisen auf enchondrale Ossifikation an der Separationsgrenze der Fragmente sowie das Vorhandensein von bandartigen oder gelenkkapselartigen Strukturen wurde qualitativ bewertet. Der hyaline Knorpelanteil wurde auf der Basis des Mankin Score evaluiert. Alle der histologisch untersuchten, in Abhängigkeit von ihrem Herkunftsort in Größe und Form variierenden osteochondralen Fragmente bestanden aus unterschiedlich großen Anteilen von Knochen (trabekulär und kortikal) und hyalinem Knorpel. Die anatomische Zuordnung der Fragmente anhand ihrer Form und der histologischen Kriterien gelang in ca. 80 % aller Fälle und in 100 % der Fragmente die vom lateralen Femur- oder Talusrollkamm und vom Processus extensorius des Hufbeins stammten. Anzeichen für aktiven Knochenumbau konnten am deutlichsten in den Fragmenten, die ihren Ursprung am lateralen Rollkamm des Femur und des medialen Malleolus der Tibia hatten, beobachtet werden. Das geringste Ausmaß an Knochenabbau und Knochenneubildung wurde in Fragmenten des proximoplantaren Aspektes der Fesselbeinlehne, des Sagittalkammes des Metakarpus/Metatarsus im Fesselgelenk, der Gleichbeine und bei traumatisch bedingten Fragmenten erfasst. In diesen und in den vom Processus extensorius des Hufbeins stammenden Fragmenten waren keine bzw. nur sehr geringe Anzeichen für enchondrale Ossifikation und die Anfärbbarkeit der Proteoglykane in den Fragmenten festzustellen. An der Separationsgrenze konnte fibröses Gewebe, aber keine Anzeichen für enchondrale Ossifikation beobachtet werden. Der Mankin Score war bei diesen Fragmenten, mit Ausnahme der vom Sagittalkamm des Metakarpus/Metatarsus relativ hoch. Das verhältnismäßig hohe Maß an enchondraler Ossifikation (in den Fragmenten und an der Separationsgrenze), die relativ gute Anfärbbarkeit der Proteoglykane und der verhältnismäßig niedrige Mankin Score und damit die beste Knorpelqualität bei Fragmenten aus dem Kniegelenk, dem Sagittalkamm der Tibia und dem lateralen Talusrollkamm lassen auf eine unterschiedliche Genese schließen.

Schlüsselwörter: Osteochondrosis, osteochondrale Fragment, Knochenumbau, enchondrale Ossifikation, Histologie

Introduction

The high incidence of osteochondrosis in the horse (up to 25 %) (*Jeffcott* 1993, *van Weeren* 2006b), resulting impact on economy and welfare (*Jeffcott* 1991, *Jeffcott* and *Henson* 1998) and the not totally understood aetiology (*Ekman* et al. 2009) resulting in limited therapeutic and preventive strategies give reason for ongoing research in this field. Generally accepted is the gross pathogenic mechanism of osteochondrosis, representing a disturbance of the process of enchondral ossification at the articular-epiphyseal cartilage complex and the growth plate in young and growing animals (*Ekman* et al. 2009, *van Weeren* 2006b). The initial stage of the disease (osteochondrosis latens) is characterized histologically through focal chondronecrosis of the resting zone with adjacent vascular necrosis. The later stages of the disease, osteochondrosis manifesta, characterized through focally impaired enchondral ossification and cartilage retention and osteochondrosis dissecans with cleft formation through the necrotic cartilage are clinically detectable through radiological examination (*Ekman* et al. 2009).

There is general agreement that osteochondrosis is a multifactorial disease. Beside failure of vascularisation and biomechanical influences, nutritional factors (energy intake and mineral supply) and genetics have all been mentioned as causative factors (*van Weeren* 2006a, b). Whereas failure of vascularisation or biomechanical influences (including exercise) are weighted to be the most important factors in the more recent literature (*Olstad* et al. 2007, *van Weeren* 2006a, *Ytrehus* et al. 2007) other mentioned factors are weighted different and may just have a supportive function.

Osteochondrosis as the underlying cause for osteochondral fragment formation is unquestioned at certain predilection sites. At other locations the question of the causative aetiology, as with osteochondral fragments located at the palmaro-/plantaroproximal border of the proximal phalanx is more or less clearly answered. Several attempts have been made to relate these fragments to osteochondrosis (*Foerner* et al. 1987, *Nixon* 1990, *Sonnichsen* et al. 1982) or to fracture (*Barclay* et al. 1987, *Birkeland* 1972, *Bukowiecki* et al. 1986, *Dalin* et al. 1993, *Nilsson* and *Olsson* 1973, *Nixon* and *Pool* 1995, *Pettersson* and *Ryden* 1982, *Sandgren* 1988).

Studies focus on the early stage of the disease (osteochondrosis latens) to investigate the aetiology of osteochondrosis. However, besides early studies limited to the description of the different types of tissue involved in the formation of osteochondral fragments (*Dalin* et al. 1993, *Nixon* and *Pool* 1995, *Pool* 1993, *Yovich* et al. 1985) and one more recent study comparing dorsal osteochondral fragments of the fetlock joint to osteochondrosis dissecans of the intermediate ridge of the tibia and the lateral femoral ridge by clinical, radiological and histological examination (*Declerq* et al. 2006), numbers of histological studies taking a closer look to events taking part in certain tissue fractions or for possible disease characteristics appear to be limited.

The aim of the present study was to investigate histological properties of different tissue fractions of arthroscopically removed osteochondral fragments more in detail and thereby to identify possible characteristics, which may allow a causative and regional allocation. The study was based on the hypothesis that histological characteristics of the fragments would allow distinction into those with disturbed enchondral ossification (OCD) and those with traumatic fractures as the initiating cause. It was expected that changes would be similar in osteochondral fragments of the same origin at the distal extremities of horses affected with OCD.

