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Keywords:	sheep, cartilage, knee, osteoarthritis, ageing
Abstract:	Though the ovine stifle is commonly used to study osteoarthritis, there is limited information about the age-related morphometric changes of the tidemark. The objective of this study was to document the number of tidemarks in the stifle of research sheep without clinical signs of osteoarthritis and of various ages (n = 80). Articular cartilage of the medial and lateral tibial condyles and of the medial and lateral femoral condyles was assessed by histology: (1) to count the number of tidemark; and (2) to assess the OARSI (OsteoArthritis Research Society International) score for structural changes of cartilage. The number of tidemarks varied between anatomical regions respectively from 4.2 in the medial femoral condyle to 5.0 in the lateral tibial condyle. The axial part showed a significant higher number of tidemarks than the abaxial part, for all regions except the medial tibial condyle. While the tidemark count strongly correlated to age (Spearman Correlation coefficient=0.70; 95% confidence interval 0.67 to 0.73; P<0.0001), the OARSI score was weakly correlated to age in our cohort of sheep (Spearman Correlation coefficient=0.25; 95% confidence interval 0.19 to 0.30; P<0.0001). Interestingly, no tidemark was seen in the three animals aged 6 months. Our data indicate that the number of tidemarks increases with age and vary with anatomical region. The regional variation also revealed a higher number of tidemarks in the tibia than in the femur. This could be attributed to the local variation in cartilage response to strain and to the

	difference in chondrocyte biology and density.

Age-related morphometric changes of the tidemark in the ovine stifle.

Running title: Tidemark in the ovine stifle

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Summary

Though the ovine stifle is commonly used to study osteoarthritis, there is limited information about the age-related morphometric changes of the tidemark. The objective of this study was to document the number of tidemarks in the stifle of research sheep without clinical signs of osteoarthritis and of various ages (n = 80). Articular cartilage of the medial and lateral tibial condyles and of the medial and lateral femoral condyles was assessed by histology: (1) to count the number of tidemark; and (2) to assess the OARSI (OsteoArthritis Research Society International) score for structural changes of cartilage.

The number of tidemarks varied between anatomical regions respectively from 4.2 in the medial femoral condyle to 5.0 in the lateral tibial condyle. The axial part showed a significant higher number of tidemarks than the abaxial part, for all regions except the medial tibial condyle. While the tidemark count strongly correlated to age (Spearman Correlation coefficient=0.70; 95% confidence interval 0.67 to 0.73; P<0.0001), the OARSI score was weakly correlated to age in our cohort of sheep (Spearman Correlation coefficient=0.25; 95% confidence interval 0.19 to 0.30; P<0.0001). Interestingly, no tidemark was seen in the three animals aged 6 months. Our data indicate that the number of tidemarks increases with age and vary with anatomical region. The regional variation also revealed a higher number of tidemarks in the tibia than in the femur. This could be attributed to the local variation in cartilage response to strain and to the difference in chondrocyte biology and density.

Key words: sheep – cartilage – stifle – osteoarthritis - ageing

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Introduction

Osteoarthritis is a degenerative process of the diarthrodial (synovial) joint characterized by progressive degeneration of the articular cartilage, combined with subchondral bone sclerosis and osteophyte formation, leading to reduced joint function (Grynpas, Albert, Katz, Lieberman, Pritzker, 1991; McIlwraith, 1996, p.34). Histology is considered as a gold standard technique to assess normality of cartilage, disease development (Lahm, Kreuz, Oberst, Haeberstroh, Uhl et al., 2006; Wucherer, Ober, Cozemius, 2012; Zamli, Adams, Tarlton, Sharif, 2013), and efficacy of treatments (Huang, Simonian, Norman, Clark, 2004; Hoeman, Hurtig, Rossomacha, Sun, Chevrier et al., 2005; Zscharnak, Hepp, Richter, Aigner, Schultz et al., 2010) in research studies on osteoarthritis.

Different scoring scales have been described for microscopic assessment of cartilage, based on several histological criteria such as the Mankin score, the "modified Mankin score" (Thomas, Fuller, Whittles, Sharif, 2007; Piskin, Gulbahar, Tomak, Gukman, Hokelek et al., 2007; Daubs, Markel, Manley, 2006), and the ICRS (International Cartilage Repair Society) -II scoring scale (Mainil-Varlet, Van Damme, Nesic, Knutsen, Kandel, Roberts et al., 2010). Species-specific scoring scales have been proposed by the Osteoarthritis Research Society International (OARSI) histopathology initiative to ensure comparison between studies using animal models of osteoarthritis, in mice (Glasson, Chambers, Van Den Berg, Little, 2010), rats (Gerwin, Bendele, Glasson, Carlson, 2010), guinea pigs (Kraus, Huebner, DeGroot, Bendele, 2010), rabbits (Laverty, Girard, Williams, Hunziker, Pritzker, 2010), dogs (Cook, Kuroki, Visco, Pelletier, Schulz et al., 2010), horses (McIlwraith, Frisbie, Kawcak, Fuller, Hurtig et al., 2010), goats and sheep (Little, Smith, Cake, Read, Murphy et al., 2010). For example in sheep, the histopathological assessment includes the following parameters: cartilage structure, percentage of the surface area affected by structural damage, chondrocyte density, cell cloning, interterritorial Toluidine blue staining, and tidemark variations.

