The Knee 23 (2016) 214–220

Contents lists available at ScienceDirect

The Knee

Surgical induced models of joint degeneration in the ovine stifle: Magnetic resonance imaging and histological assessment☆

Joaquin Moya-Angeler [a,*], moyaangelerj@hss.edu, Jimena Gonzalez-Nieto [b], Joaquin Sanchez Monforte [b], Jose R. Altonaga [c], Javier Vaquero [d], Francisco Forriol [e]

[a] Orthopedic Surgery, Hospital for Special Surgery, 535 E 71st, New York 10021, NY, USA
[b] Radiology Department, Hospital Clinico San Carlos, Calle Prof Martín Lagos S/N, 28040 Madrid, Spain
[c] Surgery Department, Facultad de Veterinaria Universidad de Leon, Facultad de Veterinaria, 25, 24004 León, Spain
[d] Orthopedic Department, Hospital Gregorio Marañon, Calle del Dr. Esquerdo, 46, 28007 Madrid, Spain
[e] School of Medicine, Universidad San Pablo CEU, Ctra. Boadilla del Monte, Km. 5 300, 28925 Alcorcon, Madrid, Spain

ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form 28 October 2015
Accepted 7 November 2015

Keywords:
Osteoarthritis
Sheep
Knee
MR
SMOAKS
Score

ABSTRACT

Background: The purposes of this study were to (1) validate and assess the reliability of a modified magnetic resonance semi-quantitative score (sheep Magnetic Resonance osteoarthritis Knee Score (sMOAKS)) to evaluate joint degeneration in the ovine knee and to (2) investigate whether the transection of the anterior cruciate ligament (ACL), isolated or in combination with meniscal injuries, reproduce the degenerative changes described in the meniscectomized sheep.
Methods: Twenty sheep were randomly subjected to one of the following injuries to induce osteoarthritis (OA): ACL transection (ACLt), mid-body transection of the medial meniscus, ACLt combined with complete medial meniscectomy and complete medial meniscectomy. OA assessment was performed eight weeks postoperatively with sMOAKS, Mankin and Osteoarthritis Research Society International (OARSI) histological scores.
Results: sMOAKS showed very good to excellent reliability (kappa = 0.61 to 1.0) for the majority of features evaluated. sMOAKS revealed small differences between groups ($p < 0.05$) being the ACLt group the most affected. We observed a strong positive correlation between the three scales in the evaluation of femoro-tibial articular cartilage (AC) ($r = 0.829$, $r = 0.917$, $r = 0.879$).
Conclusions: sMOAKS is a reliable semi-quantitative Magnetic Resonance (MR) scale to evaluate and quantify the effect of different OA induction lesions in the ovine knee and presents a high correlation with Mankin and OARSI scales in the evaluation of femoro-tibial AC. Although minor differences were observed between the different surgical procedures for the induction of OA, ACLt proved to be the intervention that produced the highest amount of degeneration eight weeks postoperatively.
Level of Evidence: II

1. Introduction

Osteoarthritis (OA) is a disease of the whole joint that not only affects articular cartilage but also the sub-chondral bone, ligaments, muscles, menisci, synovium, and joint fluid [1]. Currently, animal models are the only practical way to examine the processes involved in the initiation and progression of this disease [2–6]. However, no single gold standard animal model has been defined and each animal presents different advantages and disadvantages. In addition, the evaluation of the progression of the disease with non-invasive measures has become particularly important in this field since most of the patients remain asymptomatic until relatively late in the disease process. Magnetic resonance (MR) has been proven to be extremely useful for the study of the initial changes in OA as well as to evaluate other structures of the joint involved in the disease. Several MR scales have been developed for the assessment of OA in humans; however, none of these have been proven to be useful in animals [4–15] where subtle anatomical differences make these systems difficult to apply.

The ovine knee is considered a consistent model of OA that closely resembles humans [16]. Moreover, meniscectomy in the adult sheep has shown to reproduce the pathological changes in articular cartilage (AC) and sub-chondral bone that have been described for early OA in

☆ This work was mainly performed in the Surgical Department of the Facultad de Veterinaria of Leon University in combination with CEU San Pablo University.

