

## RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

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

Hontoir, Fanny; Pirson, Romain; Simon, Vincent; Clegg, Peter D.; Nisolle, Jean-François; Kirschvink, Nathalie; Vandeweerdt, Jean-Michel

*Published in:*  
Anatomia, Histologia, Embryologia

*DOI:*  
[10.1111/ahe.12449](https://doi.org/10.1111/ahe.12449)

*Publication date:*  
2019

*Document Version*  
Early version, also known as pre-print

#### [Link to publication](#)

*Citation for published version (HARVARD):*  
Hontoir, F, Pirson, R, Simon, V, Clegg, PD, Nisolle, J-F, Kirschvink, N & Vandeweerdt, J-M 2019, 'Age-related morphometric changes of the tidemark in the ovine stifle', *Anatomia, Histologia, Embryologia*, vol. 48, no. 4, pp. 366-374. <https://doi.org/10.1111/ahe.12449>

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

Journal:	<i>Anatomia, Histologia, Embryologia</i>
Manuscript ID	Draft
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	n/a
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Keywords:	sheep, cartilage, knee, osteoarthritis, ageing
Abstract:	<p>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.</p> <p>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&lt;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&lt;0.0001). Interestingly, no tidemark was seen in the three animals aged 6 months.</p> <p>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</p>

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	difference in chondrocyte biology and density.

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3 **1 Age-related morphometric changes of the tidemark in the ovine stifle.**  
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8 **3 Running title:** Tidemark in the ovine stifle  
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## 27 **Summary**

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

35 The number of tidemarks varied between anatomical regions respectively from 4.2 in the medial  
36 femoral condyle to 5.0 in the lateral tibial condyle. The axial part showed a significant higher  
37 number of tidemarks than the abaxial part, for all regions except the medial tibial condyle.

38 While the tidemark count strongly correlated to age (Spearman Correlation coefficient=0.70;  
39 95% confidence interval 0.67 to 0.73;  $P<0.0001$ ), the OARSI score was weakly correlated to  
40 age in our cohort of sheep (Spearman Correlation coefficient=0.25; 95% confidence interval  
41 0.19 to 0.30;  $P<0.0001$ ). Interestingly, no tidemark was seen in the three animals aged 6 months.  
42 Our data indicate that the number of tidemarks increases with age and vary with anatomical  
43 region. The regional variation also revealed a higher number of tidemarks in the tibia than in  
44 the femur. This could be attributed to the local variation in cartilage response to strain and to  
45 the difference in chondrocyte biology and density.

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

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53 **Number of figures in this manuscript: 4**

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## 53 **Introduction**

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

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

78

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

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

98

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

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3 103 Oegema et al., 1997). In the OARSI score, it is observed whether the tidemark is duplicated  
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5 104 (score 1) and whether blood vessels from the subchondral bone cross the tidemark to the  
6  
7 105 calcified cartilage (score 2) or to the hyaline cartilage (score 3).  
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11  
12 107 However, multiple tidemarks can be observed in normal joints (Oegema et al., 1997; Oettmeier  
13  
14 108 et al., 1989). The number of tidemarks has been reported to change with ageing in humans, with  
15  
16 109 an average increase from 1.5 to 2.5 in femur and humerus after the age of 60 (Lane & Bullough,  
17  
18 110 1980). Duplicated tidemarks were visible in mature normal canine femoral articular cartilage  
19  
20 111 (Oegema et al., 1997). In a study on 28 cynomolgus monkeys, as many as ten tidemarks were  
21  
22 112 observed in normal primates over 20 years old while at least two tidemarks were present in all  
23  
24 113 animals (Miller, Novatt, Hamerman, Carlson, 2004). In horses, the number of tidemarks was  
25  
26 114 higher in non-athletic than in racehorses with articular pathology (Muir, Peterson, Sample,  
27  
28 115 Scollay, Markell, 2008). In non-working and working German shepherd dogs, the tidemark  
29  
30 116 duplication in the femur and the tibia has been suggested to be related to ageing (Francuski,  
31  
32 117 Radovanović, Andrić, Krstić, Bogdanović et al., 2014).

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37 118 Since tidemark duplication and advancement could be observed in diseased but also in healthy  
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39 119 animals, it is important to document how tidemark varies with age in a population of research  
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41 120 animals. The sheep, in particular, is commonly used as a large animal model for osteoarthritis  
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43 121 (Little et al., 2010). In sheep, there is limited information about the variation of the number of  
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45 122 tidemarks (Appleyard, Burkhardt, Ghosh, Read, Cake et al., 2003; Frisbie, Cross, McIlwraith,  
46  
47 123 2006). Most of the sheep used in research are skeletally mature sheep (Huang et al., 2004;  
48  
49 124 Burger, Mueller, Wlodarczyk, Goost, Tolba et al., 2007; Dattena, Pilichi, Rocca, Mara, Casu et  
50  
51 125 al., 2009) aged between 3 and 6 years old (Hoeman et al., 2005).

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56 126 The objectives of this study were to document the variation of the number of tidemarks of the  
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58 127 stifle in a large cohort of sheep without clinical signs of osteoarthritis and of various ages.  
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**129 Materials and methods***130 Population*

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

138

*139 Gross anatomy*

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

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153

154 *Histology*

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

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

171

172 *Statistical analysis*

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

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3 177 pairs signed rank test and Friedman test were used to compare data from left and right limbs,  
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5 178 and to compare data from the different (sub-)regions of each limb.  
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8 179 Kruskal-Wallis test followed by a Dunn's multiple comparison test enabled to test difference  
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10 180 between age groups for tidemark count and OARSI scoring. Mean tidemark count and mean  
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12 181 OARSI scores of both limbs was considered for each sheep. Correlation between age and  
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14 182 tidemark number or OARSI scoring of the sheep was assessed using the Spearman's rank order  
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17 183 test. Correlation was considered very weak (0.00-0.19), weak (0.20-0.39), moderate (0.40-  
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19 184 0.59), strong (0.60-0.79) and very strong (0.80-1.00) depending on the absolute value of the  
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21 185 coefficient.  
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## 26 187 **Results**

### 28 188 *Gross anatomy*

30 189 Macroscopic assessment of cartilage for the 1280 anatomic areas revealed 911 zones of intact  
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33 190 cartilage (71.2%), 315 score-1 lesions (24.6%), 50 score-2 lesions (3.9%) and 4 score-3 lesions  
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35 191 (0.3%). Score-2 and -3 erosions were found in 11 of the 80 sheep (13.75%). No score-4 lesion  
36  
37 192 was found. No signs of joint inflammation (effusion, synovitis) and no osteophyte was detected  
38  
39 193 at gross anatomy.  
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41 194

### 44 195 *Histology*

46 196 Thirty slides presented artifacts (folding, shredding, splitting) preventing tidemark count. Thus,  
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48  
49 197 1250 of the 1280 sub-regions were appropriately assessed.

51 198 There was no significant difference between left and right limbs for tidemark count ( $P=0.5898$ ),  
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53 199 and for OARSI scores ( $P=0.2761$ ). The tidemark count ( $P<0.0001$ ) showed difference upon  
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56 200 (sub-)regions. The axial sub-region had a significant higher number of tidedmarks than the  
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58 201 abaxial sub-region, for all regions except in the medial tibial condyle (Figure 3). The number  
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3 202 of tidemarks in the four regions was ranked as MFC < LFC < MTC < LTC, with an average  
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5 203 number of 4.2, 4.5, 4.8 and 5.0, respectively; those differences were statistically significant,  
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7 204 except between MFC and LFC.