The tidemark is the limit between the hyaline cartilage and the calcified cartilage (Meachim & Allibone, 1984; Oegema, Carpenter, Hofmeister, Thompson, 1997; Burr, 2004). At microscopy, the tidemark appears as a non-cellular line of about 10 µm strongly stained with hematoxylin-eosin, or toluidine blue (Lyons, Stoddart, McClure, McClure, 2005). A trilaminar organization has been demonstrated by combining different histochemical staining (hematoxylin and eosin, picrosirius red, toluidine blue and safranin O), with a distal lamina (to the side of the non-calcified cartilage), a proximal lamina (to the side of the calcified-cartilage) and a central lamina. The proximal and distal laminae differ in their chemistry and, hence, in their tinctorial properties. It is therefore suggested that the central lamina is actually an artefactual zone due to the interpenetration of colorations of the proximal and the distal laminae (Lyons et al., 2005).

The general consensus is that the tidemark is the result of accumulation of non-specific molecules at the interface of calcified and hyaline cartilage caused by discontinuous mineralization (Oegema et al., 1997). The tidemark seems to be derived from apoptotic chondrocytes, and to include several molecules such as phospholipides, alkaline phosphatase, type X collagen, adenosine triphosphatase, deoxyribonucleic acid, lectins, and High Mobility Group Box chromosomal protein 1 (HMGB1) (Lyons et al. 2005; Simkin 2012). Chondrocytes are not present in the tidemark but a few can be partially embedded in its mineralizing side (Lyons et al., 2005).

Tidemark alterations, i.e. duplication, advancement and vascular invasion have been associated to disease such as rheumatoid arthritis (Fassbender, Seibel, Hebert, 1992; Suber & Rosen, 2009) or osteoarthritis (Oettmeier, Abendroth, Oettmeier, 1989; Bonde et al., 2005; Hulth, 1993; Suri, Gill, Massena de Camin, Wilson, McWilliams et al., 2007; Bullough & Jagannath, 1983;

Oegema et al., 1997). In the OARSI score, it is observed whether the tidemark is duplicated (score 1) and whether blood vessels from the subchondral bone cross the tidemark to the calcified cartilage (score 2) or to the hyaline cartilage (score 3).

However, multiple tidemarks can be observed in normal joints (Oegema et al., 1997; Oettmeier et al., 1989). The number of tidemarks has been reported to change with ageing in humans, with an average increase from 1.5 to 2.5 in femur and humerus after the age of 60 (Lane & Bullough, 1980). Duplicated tidemarks were visible in mature normal canine femoral articular cartilage (Oegema et al., 1997). In a study on 28 cynomolgus monkeys, as many as ten tidemarks were observed in normal primates over 20 years old while at least two tidemarks were present in all animals (Miller, Novatt, Hamerman, Carlson, 2004). In horses, the number of tidemarks was higher in non-athletic than in racehorses with articular pathology (Muir, Peterson, Sample, Scollay, Markell, 2008). In non-working and working German shepherd dogs, the tidemark duplication in the femur and the tibia has been suggested to be related to ageing (Francuski, Radovanović, Andrić, Krstić, Bogdanović et al., 2014).

Since tidemark duplication and advancement could be observed in diseased but also in healthy animals, it is important to document how tidemark varies with age in a population of research animals. The sheep, in particular, is commonly used as a large animal model for osteoarthritis (Little et al., 2010). In sheep, there is limited information about the variation of the number of tidemarks (Appleyard, Burkhardt, Ghosh, Read, Cake et al., 2003; Frisbie, Cross, McIlwraith, 2006). Most of the sheep used in research are skeletally mature sheep (Huang et al., 2004; Burger, Mueller, Wlodarczyk, Goost, Tolba et al., 2007; Dattena, Pilichi, Rocca, Mara, Casu et al., 2009) aged between 3 and 6 years old (Hoeman et al., 2005).

The objectives of this study were to document the variation of the number of tidemarks of the stifle in a large cohort of sheep without clinical signs of osteoarthritis and of various ages.