* Corresponding author at: Hospital for Special Surgery, 475th E 72nd Street Ground Floor, New York 10021, NY, USA.
E-mail addresses: jmoyaangeler@gmail.com (J. Moya-Angeler), jimenagn@gmail.com (J. Gonzalez-Nieto), yoki_atl@hotmail.com (J. Sanchez Monforte), dmajrm@unileon.es (J.R. Altonaga), vaquerocot@gmail.com (J. Vaquero), fforriol@mac.com (F. Forriol).

http://dx.doi.org/10.1016/j.knee.2015.11.017
0968-0160/

human joints [17]. The meniscectomized ovine model has provided valuable insights into the temporal changes that occur in AC and subchondral bone during the development and progression of the disease [18] with evidence of osteoarthritic findings ranging from two weeks to one year after the induction of OA [19–22]. The transection of the anterior cruciate ligament (ACLt) has not been commonly performed to induce OA in the ovine knee, and the few existing reports described very limited mild cartilage damage in these species [23,24]. There is; however, an absence of comparative prospective studies evaluating the effect of different meniscal procedures and or ACLt in the same animal model.

The objectives of this study were; therefore, to (1) validate and assess the reliability of a modified magnetic resonance semi-quantitative score to evaluate joint degeneration in the ovine knee (sheep Magnetic Resonance OA Knee Score (sMOAKS)) and to (2) investigate whether the transection of the anterior cruciate ligament (ACL), isolated or in combination with different meniscal injuries, would reproduce the degenerative changes that have been described in the meniscectomized laboratory sheep. Our hypothesis is that the degenerative changes observed would be significantly different among these four ovine models eight weeks after surgery.

2. Material and methods

Twenty merino female sheep, all of three years of age, were (mean body weight 40 kg ± 7.5 (*SD*)) selected for uniformity of size and conformation for this study. Sheep were kept in-group in individual cages and were fed with a standard diet and water ad libitum. All animals were healthy and free of any other disease. Our institutional animal care committee approved all procedures performed.

Sheep were randomly subjected to one of the following four variant intraarticular injuries to induce OA ($n = 5$ per treatment): (1) anterior cruciate ligament transection (ACLt), (2) mid-body transection of the medial meniscus (TMM), (3) ACLt with a complete medial meniscectomy (CMM) and (4) isolated complete medial meniscectomy (iCMM).

The induction of anesthesia was performed with intra-muscular diazepam and ketamine using halothane throughout the surgical procedure. All knees were approached through a standard central incision and a common medial parapatellar arthrotomy [25]. Electrocautery was used as required in order to prevent major bleeding. A thorough examination of the knee was then performed to assess all compartments making sure that there were no previous cartilage damages or other abnormalities. When indicated, the ACL was elevated in its mid-portion with the aid of a surgical hook and then transected with an 11 surgical blade. If a meniscal injury had to be performed, this was done while forcing the knee into valgus and minimal external rotation. With the aim of a Kocher forceps, the medial meniscus was completely removed when indicated. Otherwise, a complete mid-body transection of the medial meniscus was performed with an 11 blade. The capsule and the skin were closed in layers with absorbable sutures.

After a brief recovery period (seven to 24 h) in individual cages, all sheep were allowed free range of motion on irrigated pasture for the remainder eight-week trial period. All sheep underwent sedation eight weeks after the operation. Subsequently, they all received the MR which were performed on a three Tesla MR machine (Siemens Trio, Erlangen, Germany). The imaging protocol included sagittal spin-echo proton density and T2-weighted images (three millimeter slice thickness and a matrix of 256 × 192 pixels), coronal and axial spin-echo fat-suppressed proton density and T1-weighted images (same thickness and matrix). Acquisition parameters were: repetition time was 4000 ms, echo time was 24 ms, slice thickness was one to three millimeters, interslice gap was 0.2 mm, number of excitation was two, size of field view was 12 cm and echo train length between 10 and 14. Subsequently, all sheep were euthanized and the knee joints were opened and prepared for later histological preparation.