Materials and Methods

Osteochondral fragments from horses referred for arthroscopy or at necropsy were collected within a one year period. Before surgery a clinical and radiological examination was carried out. The degree of lameness was recorded according to the AAEP grading system (grade 0–5, grade 0 represents no perceptible lameness, grade 5 accounts for minimal to non weight bearing in motion or at rest). Radiological signs of osteoarthritis in the affected joints were recorded as present or absent. Osteochondral fragments where fixed in 4% buffered formalin for at least 36 hours, dehydrated in an ascending series of alcohol (50-100%) and defatted with xylene. After infiltration of the samples with liquid polymethylmetacrylate (PMMA) in an air-evacuated container at 4 C° for 14 days, they were polymerised with the PMMA in ready to cut sample blocks (*Engelhardt* and *Gasser* 1995, *Leutenegger* et al. 1999). Thin sections of 5 µm were stained with toluidine blue and van Kossa silverstaining counterstained with Mc Neal's tetrachrome (*Engelhardt* and *Gasser* 1995). Thick sections of about 30–40 µm were surface stained with toluidine blue. Histological evaluation by light microscopy was carried out by two blinded observers (BvR, MH). The reviewers tried to allocate the fragments to the different joints and within the joint (e.g. fetlock) to the specific locations (dorsodistal aspect of MC III / MT III, dorsoproximal or plantaroproximal aspect of P1) according to their shape, size and soft tissue attachments, but also according to the sum of histological appearances. Within the osteochondral fragments bone remodelling (trabecular and cortical), enchondral ossification and proteoglycan staining properties where graded quantitatively (grade 0=none, grade 1= little, grade 2=median, grade 3= high). The appearance of fibrous tissue and enchondral ossification activity at the separation border as well as ligamentous attachments (ligaments or part of the joint capsule) where graded qualitatively (existence: 0= no, 1= yes). The articular cartilage of the fragments was evaluated applying the Mankin score (*Mankin* et al. 1971) (Table 1), resulting in low total scores being better than high scores.

Results were analyzed using specialised software for statistical analysis (SPSS 16.0 for MacIntosh). Mean values and standard deviations were calculated and overall differences between groups were analysed with a factorial Univariate Analysis of Variance (ANOVA). Differences between individual groups were assessed with post hoc tests according to Bonferroni. Last, correlations were calculated according to Pearsons. Significance was set with $p<0.05$.

Results

All together, 76 osteochondral fragments out of 72 joints from 51 horses were evaluated. Mean age of horses was 6 years (6 month–to 18 years, median 4 years). Aside from 18

Table 1 Mankin score of the articular cartilage

location	dist. interph.	fetlock				hock				stifle
	P. ext.	dorsodist. MX III	dorsoprox. P1	plantaro-prox. P1	prox. sesamoid bone	sagittal ridge tibia	lat. trochlea talus	med. malleolus tibia	trauma tibia	lat. trochlea femur
structure normal (0) surface irregularities (1) pannus and surface irregularities (2) clefts to transition zone (3) clefts to radial zone (4) clefts to calcified zone (5) complete disorganization (6)	5,78 ± 0,67	4 ± 1,63	3,61 ± 1,85	6 ± 0	4,75 ± 1,89	3,31 ± 1,49	3,6 ± 2,3	6 ± 0	6 ± 0	2,63 ± 0,92
cells normal (0) diffuse hypercellularity (1) cloning (2) hypocellularity (3)	2,78 ± 0,67	1,75 ± 0,96	1,56 ± 0,86	3 ± 0	3 ± 0	1,92 ± 0,76	2 ± 1,0	3 ± 0	3 ± 0	1,38 ± 0,52
metachromasie normal (0) slight reduction (1) moderate reduction (2) severe reduction (3) no dye noted (4)	2,44 ± 1,01	2,5 ± 0,58	1,89 ± 0,76	3,4 ± 0,89	2,75 ± 1,5	2,23 ± 0,44	2,4 ± 1,14	3,5 ± 0,71	4 ± 0	1,88 ± 0,35
tidemark inegrity intact (0) crossed by blood vessels (1)	0,89 ± 0,33	0,75 ± 0,5	0,72 ± 0,46	1 ± 0	0,75 ± 0,5	0,85 ± 0,38	0,6 ± 0,55	1 ± 0	1 ± 0	0,88 ± 0,35
total Mankin (0-14)	11,89± 1,76	9 ± 2,71	7,78 ± 3,42	13,4 ± 0,89	11,25 ± 3,78	8,31 ± 2,25	8,6 ± 4,51	13,5 ± 0,71	14 ± 0	6,75 ± 1,67

Table 2 Distribution of osteochondral fragments

affected joint	number of horses	number of joints	affected structure	number of fragments	forelimb	hindlimb
stifle	6	8	lateral trochlear ridge femur	10	0	10
hock	16	24	sagittal ridge tibia	15	0	15
			lateral trochlear ridge talus	6	0	6
			medial malleolus tibia	3	0	3
			lateral malleolus tibia	1	0	1
fetlock	22	31	dorsodistal MC III / MT III	4	2	2
			dorsoproximal P1	19	14	5
			palmaro-/plantaroproximal P1	5	0	5
			proximal sesamoid bone	4	1	3
distal interphalangeal	7	9	processus extensorius P3	9	8	1
total	51	72	76	76	25	51

Swiss Warmbloods and 14 German Warmbloods, 4 Frisians, 3 French Warmbloods, 2 Irish and 2 Russian Warmbloods and 2 Arabians 1 Dutch Warmblood, 1 Belgian Warmblood, 1 Czech Warmblood, 1 Swiss Freiberger, 1 Shire and 1 Noriker were included into the study. Of those, 49 horses were presented for arthroscopic surgery. One 18 years old horse was euthanized because of P2 fracture of the contralateral limb. The other horse euthanized was a 1-year old Frisian foal with bilateral, progressive and highly destructive osteochondrosis dissecans of the lateral trochlear ridge in both tarsal joints (Fig.1g). Osteochondral fragments investigated originated from the stifle, the hock, the fetlock and the distal interphalangeal joint (Table 2, Fig. 1). Clinically, 35 (70 %) of the horses that underwent arthroscopy for fragment removal showed lameness (30 % grade 1/5, 40 % grade 2/5) correlated to, or in cases with bilateral affection to at least one of the limbs. Radiologic signs of osteoarthritis were found in 24 joints, whereof 20 were fetlock joints (data not shown).