Materials and methods

Population

Eighty pairs of hindlimbs were collected, between 2012 and 2018, from crossed Texel ewes, euthanatized for reasons other than hind limb lameness (mastitis, metritis), within six hours of euthanasia. Animals were aged between 6 months and 3 years old (N=28), 4 to 6 years old (N=31) and 7 to 11 year old (N=21). Animals had no clinical signs of osteoarthritis (lameness, articular swelling, and pain at manipulation). They had been used for teaching anatomy and were not euthanized for the purpose of the current study. The experimental protocol (KI 10/148) was approved by the local ethical committee for animal welfare.

Gross anatomy

After soft tissue dissection and joint opening, synovium and articular surfaces were assessed by one investigator in a blinded manner following OARSI recommendations (Little et al., 2010). Synovium was evaluated for macroscopic alterations (normal, slight, mild, moderate, marked and severe): discoloration, vascularity, thickening and synovial proliferation. Macroscopic scores for cartilage damages were: score 0 for intact cartilage surface; score 1 for surface roughening; score 2 for deeper defects (fibrillation, fissures) not involving the subchondral bone; score 3 for erosions down to the subchondral bone (less than 5 mm diameter); score 4 for large erosions down to the subchondral bone (more than 5 mm diameter). Scoring was performed in four areas of interest: the middle part of the medial tibial condyle (or plateau) (MTC), of the medial femoral condyle (MFC), of the lateral tibial condyle (LTC) and of the lateral femoral condyle (LFC) (Figure 1). Joint margins were observed for the presence of osteophytes. Joint surfaces were digitally photographed (Sony Alpha DSLR-A230 digital camera) with standardized lighting conditions for records (two Sony Illustar SM-300 lighting).

Histology

Four mm-thick osteochondral slabs were collected from the middle part of the medial tibial condyle (or plateau), medial femoral condyle, lateral tibial condyle and lateral femoral condyle (Figure 1). A total of 640 samples (80 sheep x 2 limbs x 4 regions) were collected. After 48-h fixation in 10% (v/v) neutral buffered formalin, samples were transferred to 70% (v/v) ethanol for further processing (Little et al., 2010). They were decalcified in DC3 (non-ionic surfactants, hydrochloric acid, EDTA, VWR International, Leuven, Belgium) for 2 days and embedded in paraffin, and then 4-μm sections were cut. Sections were deparaffinised with xylene and graded ethanol, and then stained with Toluidine blue.

Each slice was examined for cartilage structure and tidemark count. Scoring of cartilage structure followed the OARSI recommendations for histological evaluation of structural changes in ovine articular cartilage (Little et al., 2010). Each region being divided into two subregions (abaxial (Ab) and axial (Ax)), 1280 subregions were assessed (640 regions x 2). Assessments were performed in duplicates by two observers to obtain a mean score. Tidemark counts were obtained by one blinded observer in six equidistant locations per anatomical region. Mean number was calculated and recorded. Sheep, age and limb identities were blinded to histological scorers.

Statistical analysis

Statistics were performed with GraphPad Prism 7.03 (GraphPad Software, La Jolla). Statistical significance was set at 0.05. Firstly, the dataset was assessed for normality, skewness and kurtosis. Due to the moderate positive skewness, to kurtosis, and to non-normal distribution of the data, nonparametric statistics were conducted (Pearce & Frisbie, 2010). Wilcoxon matched-

pairs signed rank test and Friedman test were used to compare data from left and right limbs, and to compare data from the different (sub-)regions of each limb.

Kruskal-Wallis test followed by a Dunn's multiple comparison test enabled to test difference between age groups for tidemark count and OARSI scoring. Mean tidemark count and mean OARSI scores of both limbs was considered for each sheep. Correlation between age and tidemark number or OARSI scoring of the sheep was assessed using the Spearman's rank order test. Correlation was considered very weak (0.00-0.19), weak (0.20-0.39), moderate (0.40-0.59), strong (0.60-0.79) and very strong (0.80-1.00) depending on the absolute value of the coefficient.

Results

Gross anatomy

Macroscopic assessment of cartilage for the 1280 anatomic areas revealed 911 zones of intact cartilage (71.2%), 315 score-1 lesions (24.6%), 50 score-2 lesions (3.9%) and 4 score-3 lesions (0.3%). Score-2 and -3 erosions were found in 11 of the 80 sheep (13.75%). No score-4 lesion was found. No signs of joint inflammation (effusion, synovitis) and no osteophyte was detected at gross anatomy.

Histology

Thirty slides presented artifacts (folding, shredding, splitting) preventing tidemark count. Thus, 1250 of the 1280 sub-regions were appropriately assessed.