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10 205 The OARSI scores significantly differed with (sub-)regions (Figure 4), with the axial sub-  
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12 206 regions showing higher scores than abaxial sub-regions ( $P < 0.0001$ ). OARSI scores in the four  
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14 207 regions were ranked as LFC < LTC < MFC < MTC, with an average score of 2.0, 2.6, 5.0 and  
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16 208 5.3, respectively. The differences were not significant between regions of the same bone.

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21 210 The three age groups had significant different tidemark count ( $P < 0.0001$ ) and OARSI scores  
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23 211 ( $P = 0.0197$ ) (Table 1), with a strong positive correlation between age and the number of  
24  
25 212 tidemarks (Spearman Correlation coefficient = 0.70, 95% confidence interval 0.67 to 0.73;  $P <$   
26  
27 213 0.0001). However, the OARSI score was weakly correlated to age in our cohort of sheep  
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29 214 (Spearman Correlation coefficient = 0.25, 95% confidence interval 0.19 to 0.30;  $P < 0.0001$ ).  
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31 215 The correlation between OARSI scores and tidemark count was weak as well (Spearman  
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33 216 Correlation coefficient = 0.19, 95% confidence interval 0.13 to 0.24;  $P < 0.0001$ ). In the three  
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35 217 young animals aged 6 months, no tidemark was visible (Figure 2).

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## 39 219 **Discussion**

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42 220 In this study, the number of tidemarks increased significantly with age. Interestingly, no  
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44 221 tidemark was identified in the three sheep aged 6 months. This is in agreement with reports that  
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46 222 calcified cartilage layer does not begin to develop until well into the first year postpartum  
47  
48 223 (Martinelli, Eurell, Les, Fyhrie, Bennett, 2002). In horses, functional adaptation of articular  
49  
50 224 cartilage occurs during maturation (Brama, TeKoppele, Bank, Barneveld, van Weeren, 2002).  
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52 225 Cartilage-bone interface is a dynamic area where duplication of the tidemark and thickening of  
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3 226 calcified cartilage are due to micro-trauma at the bone cartilage-interface and quick repair  
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5 227 process in response to mechanical stresses over time (Burr & Schaffler, 1997).

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7 228 The effect of constraints on tidemark duplication is also illustrated by the variation of number  
8  
9 229 of tidemarks between anatomical regions. Constraints are higher in the medial compartment  
10  
11 230 due to the asymmetry of load bearing and contact area in the stifle (Thomas, Resnick, Alazraki,  
12  
13 231 Daniel, Greenfield, 1975; Baliunas Hurwitz, Ryals, Karrar, Case et al., 2002; Lee-Shee, Dickey,  
14  
15 232 Hurtig, 2007; Taylor, Poepplau, Konig, Ehrig, Zachow, 2011). This is associated with a higher  
16  
17 233 deterioration of cartilage and higher OARSI scores in those anatomical regions, as  
18  
19 234 demonstrated by studies in sheep (Vandeweerd, Hontoir, Kirschvink, Clegg, Nisolle et al.,  
20  
21 235 2013; Hontoir, Clegg, Simon, Kirschvink, Nisolle et al., 2017), and man (Arøen, Løken, Heir,  
22  
23 236 Alvik, Ekeland et al., 2004; Neogi, Felson, Niu, Lynch, Nevitt et al., 2009; Flanigan, Harris,  
24  
25 237 Trinh, Siston, Brophy, 2010). In the current study, OARSI scores were also higher in the medial  
26  
27 238 tibial and femoral condyles than in the lateral tibial and femoral condyles, with the axial side  
28  
29 239 being more affected.

30  
31 240 In the current study, the number of tidemarks was higher in the tibia than in the femur. A  
32  
33 241 difference in number of tidemarks has also been described in dogs (Francuski et al., 2014). In  
34  
35 242 femoral cartilage, tidemark multiplication was more frequently observed in working dogs than  
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37 243 in non-working dogs, whilst in the tibial cartilage it was more frequently observed in non-  
38  
39 244 working dogs. This particularity has not been described elsewhere. However, regional  
40  
41 245 differences of cartilage mechanobiology and cell biology could account for change in tidemark  
42  
43 246 number. Mechanically, the cartilage strain is not homogeneous through the joint after exercise:  
44  
45 247 for example, in human, the cartilage strain (percentage of thickness change) is higher in the  
46  
47 248 tibia (30%) compared to the femur (20%) after a 30-minutes jogging (Moscher, Smith, Collins,  
48  
49 249 Liu, Hancy et al., 2005; Sanchez-Adams, Leddy, McNulty, O'Connor, Guilak, 2014). Moreover,  
50  
51 250 the cartilage response to loading is different for tibial and femoral cartilage. *In vivo* assessment  
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3 251 of cartilage response to load has been performed in human using compositional imaging, this  
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5 252 technique revealed that tibial cartilage showed an homogeneous response for deep and  
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7 253 superficial layers, whilst the femur showed an opposite response for both layers, suggesting a  
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9 254 transport of water to the deep zone of cartilage in the femur, in opposition to the general squeeze  
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11 255 of water of both tibial layers (Souza, Kumar, Calixto, Singh, Schooler et al., 2014).  
12  
13 256 Biologically, tibial and femoral cartilage shows different pattern, with higher  
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15 257 glycosaminoglycans and collagen content, higher chondrocyte density and proliferation rate in  
16  
17 258 the femur than in the tibia (Stenhamre, Slynarski, Petrén, Tallheden, Lindahl, 2008). It should  
18  
19 259 be reminded here that chondrocyte reaction to mechanical load varies from enhanced matrix  
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21 260 synthesis (anabolism) to catabolism, apoptosis and necrosis depending on the frequency, the  
22  
23 261 amplitude, or the strain-scheme for example (Sanchez-Adams et al., 2014; Bleuel, Zacke,  
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25 262 Brüggemann, Niehoff, 2015; Iijima, Ito, Nagai, Tajino, Yamaguchi et al., 2017). As the  
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27 263 tidemark originates from the chondrocytes activity (Havelka, Horn, Spohrová, Valouch, 1984)  
28  
29 264 and apoptosis (Simkin, 2012), the higher number of tidemarks in the tibia could be explained  
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31 265 by the combination of higher strain and lower cell yield in the tibia compared to the femur.  
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40 267 The correlation between the number of tidemarks and the OARSI score was weak in our sheep  
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42 268 population. In a recent research study in man, the tidemark count poorly and non-significantly  
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44 269 correlated to the human OARSI scores in the middle part of 42 lateral tibial condyles, with  
45  
46 270 OARSI scores ranging from 0 (normal) to 4 (superficial delamination to mid-zone erosion).  
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48 271 (Deng, Wang, Yin, Chen, Guo et al., 2016). These results support the idea, also proposed by  
49  
50 272 other authors (Lane & Bullough, 1980; Bonde et al., 2005; Oegema et al., 1997; Muir et al.,  
51  
52 273 2008; Francuski et al., 2014), that tidemark multiplication is not a unique feature of  
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54 274 osteoarthritis and can be found in normal animals. OARSI scores in the current study were low.  
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3 275 In addition, we found no osteophytes, a feature of osteoarthritis (Little et al., 2010; Cake, Read,  
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5 276 Corfield, Daniel, Burkhardt et al., 2013).