2.1. Imaging assessment

The MOAKS (MRI Osteoarthritis Knee Score) semi-quantitave-scale [8] was selected for OA assessment; however, we modified it in order to make it applicable to the ovine knee. The three main modifications included: a different sub-regional delineation (considering the patellofemoral joint separate from the anterior part of the femur), the exclusion of the evaluation of meniscus and ligaments (as these were intentionally injured) and a system to quantify the degenerative changes. We added the suffix “s” to refer to this scale as the sheep-MOAKS (sMOAKS). The final sMOAKS scores were classified and quantified for classification (Table 1).

2.2. Subregional division and delineation

The knee joint was divided into 12 sub-regions (Figure 1) (Figure 2) for scoring AC and bone marrow lesions (BMLs).

1. The femoro-tibial articular surfaces were divided into medial and lateral compartments: femoro-tibial medial joint and femoro-tibial lateral joint (FTMJ and FTLJ) (Figure 1). These regions were further subdivided into four sub-regions (on the sagittal view), anterior-medial and posterior-medial femur (AMF and PMF), anterior-medial and posterior-medial tibia (AMT and PMT), anterior-lateral and posterior-lateral femur (ALF and PLF) and anterior-lateral and posterior-lateral tibia (ALT and PLT). The landmarks to delineate these regions involved a tangential line parallel to the femoral surface starting at the level of the insertion of the *extensor digitorum longus* (EDL) tendon (same reference for the medial side) and a line perpendicular to that, dividing the condyle proportionally into two halves. Based on these subdivisions, the FTMJ comprised regions AMF, PMF, AMT and PMT and the FTLJ comprised regions ALF, PLF, ALT and PLT.
2. The patellofemoral joint (PFJ) (Figure 2) was divided into eight sub-regions that were evaluated in the axial and sagittal views. The medial and lateral patella (MP and LP) and the medial and lateral trochlea (MTr and LTr) were assessed on the axial view, whereas the superior and inferior patella (SP and IP) and the superior and inferior trochlea (STr and ITr) were evaluated on the sagittal view. These were divided according to the apex of the patella with a line, dividing both medial and lateral sides, perpendicular to the joint line including the apex in the lateral side. The trochlea was also divided according to this line. Based on these subdivisions the PFJ comprised regions: MP, LP, MTr, LTr, SP, IP, STr and ITr.
   The original MOAKS; however, divides the knee joint into 14 sub-regions for scoring AC and BMLs, adding the sub-spinous region for BML scoring. Since MOAKS defines the trochlea as the femoral articular surface of the patellofemoral joint (PFJ), the femur is divided into six sub-regions (medial and lateral plus the corresponding sub-regions in the tibia — total of 12 sub-regions) and the patella is divided into two sub-regions (total of 14 sub-regions). This; however, cannot be applied to the ovine knee given the anatomical differences between both knees species (lack of full extension and the tripartition of the patellofemoral joint and both condyles with a

Table 1
Combined sMOAKS score for the whole knee. FTMJ: femoro-tibial medial joint. FTLJ: femoro-tibial lateral joint. PFJ: patella-femoral joint. BML: bone marrow lesions.

	FTMJ	FTLJ	PFJ	Total
Cartilage	24	24	24	72
BML/cyst	36	36	36	108
Osteophytes	12	12	24	48
Synovitis				3
Effusion				3
Total compartment	72	72	84	
Total				234