There was no significant difference between left and right limbs for tidemark count ($P = 0.5898$), and for OARSI scores ($P = 0.2761$).The tidemark count ($P<0.0001$) showed difference upon (sub-)regions. The axial sub-region had a significant higher number of tidemarks than the abaxial sub-region, for all regions except in the medial tibial condyle (Figure 3). The number

of tidemarks in the four regions was ranked as MFC < LFC < MTC < LTC, with an average number of 4.2, 4.5, 4.8 and 5.0, respectively; those differences were statistically significant, except between MFC and LFC.

The OARSI scores significantly differed with (sub-)regions (Figure 4), with the axial sub-regions showing higher scores than abaxial sub-regions (P<0.0001). OARSI scores in the four regions were ranked as LFC < LTC < MFC < MTC, with an average score of 2.0, 2.6, 5.0 and 5.3, respectively. The differences were not significant between regions of the same bone.

The three age groups had significant different tidemark count (P<0.0001) and OARSI scores (P=0.0197) (Table 1), with a strong positive correlation between age and the number of tidemarks (Spearman Correlation coefficient = 0.70, 95% confidence interval 0.67 to 0.73; P < 0.0001). However, the OARSI score was weakly correlated to age in our cohort of sheep (Spearman Correlation coefficient = 0.25, 95% confidence interval 0.19 to 0.30; P < 0.0001). The correlation between OARSI scores and tidemark count was weak as well (Spearman Correlation coefficient = 0.19, 95% confidence interval 0.13 to 0.24; P < 0.0001). In the three young animals aged 6 months, no tidemark was visible (Figure 2).

Discussion

In this study, the number of tidemarks increased significantly with age. Interestingly, no tidemark was identified in the three sheep aged 6 months. This is in agreement with reports that calcified cartilage layer does not begin to develop until well into the first year postpartum (Martinelli, Eurell, Les, Fyhrie, Bennett, 2002). In horses, functional adaptation of articular cartilage occurs during maturation (Brama, TeKoppele, Bank, Barneveld, van Weeren, 2002). Cartilage-bone interface is a dynamic area where duplication of the tidemark and thickening of

calcified cartilage are due to micro-trauma at the bone cartilage-interface and quick repair process in response to mechanical stresses over time (Burr & Schaffler, 1997).

The effect of constraints on tidemark duplication is also illustrated by the variation of number of tidemarks between anatomical regions. Constraints are higher in the medial compartment due to the asymmetry of load bearing and contact area in the stifle (Thomas, Resnick, Alazraki, Daniel, Greenfield, 1975; Baliunas Hurwitz, Ryals, Karrar, Case et al., 2002; Lee-Shee, Dickey, Hurtig, 2007; Taylor, Poepplau, Konig, Ehrig, Zachow, 2011). This is associated with a higher deterioration of cartilage and higher OARSI scores in those anatomical regions, as demonstrated by studies in sheep (Vandeweerd, Hontoir, Kirschvink, Clegg, Nisolle et al., 2013; Hontoir, Clegg, Simon, Kirschvink, Nisolle et al., 2017), and man (Arøen, Løken, Heir, Alvik, Ekeland et al., 2004; Neogi, Felson, Niu, Lynch, Nevitt et al., 2009; Flanigan, Harris, Trinh, Siston, Brophy, 2010). In the current study, OARSI scores were also higher in the medial tibial and femoral condyles than in the lateral tibial and femoral condyles, with the axial side being more affected.

In the current study, the number of tidemarks was higher in the tibia than in the femur. A difference in number of tidemarks has also been described in dogs (Francuski et al., 2014). In femoral cartilage, tidemark multiplication was more frequently observed in working dogs than in non-working dogs, whilst in the tibial cartilage it was more frequently observed in non-working dogs. This particularity has not been described elsewhere. However, regional differences of cartilage mechanobiology and cell biology could account for change in tidemark number. Mechanically, the cartilage strain is not homogeneous through the joint after exercise: for example, in human, the cartilage strain (percentage of thickness change) is higher in the tibia (30%) compared to the femur (20%) after a 30-minutes jogging (Moscher, Smith, Collins, Liu, Hancy et al., 2005; Sanchez-Adams, Leddy, McNulty, O'Conor, Guilak, 2014). Moreover, the cartilage response to loading is different for tibial and femoral cartilage. *In vivo* assessment