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10 278 Since there was no osteoarthritic sheep in the current research population, it is not possible to  
11  
12 279 infer on the association between OA and the number of tidemarks. The use of the sheep as an  
13  
14 280 animal model for osteoarthritis requires the surgical induction of the disease to ensure the  
15  
16 281 development of moderate to severe cartilage damages (Little et al., 2010). For example, in a  
17  
18 282 lateral meniscectomy model, average OARSI scores can reach up to 16 +/-3 for cartilage (with  
19  
20 283 erosion of cartilage and loss of proteoglycans to the mid/deep zone), associated to moderate  
21  
22 284 synovitis and osteophytes in the lateral femoral and tibial condyles (Gelse, Körber, Schöne,  
23  
24 285 Raum, Koch, 2017). Obviously such cases were not identified in the current population.

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26  
27 286 One could argue that the decalcification process is a limitation of the current study and would  
28  
29 287 impair assessment of the tidemark. The tidemark is basically seen as the limit between the  
30  
31 288 calcified cartilage and the hyaline cartilage (Meachim & Allibone, 1984; Oegema et al., 1997;  
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33 289 Burr, 2004; Lyons et al., 2005). However, the tidemark is not only featured by presence of  
34  
35 290 calcium deposition; it contains multiple molecules (phospholipids, alkaline phosphatase,  
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37 291 adenosine triphosphatase, DNA, lectins) revealed by a wide range of histologic stains  
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39 292 (Dmitrovsky, Lane and Bullough, 1978; Havelka et al., 1984; Oettmeir et al., 1989; Lyons et  
40  
41 293 al., 2005). Furthermore, we have purposely conducted the study according to the OARSI  
42  
43 294 recommendation for assessment of cartilage and osteochondral junction in ovine, i.e. with a  
44  
45 295 decalcification step during the histological processing of osteochondral samples (Little et al.,  
46  
47 296 2010). Another limitation is the lack of one-year old sheep to determine the apparition of the  
48  
49 297 first tidemark. Those animals are not frequently available for research since they are young  
50  
51 298 skeletally mature animal at the beginning of their reproductive career, and therefore not likely  
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53 299 to be reformed.  
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5 301 **Conclusion**  
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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.

FOR Review Only

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5 313 **Acknowledgements**6  
7 314 We acknowledge Nadine Antoine and Joelle Piret for their help in histology.8  
9 31510  
11 316 **Conflict of interest statement**12  
13 317 None of the authors of this paper has a financial or personal relationship with people or  
14  
15 318 organizations that could inappropriately influence or bias the content of the paper.16  
17 31918  
19 320 **Funding Information**20  
21 321 This study was supported by the University of Namur (UNamur), NARILIS (Namur Research  
22  
23 322 Institute for Life Science).24  
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27 324 **References**28  
29 325 Appleyard, R.C., Burkhardt, D., Ghosh, P., Read, R., Cake, M., Swain, M.V., & Murrell, G.A.  
30  
31 326 (2003). Topographical analysis of the structural, biochemical and dynamic biomechanical  
32  
33 327 properties of cartilage in an ovine model of osteoarthritis. *Osteoarthritis and Cartilage*, 11, 65-  
34  
35 328 77. <https://doi.org/10.1053/joca.2002.0867>.36  
37 329 Arøen, A., Løken, S., Heir, S., Alvik, E., Ekeland, A., Granlund, O.G., & Engebretsen, L.  
38  
39 330 (2004). Articular cartilage lesions in 993 consecutive knee arthroscopies. *American Journal of*  
40  
41 331 *Sports Medicine*, 32, 211–215. <https://doi.org/10.1177/0363546503259345>.42  
43 332 Baliunas, A.J., Hurwitz, D.E., Ryals, A.B., Karrar, A., Case, J.P., Block, J.A., & Andriacchi,  
44  
45 333 T.P. (2002). Increased knee joint loads during walking are present in subjects with knee  
46  
47 334 osteoarthritis. *Osteoarthritis and Cartilage*, 10, 573-579. doi:10.1053/joca.2002.0797.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 335 Bleuel, J., Zaucke, F., Brüggemann, GP., & Niehoff, A. (2015). Effects of cyclic tensile strain  
4  
5 336 on chondrocyte metabolism: a systematic review. *PLoS One*, 10, e0119816. doi:  
6  
7 337 10.1371/journal.pone.0119816.  
8  
9  
10 338 Bonde, H.V., Talman, M.L.M., & Kofoed, H. (2005). The area of the tidemark in osteoarthritis:  
11  
12 339 a three-dimensional stereological study in 21 patients. *Acta pathologica, microbiologica et*  
13  
14 340 *immunologica Scandinavica*, 113, 349-352. <https://doi.org/10.1111/j.1600->  
15  
16 341 0463.2005.apm\_113506.x  
17  
18  
19 342 Brama, P.A., TeKoppele, J.M., Bank, R.A., Barneveld, A., & van Weeren, P.R. (2002).  
20  
21 343 Development of biochemical heterogeneity of articular cartilage: influences of age and exercise.  
22  
23 344 *Equine Veterinary Journal*, 34, 265-269. <https://doi.org/10.2746/042516402776186146>.  
24  
25  
26 345 Bullough, P.G., & Jagannath, A. (1983). The morphology of the calcification front in articular  
27  
28 346 cartilage. *Journal of Bone and Joint Surgery*, 65B, 72-78. doi: 10.1302/0301-  
29  
30 347 620X.65B1.6337169.  
31  
32  
33 348 Burger, C., Mueller, M., Wlodarczyk, P., Goost, H., Tolba, R.H., Rangger, C., Kabir, K., &  
34  
35 349 Weber, O. (2007). The sheep as a knee osteoarthritis model: early cartilage changes after  
36  
37 350 meniscus injury and repair. *Laboratory animals*, 41, 420-431. doi:  
38  
39 351 10.1258/002367707782314265.  
40  
41  
42 352 Burr, D.B., 2004. Anatomy and physiology of the mineralized tissues: role in the pathogenesis  
43  
44 353 of osteoarthrosis. *Osteoarthritis and Cartilage*, 12, S20-S30.  
45  
46 354 <https://doi.org/10.1016/j.joca.2003.09.016>.  
47  
48  
49 355 Burr, D.B., & Schaffler, M.B. (1997). The involvement of subchondral mineralized tissues in  
50  
51 356 osteoarthrosis: quantitative microscopic evidence. *Microscopic research techniques*, 37, 343-  
52  
53 357 357. [https://doi.org/10.1002/\(SICI\)1097-0029\(19970515\)37:4<343::AID-JEMT9>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<343::AID-JEMT9>3.0.CO;2-L)  
54  
55  
56 358 Cake, M.A., Read, R.A., Corfield, G., Daniel, A., Burkhardt, D., Smith, M.M., & Little, C.B.  
57  
58 359 (2013). Comparison of gait and pathology outcomes of three meniscal procedures for induction  
59  
60