Osteochondral fragments varied in size and shape depending on their origin but even within one group. All fragments investigated histologically contained different amounts of bone (trabecular and cortical) and hyaline cartilage. Within the bone different degrees of bone resorption and new bone formation were visible. Degree of cartilage degeneration varied between locations. Depending on the origin of the fragment

Table 3 Scores of histological evaluation

	dist.	Fetlock				hock				stifle
	P. ext.	dorsodist. MX III	dorso-prox. P1	plantaroprox. P1	prox. sesamoid bone	sagittal ridge tibia	lat. trochlea talus	med. malleolus tibia	trauma tibia	lat. trochlea femur
bone remodelling										
trabecular	1,22 ± 0,44	1,00 ± 0,00	1,39 ± 0,78	1,00 ± 0,71	1,00 ± 0,00	1,64 ± 0,84	1,60 ± 0,89	2,00 ± 1,41	1,00 ± 1,41	2,38 ± 0,74
cortical	1,22 ± 0,44	1,00 ± 0,00	1,39 ± 0,78	1,00 ± 0,71	1,00 ±0,00	1,71 ± 0,83	1,80 ± 0,84	2,00 ± 1,41	1,00 ± 1,41	2,38 ± 0,74
proteoglycan staining	0,11 ± 0,33	0,25 ± 0,50	1,00 ± 1,09	0,00 ± 0,00	0,50 ± 1,00	1,71 ±0,83	1,40 ± 1,14	1,00 ± 1,41	0,00 ± 0,00	2,38 ± 0,74
enchondral ossification	0,00 ± 0,00	0,25 ± 0,50	1,06 ± 1,07	0,20 ± 0,45	0,25 ± 0,50	1,36 ± 0,93	1,40 ± 1,14	1,00 ± 1,41	0,00 ± 0,00	2,25 ± 1,08
fracture line - fibrous tissue	1,00 ± 0,00	1,00 ± 0,00	0,20 ± 0,41	1,00 ± 0,00	1,00 ± 0,00	0,07 ± 0,27	0,25 ± 0,50	1,00 ±0,00	1,00 ± 0,00	0,00 ± 0,00
fracture line - enchondr. ossific.	0,00 ± 0,00	0,00 ± 0,00	0,80 ± 0,41	0,00 ± 0,00	0,00 ±0,00	1,23 ± 0,83	1,00 ± 0,82	0,00 ± 0,00	0,00 ± 0,00	2,43 ± 0,79
lig. attachment	0,78 ± 0,44	0,75 ± 0,50	0,39 ± 0,50	1,00 ± 0,00	0,75 ± 0,50	0,00 ± 0,00	0,60 ± 0,55	0,50 ± 0,71	1,00 ± 0,00	0,00 ± 0,00

different amounts of fibrous tissue or signs of enchondral ossification were detectable at the separation border. The reviewer's attribution of fragments to the various joint allocations was correct in about 80% of the evaluated sections. Fragments of the lateral trochlear ridge of the talus and the femur, as well as those of the processus extensorius were always correctly allocated. Errors occurred mainly in the fetlock joint, where the fragments originating from the dorsodistal aspect of MC III/MT III and those from the plantaroproximal aspect of P1, or those of the dorsoproximal aspect of P1 and the plantaroproximal aspect of P1 were mixed up. Allocation to the specific sites was possible either through size and/or clear signs of ongoing extensive enchondral ossification.Results of histological evaluation are shown in Table 3.

Signs of active bone remodelling (trabecular / cortical bone), such as bone resorption by osteoclasts and new bone formation by osteoblasts/lining cells were visible at different degrees in all of the removed fragments, but not in the known traumatic avulsion fracture of the lateral malleolus (Fig. 2) in one horse. Bone remodelling was highest in fragments from the lateral trochlear ridge of the femur and the medial malle-