of cartilage response to load has been performed in human using compositional imaging, this technique revealed that tibial cartilage showed an homogeneous response for deep and superficial layers, whilst the femur showed an opposite response for both layers, suggesting a transport of water to the deep zone of cartilage in the femur, in opposition to the general squeeze of water of both tibial layers (Souza, Kumar, Calixto, Singh, Schooler et al., 2014). Biologically, tibial and femoral cartilage shows different pattern, with higher glycosaminoglycans and collagen content, higher chondrocyte density and proliferation rate in the femur than in the tibia (Stenhamre, Slynarski, Petrén, Tallheden, Lindahl, 2008). It should be reminded here that chondrocyte reaction to mechanical load varies from enhanced matrix synthesis (anabolism) to catabolism, apoptosis and necrosis depending on the frequency, the amplitude, or the strain-scheme for example (Sanchez-Adams et al., 2014; Bleuel, Zacke, Brüggemann, Niehoff, 2015; Iijima, Ito, Nagai, Tajino,Yamaguchi et al., 2017). As the tidemark originates from the chondrocytes activity (Havelka, Horn, Spohrová, Valouch, 1984) and apoptosis (Simkin, 2012), the higher number of tidemarks in the tibia could be explained by the combination of higher strain and lower cell yield in the tibia compared to the femur.

The correlation between the number of tidemarks and the OARSI score was weak in our sheep population. In a recent research study in man, the tidemark count poorly and non-significantly correlated to the human OARSI scores in the middle part of 42 lateral tibial condyles, with OARSI scores ranging from 0 (normal) to 4 (superficial delamination to mid-zone erosion). (Deng, Wang, Yin, Chen, Guo et al., 2016). These results support the idea, also proposed by other authors (Lane & Bullough, 1980; Bonde et al., 2005; Oegema et al., 1997; Muir et al., 2008; Francuski et al., 2014), that tidemark multiplication is not a unique feature of osteoarthritis and can be found in normal animals. OARSI scores in the current study were low.

In addition, we found no osteophytes, a feature of osteoarthritis (Little et al., 2010; Cake, Read, Corfield, Daniel, Burkhardt et al., 2013).

Since there was no osteoarthritic sheep in the current research population, it is not possible to infer on the association between OA and the number of tidemarks. The use of the sheep as an animal model for osteoarthritis requires the surgical induction of the disease to ensure the development of moderate to severe cartilage damages (Little et al., 2010). For example, in a lateral meniscectomy model, average OARSI scores can reach up to 16 +/-3 for cartilage (with erosion of cartilage and loss of proteoglycans to the mid/deep zone), associated to moderate synovitis and osteophytes in the lateral femoral and tibial condyles (Gelse, Körber, Schöne, Raum, Koch, 2017). Obviously such cases were not identified in the current population.

One could argue that the decalcification process is a limitation of the current study and would impair assessment of the tidemark. The tidemark is basically seen as the limit between the calcified cartilage and the hyaline cartilage (Meachim & Allibone, 1984; Oegema et al., 1997; Burr, 2004; Lyons et al., 2005). However, the tidemark is not only featured by presence of calcium deposition; it contains multiple molecules (phospholipids, alkaline phosphatase, adenosine triphosphatase, DNA, lectins) revealed by a wide range of histologic stains (Dmitrovsky, Lane and Bullough, 1978; Havelka et al., 1984; Oettmeir et al., 1989; Lyons et al., 2005). Furthermore, we have purposely conducted the study according to the OARSI recommendation for assessment of cartilage and osteochondral junction in ovine, i.e. with a decalcification step during the histological processing of osteochondral samples (Little et al., 2010). Another limitation is the lack of one-year old sheep to determine the apparition of the first tidemark. Those animals are not frequently available for research since they are young skeletally mature animal at the beginning of their reproductive career, and therefore not likely to be reformed.

Conclusion

Documentation of animal models is a concern in research and should be pursued to ensure accurate evaluation of the model and of the tested hypothesis. In the current study, we demonstrated that the multiplication of the tidemark is associated to ageing in the stifles of our sheep population aged between 6 months and 11 years old, without clinical signs of osteoarthritis. The tidemark count was weakly correlated to OARSI scores, confirming that tidemark count is not a feature of osteoarthritis. This might have implications in the interpretation of the OARSI histological score in sheep. Indeed, ageing seems to be more relevant to tidemark count than osteoarthritis progression in the sheep, as well as in man and dogs.

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Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with people or organizations that could inappropriately influence or bias the content of the paper.

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Table 1: Tidemark count and OARSI score values (median and interquartile range) for the three age groups.

	6 months to 3 years old (N = 28)	4 to 6 years old (N = 31)	7 to 11 years old (N = 21)
Tidemark count			
Median	2.67	4.33	6.67
Range	(1.33 – 4.00)	(3.33 – 5.50)	(5.30 – 8.08)
OARSI Scores			
Median	1.50	2.00	3.00
Range	(1.00 – 3.00)	(1.00 – 5.00)	(1.00 – 7.00)

N= number of sheep. Mean tidemark count and OARSI scoring of both limbs were considered for each sheep.