- 1  
2  
3 360 of knee osteoarthritis in sheep. *Osteoarthritis and Cartilage*, 21, 226-36. doi:  
4  
5 361 10.1016/j.joca.2012.10.001.  
6  
7 362 Clark, J.M., & Huber, J.D. (1990). The structure of the human subchondral plate. *Journal of*  
8  
9 363 *Bone and Joint Surgery Britain*, 72, 866-873. doi: 10.1302/0301-620X.72B5.2211774.  
10  
11 364 Cook, J.L., Kuroki, K., Visco, D., Pelletier, J.-P., Schulz, L., & Lafeber, F.P.J.G. (2010). The  
12  
13 365 OARSI histopathology initiative - recommendations for histological assessments of  
14  
15 366 osteoarthritis in the dog. *Osteoarthritis and Cartilage*, 18: S66-S79. doi:  
16  
17 367 10.1016/j.joca.2010.04.017.  
18  
19 368 Dattena, M., Pilichi, S., Rocca, S., Mara, L., Casu, S., Masala, G., Manunta, L., Manunta, A.,  
20  
21 369 Passino, E.S., Pool, R.R., & Cappai, P. (2009). Sheep embryonic stem-like cells transplanted  
22  
23 370 in full-thickness cartilage defects. *Journal of tissue engineering and regenerative medicine*, 3,  
24  
25 371 175-187. doi: 10.1002/term.151.  
26  
27 372 Daubs, B.M., Markel, M.D., & Manley, P.A. (2006). Histomorphometric analysis of articular  
28  
29 373 cartilage, zone of calcified cartilage, and subchondral bone plate in femoral heads from  
30  
31 374 clinically normal dogs and dogs with moderate or severe osteoarthritis. *American Journal of*  
32  
33 375 *Veterinary Research*, 67, 1719-1724. <https://doi.org/10.2460/ajvr.67.10.1719>.  
34  
35 376 Deng, B., Wang, F., Yin, L., Chen, C., Guo, L., Chen, H., Gong, X., Li, Y., & Yang, L. (2016).  
36  
37 377 Quantitative study on morphology of calcified cartilage zone in OARSI 0-4 cartilage from  
38  
39 378 osteoarthritic knees. *Current Research in Translational Medicine*, 64, 149-154. doi:  
40  
41 379 10.1016/j.retram.2016.01.009.  
42  
43 380 Dmitrovsky, E., Lane, L.B., & Bullough, P.G. (1978). The characterization of the tidemark in  
44  
45 381 human articular cartilage. *Metabolic Bone Disease and Related Research*, 1, 115-118.  
46  
47 382 [https://doi.org/10.1016/0221-8747\(78\)90047-4](https://doi.org/10.1016/0221-8747(78)90047-4).  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 383 Fassbender, H.G., Seibel, M., & Hebert, T. (1992). Pathways of destruction in metacarpal and  
4  
5 384 metatarsal joints of patients with rheumatoid arthritis. *Scandinavian Journal of Rheumatology*,  
6  
7 385 21, 10-16. <https://doi.org/10.3109/03009749209095055>.  
8  
9  
10 386 Flanigan, D.C., Harris, J.D., Trinh, T.Q., Siston, R.A., & Brophy, R.H. (2010). Prevalence of  
11  
12 387 chondral defects in athletes' knees: a systematic review. *Medicine and science in sports and*  
13  
14 388 *exercise*, 42, 1795-801. doi: 10.1249/MSS.0b013e3181d9eea0.  
15  
16  
17 389 Francuski, J.V., Radovanović, A., Andrić, N., Krstić, V., Bogdanović, D., Hadžić, V.,  
18  
19 390 Todorović, V., Lazarević Macanović, M., Sourice Petit, S., Beck-Cormier, S., Guicheux, J.,  
20  
21 391 Gauthier, O., & Kovacević Filipović, M. (2014). Age-related changes in the articular cartilage  
22  
23 392 of the stifle joint in non-working and working German shepherd dogs. *Journal of comparative*  
24  
25 393 *pathology*, 151, 363-374. doi: 10.1016/j.jcpa.2014.09.002.  
26  
27  
28 394 Frisbie, D.D., Cross, M.W., & McIlwraith, C.W. (2006). A comparative study of articular  
29  
30 395 cartilage thickness in the stifle of animal species used in human pre-clinical studies compared  
31  
32 396 to articular cartilage thickness in the human knee. *Veterinary and Comparative Orthopaedics*  
33  
34 397 *and Traumatology*, 19, 142-146. doi:10.1055/s-0038-1632990.  
35  
36  
37 398 Gelse, K., Körber, L., Schöne, M., Raum, K., Koch, P., Pachowsky, M., Welsch, G., & Breiter,  
38  
39 399 R. (2017). Transplantation of Chemically Processed Decellularized Meniscal Allografts.  
40  
41 400 *Cartilage*, 8, 180-190. doi: 10.1177/1947603516646161.  
42  
43  
44 401 Gerwin, N., Bendele, A.M., Glasson, S., & Carlson, C.S. (2010). The OARSI histopathology  
45  
46 402 initiative - recommendations for histological assessments of osteoarthritis in the rat.  
47  
48 403 *Osteoarthritis and Cartilage*, 18: S24-S34. doi: 10.1016/j.joca.2010.05.030.  
49  
50  
51 404 Glasson, S.S., Chambers, M.G., Van Den Berg, W.B., & Little, C.B. (2010). The OARSI  
52  
53 405 histopathology initiative - recommendations for histological assessments of osteoarthritis in the  
54  
55 406 mouse. *Osteoarthritis and Cartilage*, 18: S17-S23. doi: 10.1016/j.joca.2010.05.025.  
56  
57  
58  
59  
60

- 1  
2  
3 407 Grynblas, M., Albert, B., Katz, I., Lieberman, I., & Pritzker, K.P.H. (1991). Subchondral bone  
4  
5 408 in osteoarthritis. *Calcified Tissue International*, 49, 20–26. Doi: 10.1007/BF02555898.  
6  
7 409 Havelka, S., Horn, V., Spohrová, D., & Valouch, P. (1984). The calcified-non calcified cartilage  
8  
9 410 interface: the tidemark. *Acta Biologica Hungary*, 35, 271-279.  
10  
11  
12 411 Hoeman, C.D., Hurtig, M., Rossomacha, E., Sun, J., Chevrier, A., Shive, M.S., & Buschmann,  
13  
14 412 M.D. (2005). Chitosan-Glycerol Phosphate/Blood Implants improve Hyaline Cartilage Repair  
15  
16 413 in Ovine Microfracture Defects. *The Journal of Bone And Joint Surgery*, 87, 2671-2686.  
17  
18 414 doi:10.2106/JBJS.D.02536.  
19  
20  
21 415 Hoemann, C., Kandel, R., Roberts, S., Saris, D.B.F., Creemers, L., Mainil-Varlet, P., Méthot,  
22  
23 416 S., Hollander, A.P., & Buschmann, M.D. (2011). International Cartilage Repair Society (ICRS)  
24  
25 417 Recommended Guidelines for Histological Endpoints for Cartilage Repair Studies in Animal  
26  
27 418 Models and Clinical Trials. *Cartilage*, 2, 153– 172. doi: 10.1177/1947603510397535.  
28  
29  
30 419 Hontoir, F., Clegg, P., Simon, V., Kirschvink, N., Nisolle, J.-F., & Vandeweerdt, J.-M. (2017).  
31  
32 420 Accuracy of computed tomographic arthrography for assessment of articular cartilage defects  
33  
34 421 in the ovine stifle. *Veterinary Radiology and Ultrasound*, 58, 512-523. doi: 10.1111/vru.12504.  
35  
36  
37 422 Huang, F.S., Simonian, P.T., Norman, A.G., & Clark, J.M. (2004). Effects of small  
38  
39 423 incongruities in a sheep model of osteochondral autografting. *The American Journal of sports*  
40  
41 424 *medicine*, 32, 1842-1848. <https://doi.org/10.1177/0363546504264895>.  
42  
43  
44 425 Hulth, A. (1993). Does osteoarthrosis depend on growth of the mineralized layer of cartilage?  
45  
46 426 *Clinic Orthopaedics Related Research*, 287, 19–24. doi: 10.1097/00003086-199302000-00004.  
47  
48  
49 427 Iijima, H., Ito, A., Nagai, M., Tajino, J., Yamaguchi, S., Kiyan, W., Nakahata, A., Zhang, J.,  
50  
51 428 Wang, T., Aoyama, T., Nishitani, K., & Kuroki, H. (2017). Physiological exercise loading  
52  
53 429 suppresses post-traumatic osteoarthritis progression via an increase in bone morphogenetic  
54  
55 430 proteins expression in an experimental rat knee model. *Osteoarthritis and Cartilage*, 25, 964-  
56  
57 431 975. doi: 10.1016/j.joca.2016.12.008.  
58  
59  
60