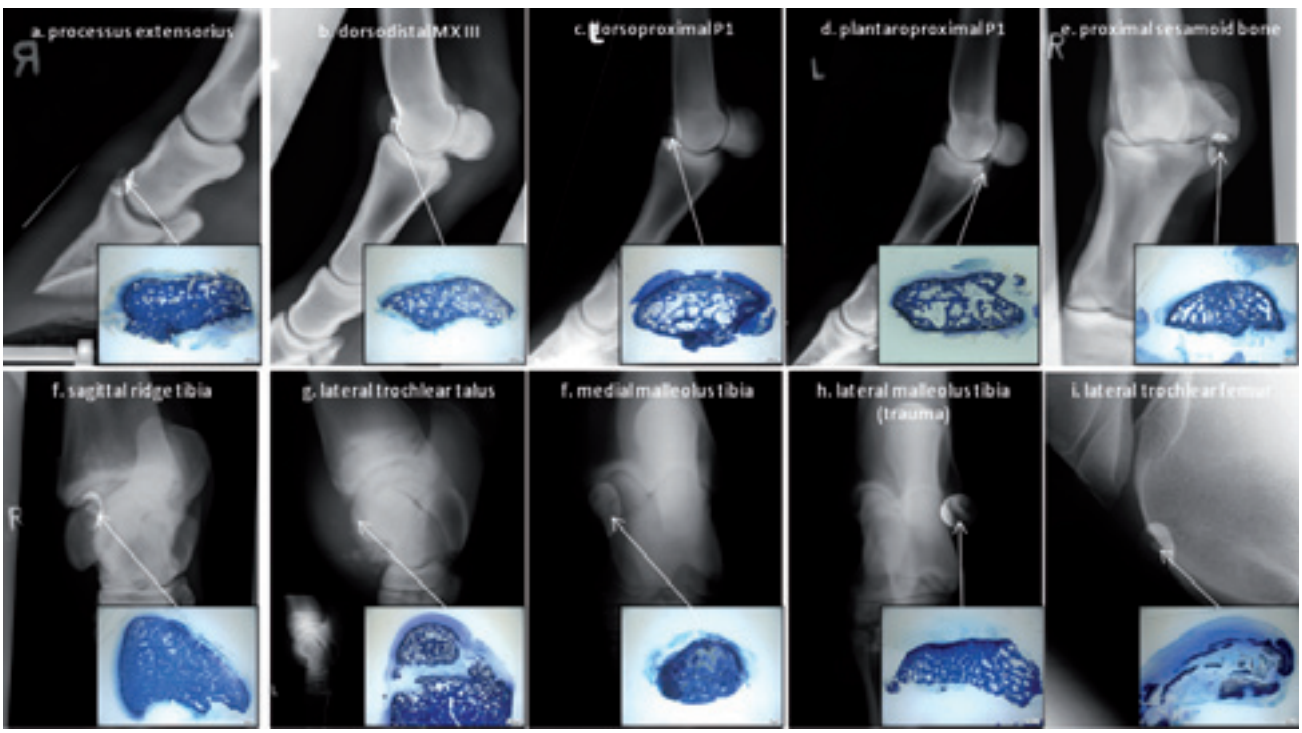

Fig. 1 Overview of different osteochondral fragments evaluated, radiographs give examples of the location of the different fragments, thick section stained with toluidin blue illustrate the different tissue types involved in fragment formation. In the x-rays of Fig. 1g the progession of radiological detectable changes can be seen. The small image (left bottom) was taken with 3 month, the large image with 12 month of age.

olus of the tibia (Fig. 3). Lowest bone remodelling activity was observed in equal measures in proximoplantar first phalanx fragments, dorsal aspect of the distal metacarpus/-tarsus and the proximal sesamoid bone. Controversially to the above mentioned avulsion fracture of the lateral malleolus the avulsion fracture of the medial malleolus at the origin of the collateral ligament showed a moderate (grade 2) bone remodelling activity. Differences in scores detected between the different groups were not statistically significant.

Enchondral ossification was most obvious in osteochondral fragments from the lateral trochear ridge of the femur in the stifle (Fig. 4a). Decreasing scores were visible at the lateral trochlear ridge of the talus and the sagittal ridge of the tibia in the hock. Only mild degrees of enchondral ossification could be detected in fragments from the proximodorsal border of P1 and from the medial malleolus. Very low levels of enchondral ossification were detected in those, originating from the dorsodistal aspect of MC III/MT III, the proximal sesamoid bone and proximoplantar from P1. No signs of enchondral ossification could be detected in fragments located at the processus extensorius of P3 and those of known traumatic origin in the hock. Differences in activity for enchondral ossification in fragments were significant between those originating from the lateral trochlear ridge of the femur and those from the distal interphalangeal joint ($p<0.0001$), from the dorsodistal aspect of MC III / MT III ($p=0.02$), plantaroproximal aspect of P1 ($p=0.006$) and from the proximal sesamoid bone ($p=0.02$). Furthermore, activity was significant higher in osteochondral fragments located at the sagittal ridge of the tibia compared to those from the coffin joint ($p=0.028$).

The proteoglycan staining as a measurement for the enchondral pathway was highest in fragments from the lateral trochlear ridge in the stifle (Fig. 4b). Levels decreased over the sagittal ridge and the lateral trochlear ridge of the tibia, which showed mild- to moderate levels, to only mild levels in fragments originating from the medial malleolus and the dorsoproximal border of P1. In those, where the source of fragment formation was the proximal sesamoid bone, the dorsodistal aspect of MC III / MT III or the region of the processus extensorius of P3 only very low levels for proteoglycan staining could be detected. No proteoglycan staining properties at all were seen in fragments located at the plantaroproximal border of P1 (Fig. 5) and those of known traumatic origin (Fig. 2). Significant differences in proteoglycan staining of osteochondral fragments were detected between those originating from the lateral trochlear ridge of the femur and those

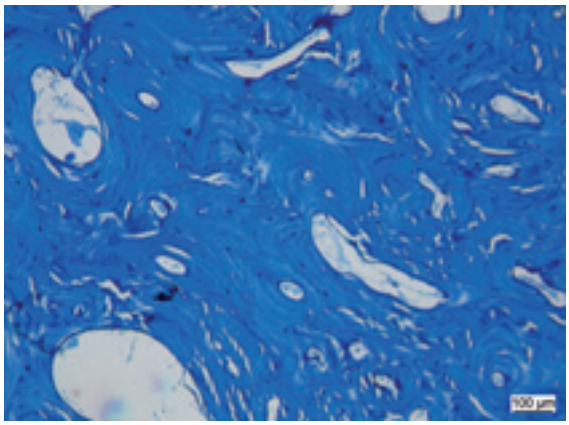

Fig. 2 Section of trabecular bone within the avulsion fragment of the lateral malleolus; no signs of active bone remodelling such as bone resorption by osteoclasts or new bone formation by osteoblasts as well as no signs for proteoglycan stainingcould be detected; toluidin blue

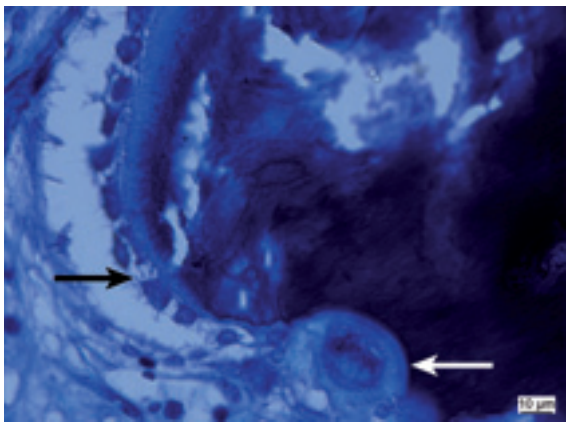

Fig. 3 Section of the medial malleolus showing bone resorption by osteoclasts (white arrow) paralleled by new bone formation by osteoblasts (black arrow); toluidin blue