The tidemark count ($P<0.0001$) and the OARSI scores ($P=0.0197$) differed significantly between groups.

Figure legends

Figure 1. Sampling sites in the middle third of the medial tibial condyle (MTC), medial femoral condyle (MFC), lateral tibial condyle (LTC) and lateral femoral condyle (LFC). Tibial slabs were centered on the intercondylar eminence (black lines). Femoral slabs were obtained in the centre of the middle third of the circumference of the condyle (black lines and dotted black box). White rectangles illustrate the histological slices that were obtained, each abaxial (Ab) and axial (Ax) part being assessed separately at microscopy. White arrows highlight cartilage

Figure 2. The osteochondral junction at histology.

A. The white line indicates non-calcified hyaline cartilage (HC); the black line is the calcified cartilage (CC).

B. White arrows indicate tidemarks.

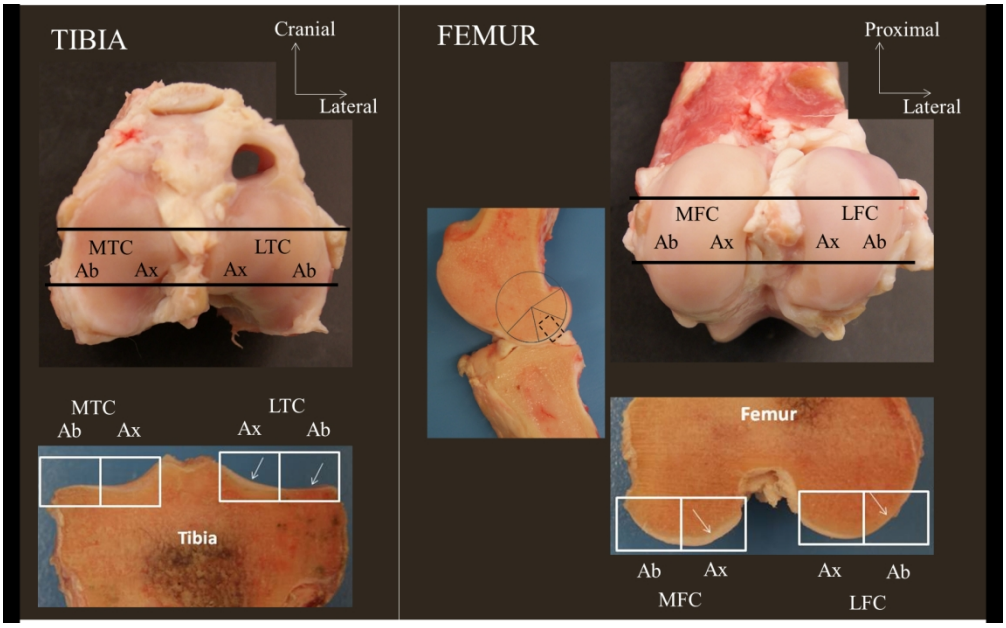
C. Histological slide showing the absence of tidemark in a sample of cartilage of the medial femoral condyle in a 6 months old sheep.

Figure 3: Number of tidemarks in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ($P<0.05$) is reached for the difference between the axial and the abaxial part of the region.

MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral femoral condyle, respectively.

Figure 4: OARSI scores in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ($P<0.05$) is reached for the difference between the axial and the abaxial part of the region.

MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral femoral condyle, respectively.