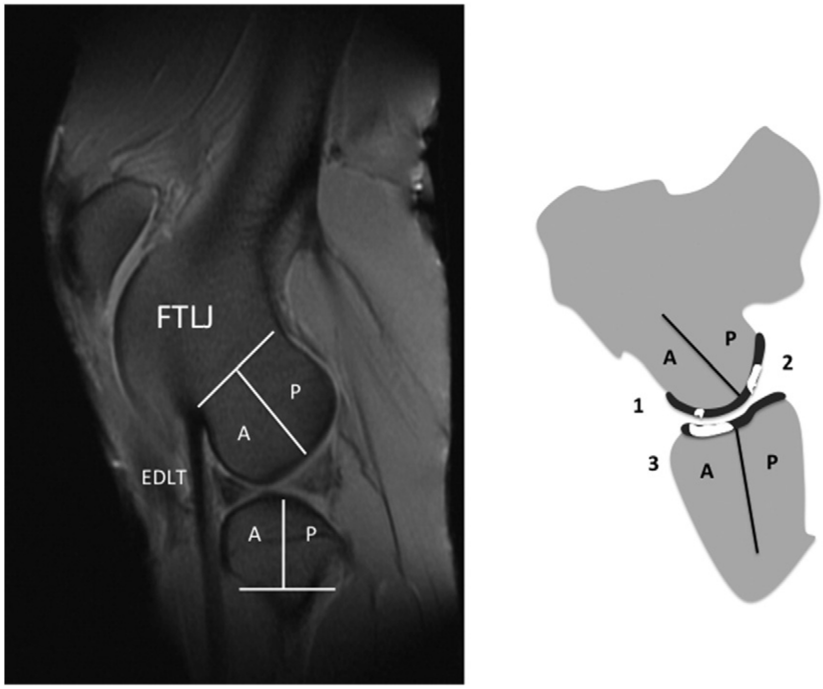

Figure 1. Regional delineation of the femoro-tibial lateral joint (FTLJ — sagittal view) and representation of the percentage of size of cartilage lesions. EDTL: *extensor digitorum longus tendon*. A: anterior. B: posterior.

prominent and slim trochlea in the ovine stifle). For this reason, we performed the evaluation of the patellofemoral compartment independently.

2.3. Scoring individual features

The scoring of the five individual features (one — AC, two — BMLs and cysts, three — osteophytes, four — Hoffa's synovitis and five — joint effusion) was based on MOAKS [15]. Since the MOAKS system was modified into a volume-oriented approach (AC and BMLs are classified according to the volume of the lesion occupying a certain specific region) when a lesion spans across more than one sub-region it has to be scored in each sub-region individually.

3. Articular cartilage

AC was scored in each of the 12 articular surface regions using *fat-suppressed T2-weighted FSE images*. AC was graded for size of any cartilage