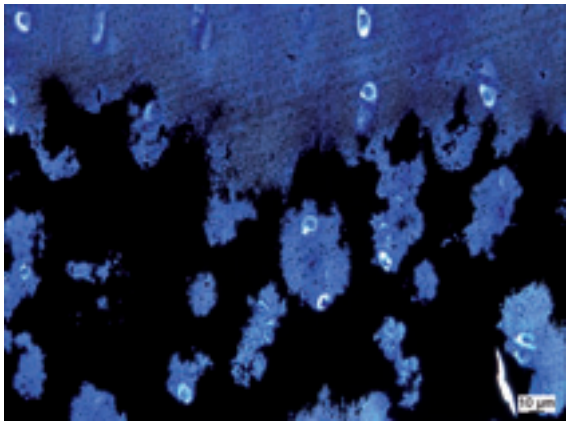

Fig. 4a Osteochondral fragment from the lateral trochlear of the femur with grade 3 enchondral ossification, *van Kossa/Mc Neal*

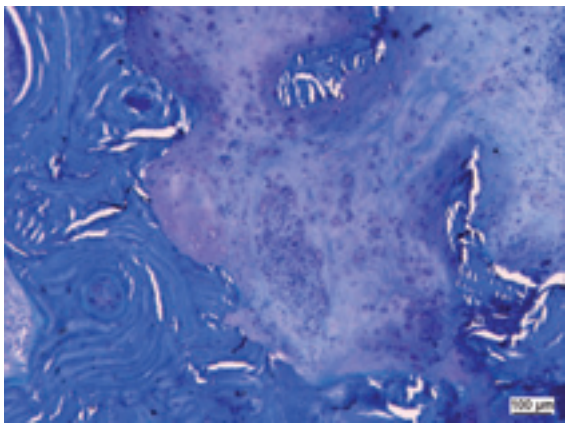

Fig. 4b osteochondral fragment from the lateral trochlear of the femur with proteoglycan staining identifiable by the pinkish color; toluidin blue

originating from the processus extensorius ($p<0.0001$), the dorsodistal aspect of MC III / MT III ($p=0.006$), the proximodorsal ($p=0.016$) and plantaroproximal aspect of P1 ($p<0.0001$), the proximal sesamoid bone ($p=0.03$) and those of known traumatic origin from the hock ($p=0.038$). Furthermore, significant differences were detected between the sagittal ridge of the tibia and the processus extensorius of P3 ($p=0.002$) and the plantaroproximal aspect of P1 ($p=0.013$) as the source of fragment formation.

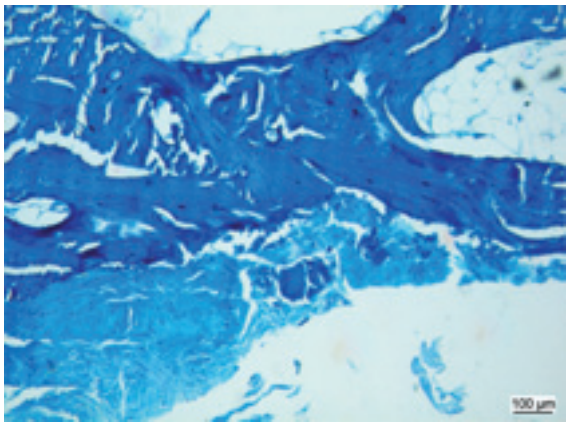

Fig. 5 in fragments from the plantaroproximal aspect of P1 no signs of proteoglycan staining could be detected; toluidin blue

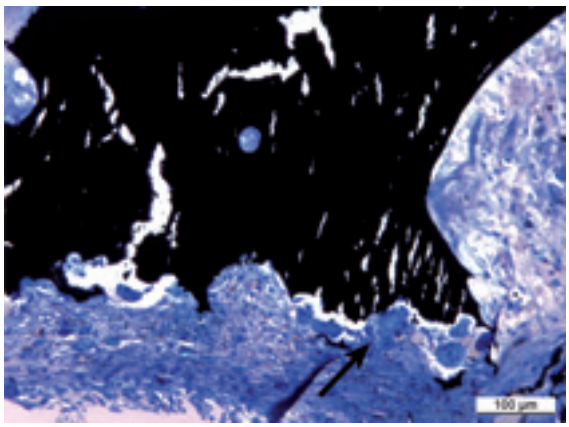

Fig. 6 Fibrous tissue covering the separation border of a fragment from the region of the processus extensorius, further more osteoclasts can be seen resorbing bone at the separation border; *van Kossa / Mc Neal*