- 1  
2  
3 432 Jeffery, A.K., Blunn, G.W., Archer, C.W., & Bentley, G. (1991). Three-dimensional collagen  
4  
5 433 architecture in bovine articular cartilage. *Journal of Bone and Joint Surgery*, 73, 795-801.  
6  
7 434 <https://doi.org/10.1016/j.joca.2017.02.673>.  
8  
9  
10 435 Kraus, V.B., Huebner, J.L., DeGroot, J., & Bendele, A. (2010). The OARSI histopathology  
11  
12 436 initiative - recommendations for histological assessments of osteoarthritis in the guinea pig.  
13  
14 437 *Osteoarthritis and Cartilage*, 18, S35-S52. <https://doi.org/10.1016/j.joca.2010.04.015>.  
15  
16  
17 438 Lahm, A., Kreuz, P., Oberst, M., Haeberstroh, J., Uhl, M., & Maier, D. (2006). Subchondral  
18  
19 439 and trabecular bone remodelling in canine experimental model of osteoarthritis. *Archives of*  
20  
21 440 *Otrthopaedic and Trauma Surgery*, 126, 582-587. doi: 10.1007/s00402-005-0077-2.  
22  
23  
24 441 Lane, L.B., & Bullough, P.G., (1980). Age-related changes in the thickness of the calcified  
25  
26 442 cartilage and the number of tidemarks in adult human articular cartilage. *The journal of bone*  
27  
28 443 *and joint surgery*, 62, 372–375. doi: 10.1302/0301-620X.62B3.7410471.  
29  
30  
31 444 Laverty, S., Girard, C.A., Williams, J.M., Hunziker, E.B., & Pritzker, K.P.H. (2010). The  
32  
33 445 OARSI histopathology initiative - recommendations for histological assessments of  
34  
35 446 osteoarthritis in the rabbit. *Osteoarthritis and Cartilage*, 18, S53-S65. doi:  
36  
37 447 10.1016/j.joca.2010.05.029.  
38  
39  
40 448 Lee-Shee, N.K., Dickey, J.P., & Hurtig, M.B. (2007). Contact mechanics of the ovine stifle  
41  
42 449 during simulated early stance in gait. An *in vitro* study using robotics. *Veterinary and*  
43  
44 450 *comparative orthopaedics and traumatology*, 20, 70-72. doi: 10.1055/s-0037-1616591.  
45  
46  
47 451 Little, C.B., Smith, M.M., Cake, M.A., Read, R.A., Murphy, M.J., & Barry, F.P. (2010). The  
48  
49 452 OARSI histopathology initiative - recommendations for histological assessments of  
50  
51 453 osteoarthritis in sheep and goats. *Osteoarthritis and Cartilage*, 18, 80-92.  
52  
53 454 <http://dx.doi.org/10.1016/j.joca.2010.04.016>.  
54  
55  
56 455 Lyons, T.J., Stoddart, R.W., McClure, S.F., & McClure, J. (2005). The tidemark of the chondro-  
57  
58 456 osseous junction of the normal human knee joint. *Journal of molecular histology*, 36, 207–215.  
59  
60 457 <https://doi.org/10.1007/s10735-005-3283-x>.

- 1  
2  
3 458 Mainil-Varlet, P., Van Damme, B., Nestic, D., Knutsen, G., Kandel, R., & Roberts, S. (2010).  
4  
5 459 A new histology scoring system for the assessment of the quality of human cartilage repair:  
6  
7 460 ICRS II. *American Journal of Sports Medicine*, 38, 880-890. doi: 10.1177/0363546509359068.  
8  
9  
10 461 Martinelli, M.J., Eurell, J., Les, C.M., Fyhrie, D., & Bennett, D. (2002). Age-related  
11  
12 462 morphometry of equine calcified cartilage. *Equine Veterinary Journal*, 34, 274-278.  
13  
14 463 <https://doi.org/10.2746/042516402776186100>.  
15  
16  
17 464 McIlwraith, C.W. (1996). *Joint Disease in the Horse*. Philadelphia, PA: Saunders.  
18  
19 465 McIlwraith, C.W., Frisbie, D.D., Kawcak, C.E., Fuller, C.J., Hurtig, M., & Cruz, A. (2010).  
20  
21 466 The OARSI histopathology initiative - recommendations for histological assessments of  
22  
23 467 osteoarthritis in the horse. *Osteoarthritis and Cartilage*, 18, S93-S105.  
24  
25 468 <https://doi.org/10.1016/j.joca.2010.05.031>.  
26  
27  
28 469 Meachim, G., & Allibone, R. (1984). Topographical variation in the calcified zone of upper  
29  
30 470 femoral articular cartilage. *Journal of Anatomy*, 139, 341-352.  
31  
32  
33 471 Miller, L.M., Novatt, J.T., Hamerman, D., & Carlson, C.S. (2004). Alterations in mineral  
34  
35 472 composition observed in osteoarthritic joints cynomolgus monkeys. *Bone*, 35, 498-506.  
36  
37 473 <https://doi.org/10.1016/j.bone.2004.03.034>.  
38  
39  
40 474 Mosher, T.J., Smith, H.E., Collins, C., Liu, Y., Hancy, J., Dardzinski, B.J., & Smith, M.B.  
41  
42 475 (2005). Change in knee cartilage T2 at MR imaging after running: a feasibility study.  
43  
44 476 *Radiology*, 234, 245-249. <https://doi.org/10.1148/radiol.2341040041>.  
45  
46  
47 477 Muir, P., Peterson, A.L., Sample, S.J., Scollay, S.C., Markell, M.D., & Kalscheur, V.L. (2008).  
48  
49 478 Exercise-induced metacarpophalangeal joint adaptation in the Thoroughbred racehorse.  
50  
51 479 *Journal of anatomy*, 213, 706–717. doi: 10.1111/j.1469-7580.2008.00996.x.  
52  
53  
54 480 Neogi, T., Felson, D., Niu, J., Lynch, J., Nevitt, M., Guermazi, A., Roemer, F., Lewis, C.E.,  
55  
56 481 Wallace, B., & Zhang, Y. (2009). Cartilage loss occurs in the same subregions as subchondral  
57  
58  
59  
60