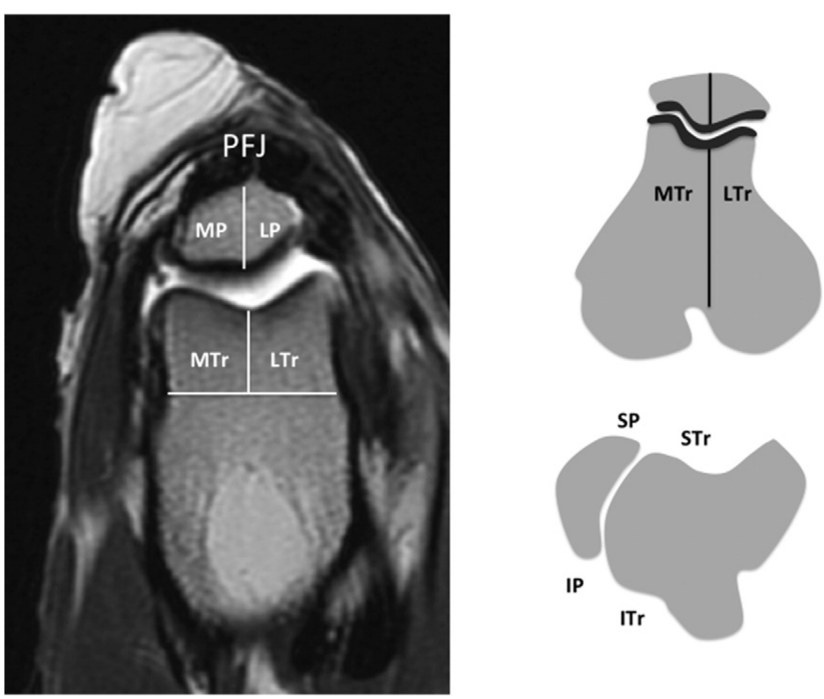

Figure 2. Regional delineation of the patella-femoral joint (PFJ — axial view). MP: medial patella. LP: lateral patella. MTr: medial trochlea. LTr: lateral trochlea. SP: superior patella. IP: inferior patella. STr: superior trochlea. ITr: inferior trochlea.

loss, as the percentage of the surface area as related to the size of each individual surface region (0: none, 1: <10% of region of cartilage surface area (CSA), 2: 10–75% of region of CSA, 3: >75% of CSA) (Figure 1) and as the percentage of loss in this sub-region that presented full-thickness loss (0: none, 1: <10% of region of CSA, 2: 10–75% of region of CSA, 3: >75% of region of CSA). The maximum cartilage scores for FTMJ, FTLJ, PFJ (four sub-regions per compartment with a maximum of six points per sub-region) and the entire knee were: 24, 24, 24 and 72 respectively.

4. BMLs and cysts

BMLs were defined as poorly marginal areas of increased signal intensity in the normally fatty epiphyseal marrow on *fat-suppressed T2-weighted FSE images*. BMLs were graded by size in regard to the total volume of the sub-region occupied by BMLs in each of the 12 articular surface regions (0: none, 1: <33% of sub-regional volume, 2: 33–66% of sub-regional volume, 3: >66% of sub-regional volume) and in number in regard to the amount of BMLs of the sub-region (0: none, 1: one BMLs, 2: two BMLs). Sub-articular cysts were identified as foci of markedly increased signal in the sub-articular bone with sharply defined rounded margins *on the fat-suppressed T2-weighted FSE images*. Cysts were graded as the percentage of the volume of each BML corresponding to a BML, as distinct from cyst (0: none, 1: <33%, 2: 33–66%, 3: >66%). The maximum BMLs and cyst scores for FTMJ, FTLJ, PFJ and the entire knee were (four sub-regions per compartment with a maximum of eight points per sub-region): 36, 36, 36, and 108 respectively.

5. Osteophytes

Osteophytes were evaluated along 16 different margins of the knee (on axial and sagittal views). These margins included: the anterior, posterior margins of the medial, lateral condyles and tibial plateau (four regions each compartment) and the medial, lateral, superior and inferior facets of the patella and trochlea (eight regions). These were graded according to size: 0: none, 1: small (<10% of the corresponding sub-region size (CSS)), 2: medium (10–20% of the CSS), and 3: large (>20% of the CSS). Maximum scores for the FTMJ, FTLJ, PFJ and the entire knee were: 12, 12, 24, and 48 respectively.

6. Hoffa's synovitis — effusion

Hoffa's synovitis was described as diffuse hyperintense signal on the sagittal image within the fat pad, whereas effusion was defined as fluid equivalent signal within the joint cavity on the axial view, both on T2/PD/IW-w fat-suppressed sequences. Scores were based on size (0: normal, 1: mild, 2: moderate, 3: severe). Effusion was defined as fluid equivalent signal in the joint cavity and it was evaluated in axial views on T2/IW/PDw as grade 0 (normal intraarticular hyperintensity), grade 1: small, grade 2: medium and grade 3: large.

sMOAKS scores were calculated for each feature in each of the three compartments (FTMJ, FTLJ, PFJ). These scores were combined to give a total score for the whole knee with a maximum score of 282 points equivalent to 100% of articular degeneration (Table 1). The evaluations were done within a time period of six weeks by two observers (JG-N) (JS-M) with over four years of experience in radiology of the musculoskeletal system (two blinded observations). A minimum interval period of four weeks were kept between observations. They received an initial training session in order to understand the modifications performed in MOAKS.