Fibrous tissue or enchondral ossification at the separation border of the fragment could not be detected in 10 out of 76 fragments. Depending on the origin of the fragment there were different amounts of fibrous tissue or signs of enchondral ossification detectable. The presence of fibrous tissue covering one border of the fragment was observed in 6 out of the 9 fragments located at the processus extensorius of P3 (Fig. 6). The separation border was not visible in histology section of the other three cases. In none of those 6 cases signs of enchondral ossification at the separation border were visible. From fragments located at the dorsodistal aspect of MC III/MT III the separation border was detectable in 2 out of 4 cases. In those two cases fibrous tissue, but no signs of enchondral ossification, were detectable. From 19 samples originating from the proximodorsal aspect of P1 the separation border was not detectable in 3 samples. In the remaining 16 cases 12 showed the presence of mild signs for enchondral ossification but no signs of fibrous tissue covering the separation border. The 4 cases with fibrous tissue present, showed no signs of enchondral bone formation. There was no correlation between the appearance of fibrous tissue or enchondral ossification and limb affected (front or hind limb). In all 5 cases, where osteochondral fragments originated from the plantaroproximal border of P1, and in the 3/4 cases originating from the proximal sesamoid bone, where the separation border could be evaluated, there was fibrous tissue but no signs for enchondral ossification. In the 3 cases, where the fragment represented a portion of the medial malleolus of the tibia only in 2 cases the separation border was visible. In those 2 cases, where 1 case represented an avulsion fracture of the origin of the collateral ligament and in the case of the traumatic avulsion fracture of the lateral malleolus fibrous tissue, but no signs for enchondral ossification, was detected by the two observers. In 14/15 cases, where fragment formation appeared at the sagittal ridge of the tibia, and in all cases of fragment formation at the lateral trochlear ridge of the femur no signs of fibrous tissue, but mainly enchondral ossification appeared at the separation border. Whereas cases from the previous mentioned groups and all but one located at the sagittal ridge of the tibia did only show mild signs for enchondral ossification, the scores were judged moderate in 2 and high in 5 cases of the stifle group (Fig.7). Due to low numbers in one group no statistical evaluation was carried out for the presence of fibrous tissue and enchondral ossification at the separation border of the fragments.

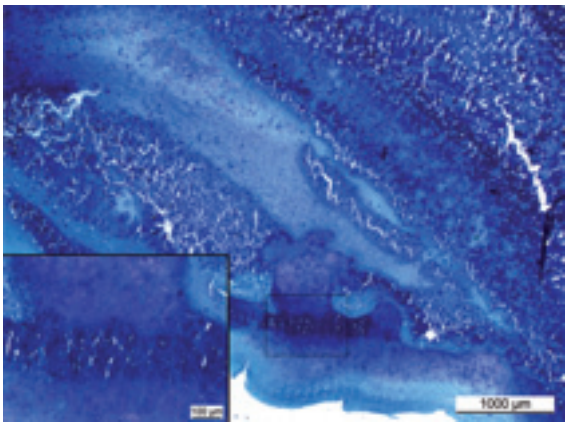

Fig. 7 example of grade 3 enchondral ossification at the separation border of a fragment from the lateral trochlear ridge of the femur; toluidin blue

Ligamentous attachments, respectively the presence of organised connective tissue, like ligaments or joint capsule, could be found in the 2 specimens of known traumatic origin of the hock, one located at the lateral malleolus and one at the medial malleolus and in all cases where fragments originated from the plantaroproximal border of P1. It appeared in decreasing frequency at osteochondral fragments located at the processus extensorius of P3 (7 out of 9 samples, 77.7 %)), the dorsodistal aspect of MC III / MT III and the proximal sesamoid bone (3/4 samples each, 75 %), the lateral trochlear ridge of the talus (3/6 samples, 50 %), the medial malleolus (1/2 samples, 50 %) and the proximodorsal aspect of P1 (9/19 samples, 42 %). No attachments of connective tissue could be detected in fragments originating from the sagittal ridge of the tibia and the lateral trochlear of the stifle. Differences in ligamentous

attachments were statistical significant between fragments from the processus extensorius and the sagittal ridge of the tibia ($p=0.001$) and the lateral trochlear of the stifle ($p=0.009$), the plantaroproximal aspect of P1 and the stifle ($p=0.002$) and the sagittal ridge of the tibia ($p<0.0001$).

Scores for the structure of the articular cartilage were highest in osteochondral fragments originating from the plantaroproximal aspect of P1, the medial malleolus of the tibia and in those with known trauma aetiology in the hock. The lowest score was detectable in fragments from the lateral trochlea of the femur. A significant difference was calculated between the processus extensorius and the dorsoproximal aspect of P1 ($p=0.034$), the sagittal ridge of the tibia ($p=0.015$) and the lateral trochlear of the femur ($p=0.003$) as the origin of fragment formation. Furthermore the difference between those from the plantaroproximal aspect of P1 and those from the stifle was statistically significant ($p=0.009$). Cell scores were again highest in those from the plantaroproximal aspect of P1, the medial malleolus and those with traumatic genesis as well as from those originating from the proximal sesamoid bone. The lowest cell score was detectable in fragments from the lateral trochlea of the femur. Cell scores differed significantly between those from the dorsoproximal aspect of P1 and the processus extensorius ($p=0.005$), the plantaroproximal aspect of P1 ($p=0.01$) and the proximal sesamoid bone ($p=0.029$), as well as between the lateral trochlea of the stifle and the processus extensorius ($p=0.008$), the plantaroproximal aspect of P1 ($p=0.01$) and the proximal sesamoid bone ($p=0.024$). Metachromasia was highest in traumatic fragments and lowest in those originating from the proximodorsal aspect of P1. The calculated significance between fragments from the dorsoproximal aspect of P1 and those of traumatic origin in the hock to those of the dorsoproximal aspect of P1 was $p=0.017$ and $P=0.032$ respectively. Tidemark integrity was judged best in fragments from the plantaroproximal aspect of P1, from the medial malleolus and from those of traumatic origin. Cartilage with the lowest Mankin scores, representing most viable cartilage, was found in fragments from the lateral trochlea of the femur (Fig. 8), the proximodorsal aspect of P1 and the sagittal ridge of the tibia. Highest scores were found in those originating from trauma (in the hock), the medial malleolus and the plantaroproximal aspect of P1 (Fig. 9). Statistical significant differences for the overall cartilage scores were detected between fragments from the dorsoproximal aspect of P1 and the processus extensorius ($p=0.024$) and the plantaroproximal aspect of P1 ($p=0.007$), from the sagittal ridge of the tibia and the plantaroproximal aspect of P1 ($p=0.038$) and from the lateral trochlear of the femur to the processus extensorius ($p=0.013$) and the plantaroproximal aspect of P1 ($p=0.004$). For more details see Table 1.