- 1  
2  
3 482 bone attrition: a within-knee subregion-matched approach from the multicentre osteoarthritis  
4  
5 483 study. *Arthritis and rheumatism*, 61, 1539-1544. doi: 10.1002/art.24824.  
6  
7  
8 484 Oegema, T.R., Carpenter, R.J., Hofmeister, F., & Thompson, R.C. (1997). The interaction of  
9  
10 485 the zone of calcified cartilage and subchondral bone in osteoarthritis. *Microscopy research and*  
11  
12 486 *technique*, 37, 324–332. [https://doi.org/10.1002/\(SICI\)1097-0029\(19970515\)37:4<324::AID-](https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<324::AID-JEMT7>3.0.CO;2-K)  
13  
14 487 [JEMT7>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<324::AID-JEMT7>3.0.CO;2-K)  
15  
16  
17 488 Oettmeier, R., Abendroth, K., & Oettmeier, S. (1989). Analyses of the tidemark on human  
18  
19 489 femoral heads. II. Tidemark changes in osteoarthrosis: a histological and histomorphometric  
20  
21 490 study in non-decalcified preparations. *Acta morphologica Hungarica*, 37, 169-180.  
22  
23  
24 491 Pearce, G.L., & Frisbie, D.D. (2010). Statistical evaluation of biomedical studies. *Osteoarthritis*  
25  
26 492 *and Cartilage* 18, S117-122. doi: 10.1016/j.joca.2010.04.014.  
27  
28  
29 493 Piskin, A., Gulbahar, M.Y., Tomak, Y., Gulman, B., Hokelek, M., Kerimoglu, S. Koksall, B.,  
30  
31 494 Alic, T., & Kabak, Y.B. (2007). Osteoarthritis models after anterior cruciate ligament resection  
32  
33 495 and medial meniscectomy in rats. A histological and immunohistochemical study. *Saudi*  
34  
35 496 *Medical Journal*, 28, 1796–1802.  
36  
37  
38 497 Sanchez-Adams, J., Leddy, H.A., McNulty, A.L., O'Connor, C.J., & Guilak, F. (2014). The  
39  
40 498 mechanobiology of articular cartilage: bearing the burden of osteoarthritis. *Current*  
41  
42 499 *Rheumatology Reports*, 16, 451. doi: 10.1007/s11926-014-0451-6. doi: 10.1007/s11926-014-  
43  
44 500 0451-6.  
45  
46  
47 501 Simkin, P.A. (2012). Consider the tidemark. *The Journal of Rheumatology*, 39, 890-892. doi:  
48  
49 502 10.3899/jrheum.110942.  
50  
51  
52 503 Souza, R.B., Kumar, D., Calixto, N., Singh, J., Schooler, J., Subburaj, K., Li, X., Link, T.M.,  
53  
54 504 & Majumdar, S. (2014). Response of knee cartilage T1rho and T2 relaxation times to *in vivo*  
55  
56 505 mechanical loading in individuals with and without knee osteoarthritis. *Osteoarthritis and*  
57  
58 506 *Cartilage*, 22, 1367-1376. doi: 10.1016/j.joca.2014.04.017.  
59  
60

- 1  
2  
3 507 Stenhamre, H., Slynarski, K., Petrén, C., Tallheden, T., & Lindahl, A. (2008). Topographic  
4  
5 508 variation in redifferentiation capacity of chondrocytes in the adult human knee joint.  
6  
7 509 *Osteoarthritis and Cartilage*, 16, 1356-1362. doi: 10.1016/j.joca.2008.03.025.  
8  
9  
10 510 Suber, T., & Rosen, A. (2009). Apoptotic cell blebs: repositories of autoantigens and  
11  
12 511 contributors to immune context. *Arthritis and Rheumatism*, 60, 2216-2219. doi:  
13  
14 512 10.1002/art.24715.  
15  
16  
17 513 Suri, S., Gill, S.E., Massena de Camin, S., Wilson, D., McWilliams, D.F., & Walsh, D.A.  
18  
19 514 (2007). Neurovascular invasion at the osteochondral junction and in osteophytes in  
20  
21 515 osteoarthritis. *Annals of Rheumatic Diseases*, 66, 1423–1428. doi: 10.1136/ard.2006.063354  
22  
23  
24 516 Taylor, W.R., Poeplau, B.M., Konig, C., Ehrig, R.M., Zachow, S., Duda, G.N., & Heller, M.O.  
25  
26 517 (2011). The medial-lateral force distribution in the ovine stifle joint during walking. *Journal of*  
27  
28 518 *Orthopaedic Research*, 29, 567-571. doi: 10.1002/jor.21254.  
29  
30  
31 519 Thomas, C.M., Fuller, C.J., Whittles, C.E., & Sharif, M. (2007). Chondrocyte death by  
32  
33 520 apoptosis is associated with cartilage matrix degradation. *Osteoarthritis and Cartilage*, 15, 27–  
34  
35 521 34. <https://doi.org/10.1016/j.joca.2006.06.012>.  
36  
37  
38 522 Thomas, R.H., Resnick, D., Alazraki, N.P., Daniel, D., & Greenfield, R. (1975). Compartmental  
39  
40 523 evaluation of osteoarthritis of the knee: a comparative study of available diagnostic modalities.  
41  
42 524 *Radiology*, 116, 585-94. <https://doi.org/10.1148/116.3.585>.  
43  
44  
45 525 Vandeweerd, J.M., Hontoir, F., Kirschvink, N., Clegg, P., Nisolle, J.F., Antoine, N., & Gustin,  
46  
47 526 P. (2013). Prevalence of Naturally Occurring Cartilage Defects in the Ovine Knee.  
48  
49 527 *Osteoarthritis and Cartilage*, 21, 1125-1131. doi: 10.1016/j.joca.2013.05.006.  
50  
51  
52 528 Wucherer, K.L., Ober, C.P., & Conzemius, M.G. (2012). The use of delayed gadolinium  
53  
54 529 enhanced magnetic resonance imaging of cartilage and T2 mapping to evaluate articular  
55  
56 530 cartilage in the normal canine elbow. *Veterinary Radiology and Ultrasound*, 53, 57-63. doi:  
57  
58 531 10.1111/j.1740-8261.2011.01867.x.  
59  
60

- 1  
2  
3 532 Zamli, Z., Adams, M.A., Tarlton, J.F., & Sharif, M. (2013). Increased Chondrocyte Apoptosis  
4  
5 533 Is Associated with Progression of Osteoarthritis in Spontaneous Guinea Pig Models of the  
6  
7 534 Disease. *Internatinal Journal of Molecular Sciences*, 14, 17729-17743. doi:  
8  
9 535 10.3390/ijms140917729.  
10  
11  
12 536 Zscharnak, M., Hepp, P., Richter, R., Aigner, T., Schultz, R., Somerson, J., Josten, C., Bader,  
13  
14 537 A., & Marquass, B. (2010). Repair of chronic osteochondral defects using predifferentiated  
15  
16 538 mesenchymal stem cells in an ovine model. *American Journal of Sports Medicine*, 38, 1857-  
17  
18 539 1869. doi: 10.1177/0363546510365296.  
19  
20  
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For Review Only

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

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

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 543 N= number of sheep. Mean tidemark count and OARSI scoring of both limbs were considered  
 544 for each sheep.  
 545 The tidemark count ( $P < 0.0001$ ) and the OARSI scores ( $P = 0.0197$ ) differed significantly  
 546 between groups.