7. Histology

The purpose of the histologic evaluation was to quantify the overall degree of cartilage degeneration and correlate it with sMOAKS. The process was done following the OARSI histopathology initiative recommendations for histological assessment of OA in the sheep [26]. Coronal osteochondral slabs of three to four millimeters thick, covering the entire width of the tibial and femoral condyles, were cut with a fine-toothed band saw. These were posteriorly fixed in a 10% (v/v) neutral buffer formalin for 48 h and then transferred to 70% (v/v) ethanol. After decalcification and paraffin embedding, five-micron sections were cut and mounted on positively charged slides, which were heated to maximize adhesion and minimize wrinkles in the cartilage. Sections were deparaffinized with xylene and graded ethanol and then stained with Masson trichrome and Safranin-O. For histological scoring one observer blinded to treatment scored each section using the Mankin [27] and OARSI histological scales [28].

7.1. Statistics

Kappa (k) statistics were used to assess inter- and intra-observer agreements in grading cartilage, bone marrow lesions, cyst, osteophytes, effusion and synovitis. A weighted kappa was used when the degree of disagreement was taken into account, and a kappa when disagreements were treated equally [29]. The presented results are for intra and inter-rater reliability (calculated using the weighted kappa (95% confidence interval (CI)), the percentage of agreement of the exercise and the total scores of the different groups. A one-way ANOVA was performed to determine differences among groups with respect to sMOAKS, Mankin and OARSI scores. The Sidake–Holm procedure was selected as post hoc analysis (when significant differences among groups were obtained) to determine which groups were different while minimizing possible type I errors. We used the Spearman's non-parametric correlation coefficient rho to correlate the scores of the sMOAKS, Mankin and OARSI scales. Calculations were performed in SPSS 16.0 (SPSS Inc., Chicago, IL). The statistical significant difference was set with a p below 0.05.

8. Results

No macroscopic abnormalities were observed at the time of surgery in any of the twenty sheep. Three sheep were excluded from the study as they presented a dislocation of the patello-femoral joint in the MR (one from Group 1, one from Group 2 and one from Group 3 subsequently).

9. sMOAKS Scale Reliability

Table 2 represents the reliability scores of the features evaluated. Intra-rater BML size (kappa = 0.57) and percentage of cyst (kappa = 0.58) presented the poorest reliability; however, the prevalence of these features were extremely low, particularly in the PFJ. Overall, these preliminary results demonstrated that the modified scoring system is reproducible.

10. Imaging assessment

Table 3 shows the mean scores and percentages for each group. Most of these scores were in the lower quarter of their possible range with the exception of Group 1. Group 1 (ACLt) was the most affected followed by

Table 2
Reliability of sMOAKS (%). Percentage of agreement. CI: confident interval.

	Intra-observer		%	Inter-observer		%
Total score	0.62	(*CI 0.39–0.72*)	0.63	0.72	(*CI 0.44–0.79*)	0.75
Cartilage						
Area	0.65	(*CI 0.47–0.77*)	0.70	0.71	(*CI 0.57–0.83*)	0.73
Deep	0.61	(*CI 0.44–0.67*)	0.59	0.65	(*CI 0.44–0.75*)	0.67
BML						
Size	0.57	(*CI 0.72–0.91*)	0.53	0.92	(*CI 0.67–0.97*)	0.95
% cyst	0.58	(*CI 0.76–0.97*)	0.58	0.88	(*CI 0.80–0.93*)	0.85
No. of lesions	0.90	(*CI 0.88–0.92*)	0.93	0.95	(*CI 0.90–0.99*)	0.97
Osteophytes	0.65	(*CI 0.65–0.92*)	0.71	0.85	(*CI 0.77–0.90*)	0.86
Synovitis	0.89	(*CI 0.65–0.92*)	0.78	0.83	(*CI 0.76–0.85*)	0.87
Effusion	0.91	(*CI 0.79–0.92*)	0.88	0.90	(*CI 0.77–0.99*)	0.83

Group 2 (mid-body transection of the medial meniscus), Group 3 (ACLt combined with complete medial meniscectomy) and Group 4 (complete medial meniscectomy). All groups presented the highest amount of OA in the medial compartment followed by the lateral compartment and the patella-femoral compartment. Table 4 represents the contribution of the different features evaluated to the overall score for all groups. Bone marrow lesions and cyst accounted for less than 1% of the overall score in all groups while cartilage lesions contributed an 80%. Osteophytes presented a 19% contribution to the overall score which were predominantly observed in the ACL transection group (Table 4).