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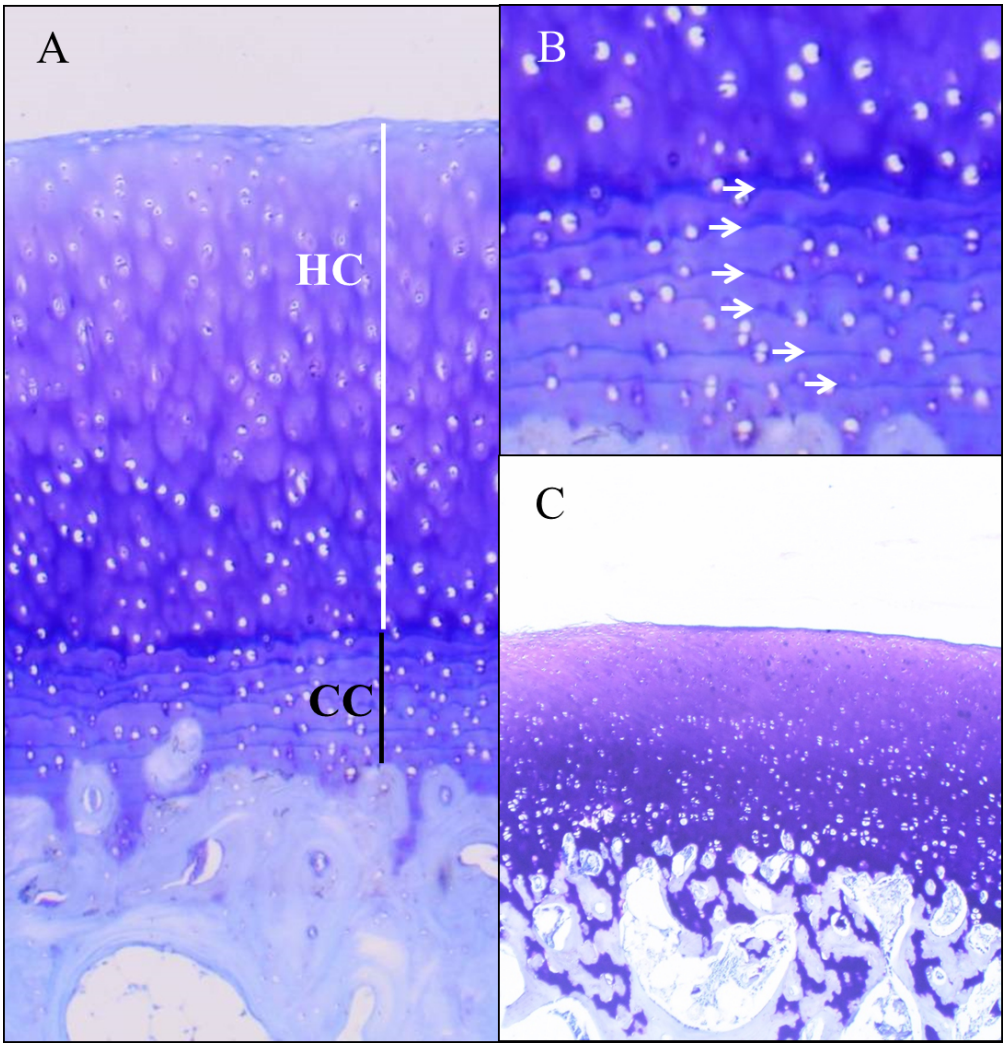

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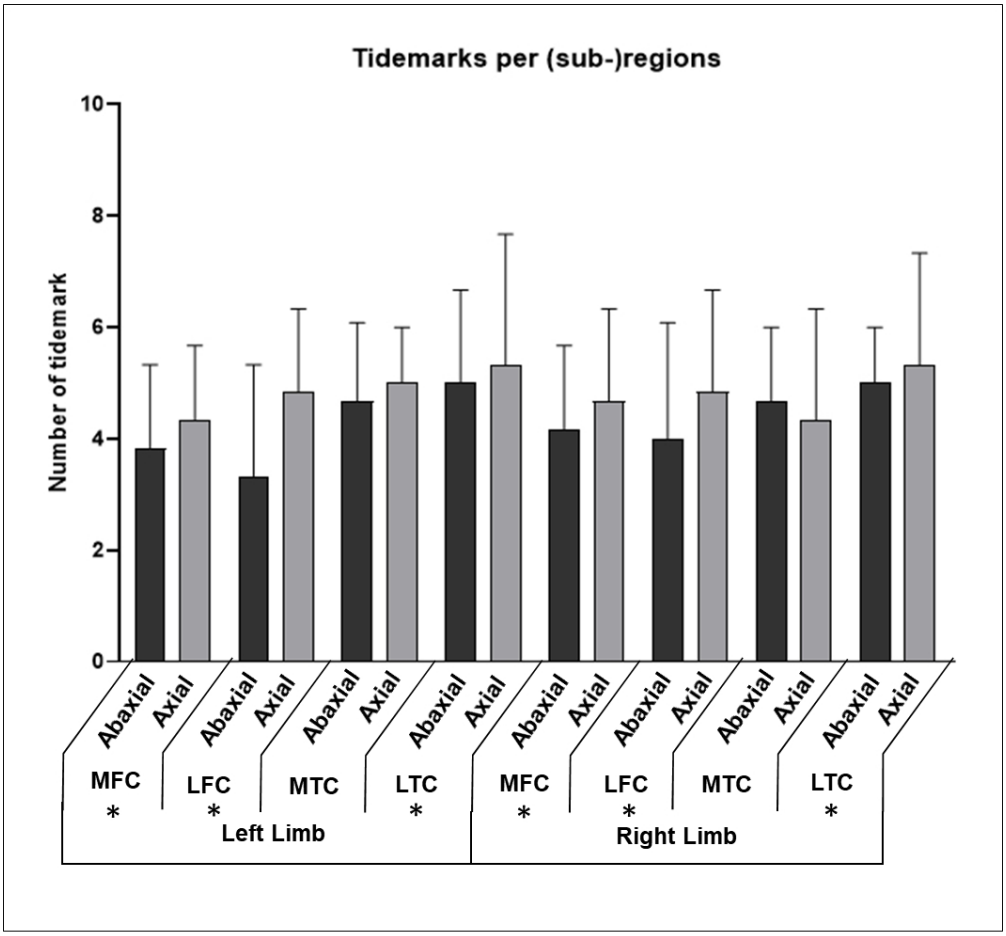
C. Histological slide showing the absence of tidemark in a sample of cartilage of the medial femoral condyle in a 6 months old sheep.