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3 548 **Figure legends**  
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5 549 **Figure 1.** Sampling sites in the middle third of the medial tibial condyle (MTC), medial femoral  
6 condyle (MFC), lateral tibial condyle (LTC) and lateral femoral condyle (LFC). Tibial slabs  
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8 550 were centered on the intercondylar eminence (black lines). Femoral slabs were obtained in the  
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10 551 centre of the middle third of the circumference of the condyle (black lines and dotted black  
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12 552 box). White rectangles illustrate the histological slices that were obtained, each abaxial (Ab)  
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14 553 and axial (Ax) part being assessed separately at microscopy. White arrows highlight cartilage  
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21 556 **Figure 2.** The osteochondral junction at histology.

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24 557 A. The white line indicates non-calcified hyaline cartilage (HC); the black line is the calcified  
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26 558 cartilage (CC).

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28 559 B. White arrows indicate tidemarks.

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30 560 C. Histological slide showing the absence of tidemark in a sample of cartilage of the medial  
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32 561 femoral condyle in a 6 months old sheep.

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37 563 **Figure 3:** Number of tidemarks in the different sub-regions for right and left limbs, expressed  
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39 564 as median and interquartile range (bar). Asterisks means that statistical significance ( $P < 0.05$ ) is  
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41 565 reached for the difference between the axial and the abaxial part of the region.

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44 566 MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral  
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46 567 femoral condyle, respectively.

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51 569 **Figure 4:** OARSI scores in the different sub-regions for right and left limbs, expressed as  
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53 570 median and interquartile range (bar). Asterisks means that statistical significance ( $P < 0.05$ ) is  
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55 571 reached for the difference between the axial and the abaxial part of the region.  
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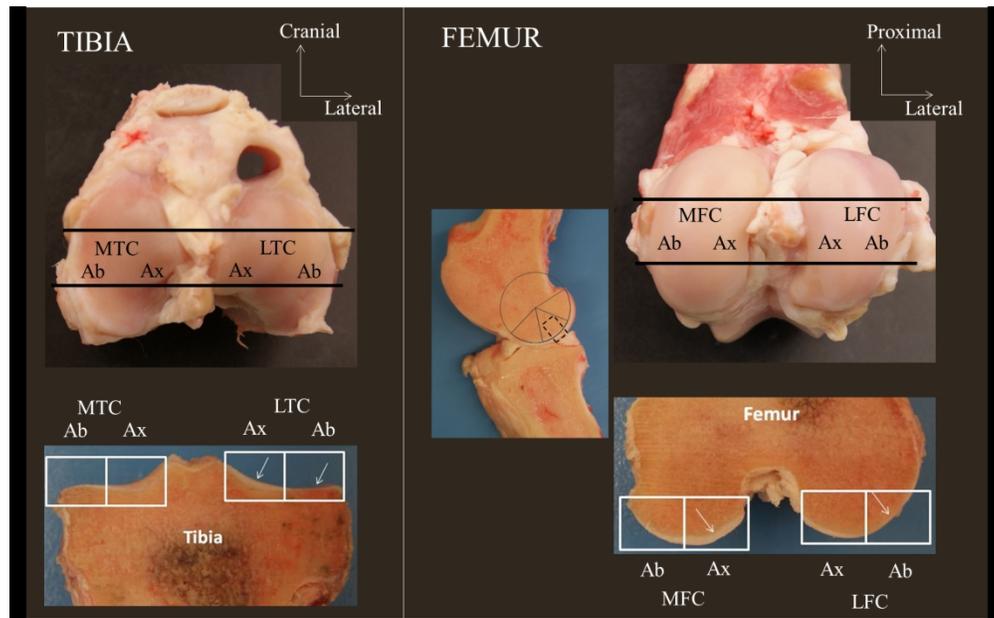


Figure 1. Sampling sites in the middle third of the medial tibial condyle (MTC), medial femoral condyle (MFC), lateral tibial condyle (LTC) and lateral femoral condyle (LFC). Tibial slabs were centered on the intercondylar eminence (black lines). Femoral slabs were obtained in the centre of the middle 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.

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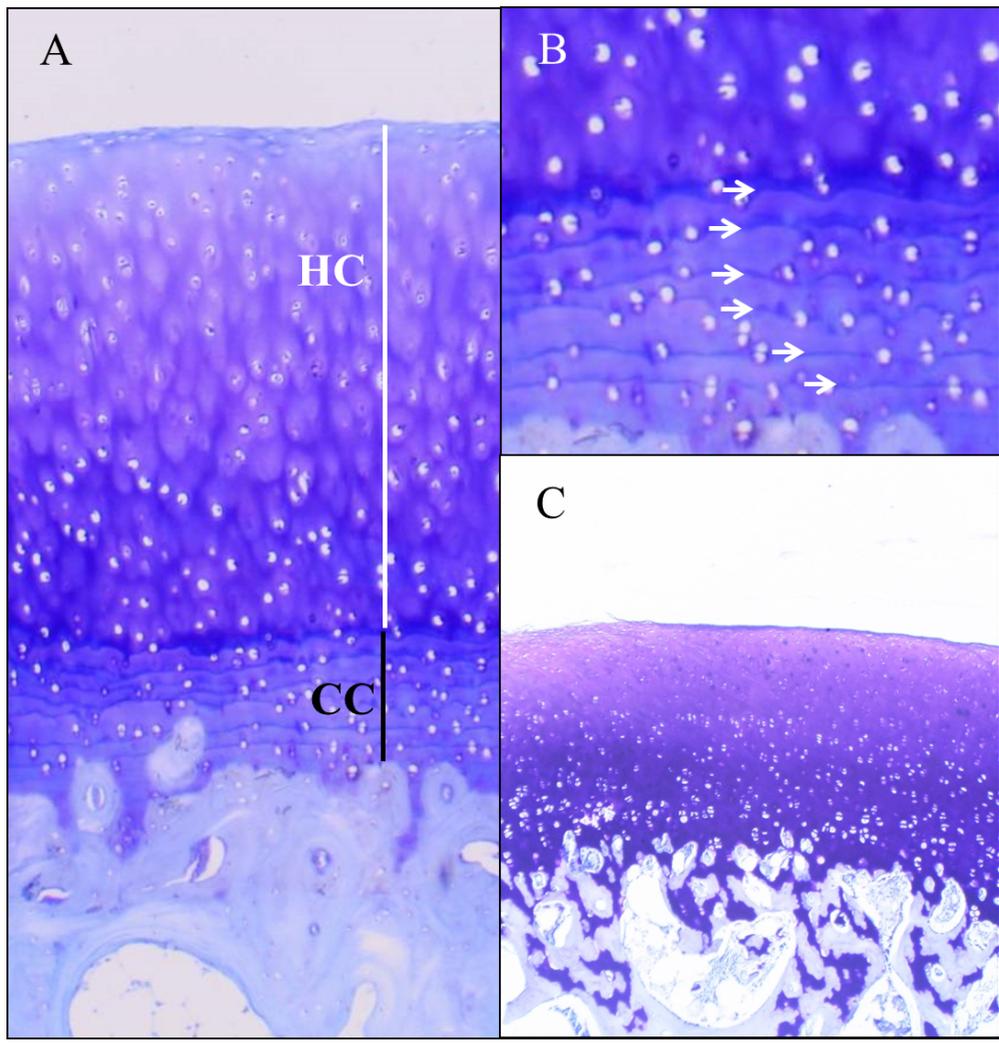