Basically there was statistic significant correlation between bone remodelling (trabecular and cortical) ($p<0.0001$), proteoglycan staining ($p<0.0001$) and the presence of enchondral ossification within the fragment, which was negatively correlated to the appearance of fibrous tissue at the fragment separation border and organized connective tissue ($p<0.0001$). In contrast this correlation was significant positively correlated to enchondral ossification at the fragment border ($p<0.0001$). High counts in total cartilage Mankin score as well as the structure of the cartilage and cartilage cell parameters were significantly negatively correlated to bone remodelling ($p<0.0001$–0.001), proteoglycan staining ($p<0.0001$) and enchondral ossification ($p<0.0001$) within the fragment. In contrast, the appearance of fibrous tissue on the separation border and ligamentous attachments was significant positively correlated to the overall Mankin score ($p<0.0001$) as well as to high counts in cartilage structure ($p<0.0001$), cells ($p<0.0001$), metachromasie ($p<0.0001$, resp. $P=0.001$) and tide mark integrity ($p=0.04$, resp. $P=0.001$). For more details see Table 4a, b.

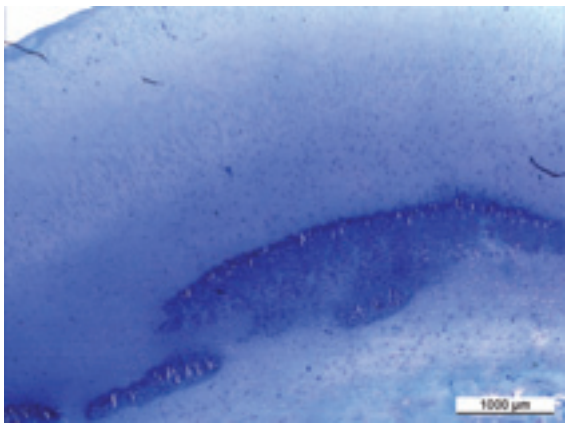

Fig. 8 fragment from the latera trochlear of the femur, example of cartilage with a relatively low Mankin Score; Toluidin blue

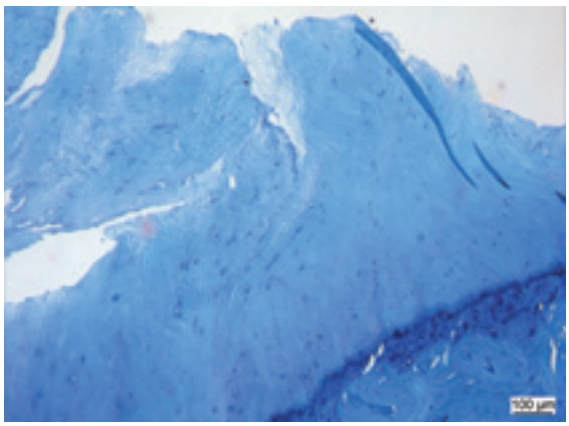

Fig. 9 Cartilage from an osteochondral fragment originating from the plantaroproximal aspect of P1, representing a relatively high Mankin score, Toluidin blue

Discussion

The median age of 4 years for horses presented for arthroscopic removal of osteochondral fragments reflects the increase of training at this age and thereby the appearance of clinical symptoms, such as synovialitis and lameness and the fact that most horse breeders sell their horses around this age. 30 % of horses were presented without any signs of lameness. In these cases osteochondral fragments where most likely detected during prepurchase examinations. Breed distribution parallels the normal caseload of the hospital, where this study was conducted. The fact that signs for osteoarthritis were detected radiograpically in 20 out of 24 fetlock joints cannot be correlated to the age of these groups. Median age in all horses with osteochondral fragments located at one of the fetlock location was 5 years, for those with additional osteoarthritis 4.5 years (data not shown). It seems to be much more likely, that an irritating effect and thereby the

Table 4a Positive correlations

variable 1	variable 2	Pearsons Coefficient	p-value
trabecular remodelling	cortical remodelling	0,981	<0,0001
	proteoglycan staining	0,835	<0,0001
	enchondral ossification	0,852	<0,0001
	enchondral ossification sep. border	0,653	<0,0001
cortical remodelling	proteoglycan staining	0,834	<0,0001
	enchondral ossification	0,839	<0,0001
	enchondral ossification sep. border	0,653	<0,0001
proteoglycan staining	enchondral ossification	0,929	<0,0001
	enchondral ossification sep. border	0,771	<0,0001
Enchondral ossification	enchondral ossification sep. border	0,788	<0,0001
fibrous tissue sep. border	ligamentous attachements	0,633	<0,0001
	cartilage structure	0,653	<0,0001
	cartilage cells	0,572	<0,0001
	cartilage metachromasie	0,429	<0,0001
	cartilage integrity tidemark	0,262	0,04
	total Mankin score	0,645	<0,0001
ligamentous attachmenets	cartilage structure	0,604	<0,0001
	cartilage cells	0,581	<0,0001
	cartilage metachromasie	0,382	0,001
	cartilage integrity tidemark	0,381	0,001
	total Mankin score	0,613	<0,0001
cartilage structure	cartilage cells	0,748	<0,0001
	cartilage metachromasie	0,614	<0,0001
	cartilage integrity tidemark	0,551	<0,0001
cartilage cells	cartilage metachromasie	0,626	<0,0001
	cartilage integrity tidemark	0,408	<0,0001
	total Mankin score	0,865	<0,0001
cartilage metachromasie	cartilage integrity tidemark	0,386	0,001
	total Mankin score	0,793	<0,0001
cartilage integrity tidemark	total Mankin score	0,612	<0,0001

induction of osteoarthritis is higher in this high motion joint. Another explanation for the high incidence of osteoarthritis in affected fetlock joints could be the genesis of osteochondral fragments. In cases where fracture is the initiating cause of fragment formation a concurrent trauma to additional joint structures cannot be excluded.