92x95mm (300 x 300 DPI)



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MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral femoral condyle, respectively.

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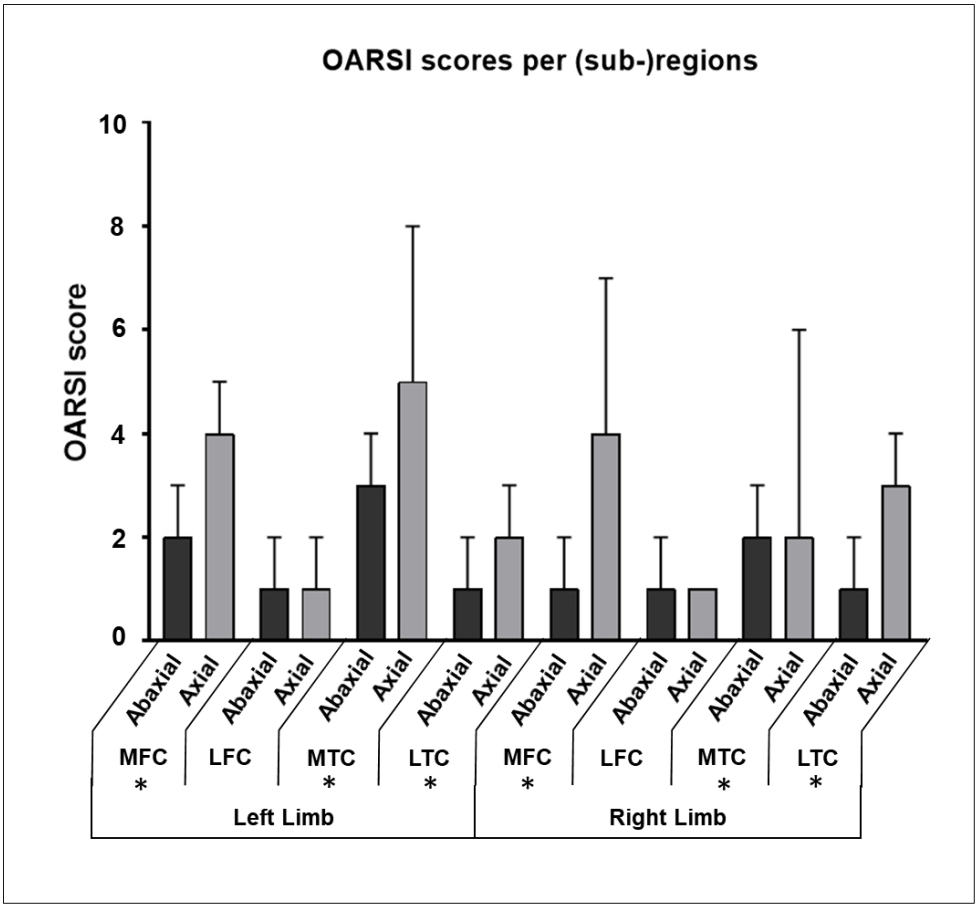

Figure 4: OARSI (OsteoArthritis Research Society International) scores in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ($P<0.05$) is reached for the difference between the axial and the abaxial part of the region. MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral femoral condyle, respectively.

92x92mm (300 x 300 DPI)

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	6 months to 3 years old (N = 28)	4 to 6 years old (N = 31)	7 to 11 years old (N = 21)
Tidemark count			
Median Range	2.67 (1.33 – 4.00)	4.33 (3.33 – 5.50)	6.67 (5.30 – 8.08)
OARSI Scores			
Median Range	1.5 (1.00 – 3.00)	2 (1.00 – 5.00)	3 (1.00 – 7.00)

N= number of sheep. Mean tidemark count and OARSI scoring of both limbs were considered for each sheep.

The tidemark count ($P<0.0001$) and the OARSI scores ($P=0.0197$) differed significantly between groups.