Figure 2. The osteochondral junction at histology.  
 A. The white line indicates non-calcified hyaline cartilage (HC); the black line is the calcified cartilage (CC).  
 B. White arrows indicate tidemarks.  
 C. Histological slide showing the absence of tidemark in a sample of cartilage of the medial femoral condyle in a 6 months old sheep.

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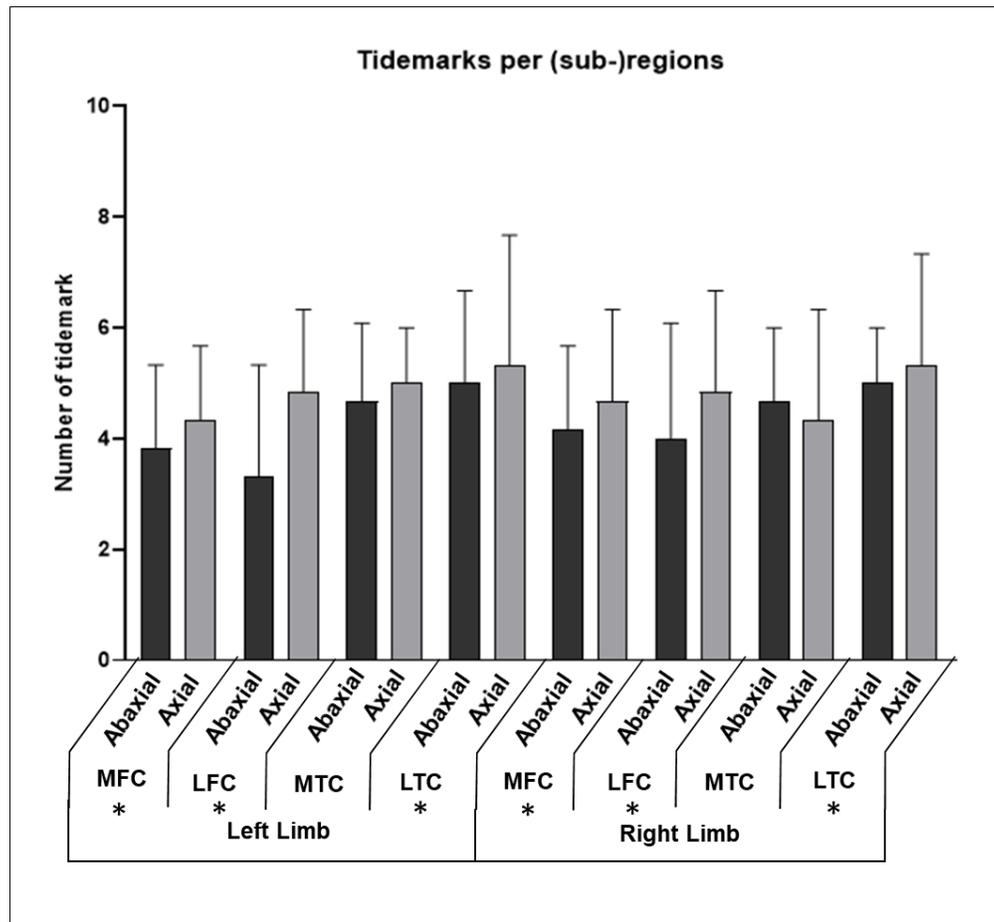


Figure 3: Number of tidemarks in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ( $P < 0.05$ ) is reached for the difference between the axial and the abaxial part of the region.

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

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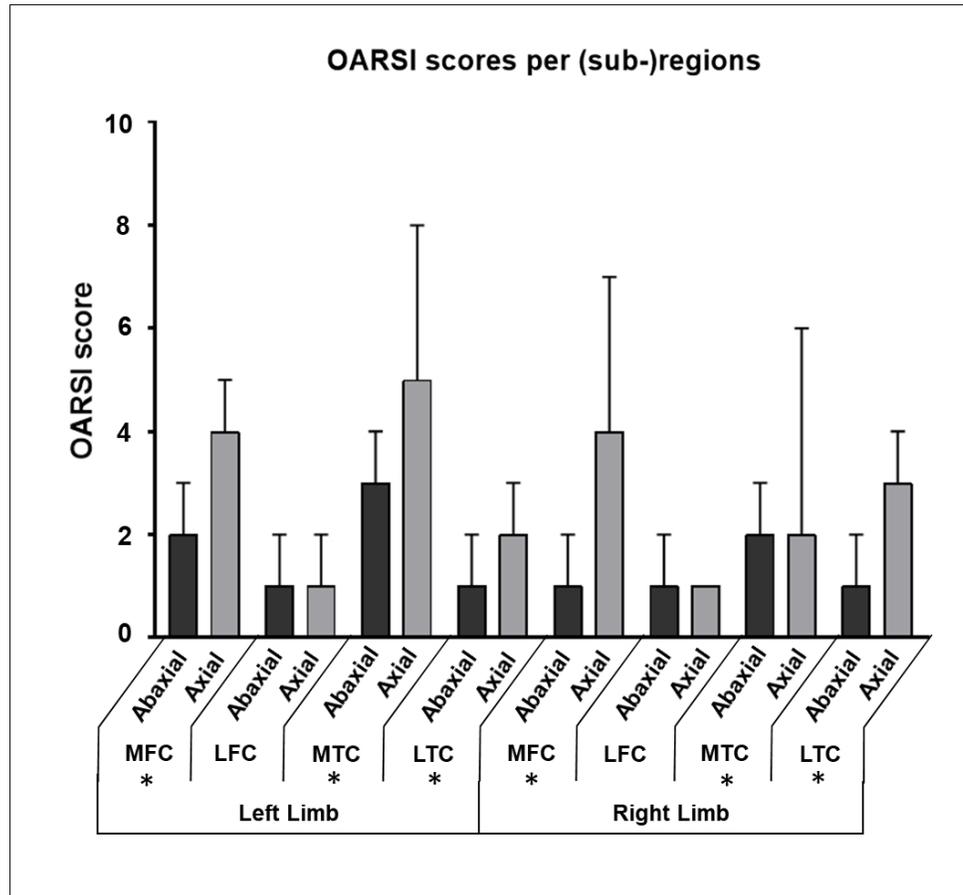


Figure 4: OARSI (OsteoArthritis Research Society International) scores in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ( $P < 0.05$ ) is reached for the difference between the axial and the abaxial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

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

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