It is known that intrarticular osteochondral fragments may show signs of degenerative or regenerative processes (*Ytrehus* et al. 2007). This fact does not exclude, that the initiating cause of fragment formation may have a further influence on those processes and thereby on detectable and maybe specific characteristics.

In osteochondral fragments of known traumatic origin enchondral ossification and proteoglycan staining within the fragments and enchondral ossification at the separation border were not detectable, whereas fibrous tissue at the separation border was always present. Analogue or close observation could be made in osteochondral fragments originating from the dorsodistal aspect of MC III / MT III, the plantaroproximal aspect of P1 and the proximal sesamoid bone as well as in those originating from the processus extensorius in the coffin joint. Even though fragments of known traumatic cause may have been removed in a shorter period from development and thereby possible alterations within the different types of tissue involved may have been limited they still parallel those seen in fragments from the dorsodistal aspect of MC III/MT III, the plantaroproximal aspect of P1, the proximal sesamoid bone and the processus extensorius. In contrast to this, enchondral ossification (within the fragment and at the separation border) and proteoglycan staining were highest in fragments of the stifle and relatively high in those from the sagittal ridge of the tibia and the lateral trochlea of the talus. These observations indicate a difference in genesis of fragment formation, where osteochondral fragments from the coffin joint, the dorsodistal aspect of MC III/MT III, the plantaroproximal aspect of P1 and the proximal sesamoid bone have to be related to fracture, whereas those in the stifle, from the sagittal ridge of the tibia and the lateral trochlea of the talus have to be related to osteochondrosis. Ligamentous attachments in the form of organized connective tissue represent remnants of ligaments or the joint capsule. Even though their presence is dependent on the anatomic location the

Table 4b Negative correlations

variable 1	variable 2	Pearsons Coefficient	p-value
trabecular remodelling	fibrous tissue	-0,453	<0,0001
	ligamentous attachments	-0,496	<0,0001
	cartilage structure	-0,484	<0,0001
	cartilage cells	-0,382	<0,0001
	cartilage metachromasie	-0,226	<0,0001
	cartilage integrity tidemark	-0,216	0,065
	total Mankin score	-0,438	<0,0001
cortical remodelling	fibrous tissue	-0,469	<0,0001
	ligamentous attachments	-0,493	<0,0001
	cartilage structure	-0,476	<0,0001
	cartilage cells	-0,365	0,001
	cartilage metachromasie	-0,202	0,085
	cartilage integrity tidemark	-0,199	0,089
	total Mankin score	-0,421	<0,0001
proteoglycan staining	fibrous tissue	-0,713	<0,0001
	ligamentous attachments	-0,63	<0,0001
	cartilage structure	-0,666	<0,0001
	cartilage cells	-0,496	<0,0001
	cartilage metachromasie	-0,361	0,002
	cartilage integrity tidemark	-0,322	0,005
	total Mankin score	-0,611	<0,0001
enchondral ossification	fibrous tissue separation border	-0,697	<0,0001
	ligamentous attachments	-0,592	<0,0001
	cartilage structure	-0,609	<0,0001
	cartilage cells	-0,523	<0,0001
	cartilage metachromasie	-0,367	0,001
	cartilage integrity tidemark	-0,234	0.045
	total Mankin score	-0,579	<0,0001
fibrous tissue sep. border	enchondral ossification sep. border	-0,76	<0,0001
enchondral ossification sep. border	ligamentous attachments	-0,567	<0,0001
	cartilage structure	-0,585	<0,0001
	cartilage cells	-0,504	<0,0001
	cartilage metachromasie	-0,286	0,024
	cartilage integrity tidemark	-0,121	0,35
	total Mankin score	-0,537	<0,0001

possibility of force transmission may explain their more frequent appearance at those locations were fragment formation is allocated to trauma.

Even though differences exist in articular cartilage texture depending on different anatomic locations, observations made parallel those made for enchondral ossification and proteoglycan staining, indicating that cartilage condition may help in aetiologic allocation of fragments. In fragments allocated to osteochondrosis the total Mankin score is relatively low (6.75 for fragments of the stifle, 8.31 for fragments from the sagittal ridge of the tibia, 8.6 for fragments of the lateral trochlear of the talus) compared to those related to fracture (14 for fragments of known traumatic origin, 13.4 for fragments of the plantaroproximal aspect of P1, 11.25 for fragments of the proximal sesamoid bone). No clear differentiation was possible on the base of the Mankin score for fragments from the dorsodistal aspect of MC III/MT III. A Mankin score of 13.5 for the articular cartilage of fragments originating from the medial malleolus would indicate an allocation to trauma as well as the appearance of fibrous tissue at the separation border in all fragments. An intermediate grade for enchondral ossification and proteoglycan staining with high standard deviation as well as low sample numbers prohibit a causative allocation. The same is true for fragments from the dorsoproximal aspect of P1. Even though 19 fragments were available for evaluation (in 16 cases the separation border was present) and enchondral ossification at the separation border was detectable in 12 out of 16 cases as well as a Mankin score of 7.78 would implement osteochondrosis as

the most likely cause of fragment formation, enchondral ossification and proteoglycan staining within the fragment were not distinctive.

Beside that low sample numbers in some groups narrow possible conclusions technical shortcomings could be improved. The fact that the separation border was not detectable in 10 out of 76 fragments is caused most likely through the preparation process of the histology sections. In some cases, especially in cases were osteochondral fragments were very small, it caused some difficulties to keep those specimens in the proper orientation during the process of PMMA polymerization. Additionally an identification mark at the separation border would have been helpful during sample processing.

To the author's knowledge this is the first study of osteochondral fragments, where histological criteria were applied to distinguish fragments according to their aetiology. The clinical relevance may be debated for the individual horse, since fragments contribute to the development of osteoarthritis independent of their aetiology. However, the outcome may be indicative for horse breeders, since osteochondrosis is still debated as hereditary developmental disease (*Ekman* et al. 2009, *van Weeren* 2006b), whereas traumatic origin is more related to activity and temperament of horses.

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