11. Histological Assessment

Average differences between groups are shown in Table 5. Group 3 (ACLt + CMM) and Group 4 (CMM) presented similar results followed by Group 2 (MMT) and Group 1 (ACLt). Differences among groups were statistically significant.

12. Correlations between sMOAKS, Mankin and OARSI assessments

We observed a strong positive correlation between the three scoring systems, sMOAKS versus Mankin ($r = 0.829$), sMOAKS versus OARSI ($r = 0.917$), Mankin versus OARSI ($r = 0.879$) in the evaluation of articular cartilage degeneration in the medial and lateral femoro-tibial compartments. Graphs and trend lines presented a similar pattern (Figure 3).

13. Discussion

The results of this study showed that sMOAKS is a valid and reliable score for the evaluation of articular cartilage in the ovine knee. However, although the three scoring systems (sMOAKS, Mankin and OARSI) were significantly correlated evaluating articular cartilage, sMOAKS yielded relatively higher scores in the ACL transection group at the expense of an increased presence of osteophytes. The differences between groups were relatively small although statistically significant.

OA research is conducted into non-invasive measurements that correlate well with histopathological changes, joint pain and disease progression. MR semi-quantitative scores are valuable methods to evaluate the multiple characteristics of the osteoarthritic knee using conventional MR acquisitions [9] in humans [8–10] [13,14]. These scales were developed based on the potential of MR to evaluate all the synovial structures involved in the degenerative process, given the understanding of OA as a disease of the whole joint [30]. The MOAKS scale was recently developed with the aim of refining the scoring system of BMLs, articular cartilage, and meniscal morphology showing very good to excellent reliability for the majority of the features evaluated [8].

However, these scales have never been utilized in the ovine knee, probably due to the anatomical differences that make these systems difficult to apply. The major differences between the human and ovine knee are: (1) the presence of the *extensor digitorum longus tendon*, (2) the attachment of the patellar tendon to the cranial pole of the patella, (3) the lack of full extension and (4) the tripartition of the patellofemoral joint and both condyles [31]. For this reason, we had to divide the femoro-tibial joint into two halves and evaluate the femoropatellar joint independently. In addition, we excluded the evaluation of the anterior cruciate ligament and the meniscus as these were intentionally injured to induce osteoarthritis. One of the limitations of MOAKS is that it does not allow the quantification of the amount of degeneration of the whole knee [14]. For this purpose, we created a quantification method in order to be able to compare the degenerative changes produced by the different OA induction lesions.

According to Landis and Koch [29] criteria, almost all measures of reliability presented in this study showed substantial agreement (0.61–0.8) or reached near almost perfect agreement (0.81–1.0). This is in accordance to what Hunter et al., [8] published in the original scale. Interestingly, the inter-observer reliability and percentage of agreement results for the evaluation of cartilage, BML and osteophytes were higher than the intra-observer reliability results. This observation could be justified by the six-week gap period in between observations and the low presence of BML. This study now provides an adapted reliable MR scale (sMOAKS) to quantify the effect of different interventions in the surgically induced OA ovine knee. Although recent studies provide data to suggest that three T MR may significantly improve the diagnostic of cartilage lesions compared with 1.5 T MR the evaluation of articular cartilage using a semi-quantitative scale as MOAKS can be also performed in a 1.5 T MR [32].

The development of OA in the human knee after ACL rupture is a well documented condition which is thought to be due to the effect of an increased anterior tibial translation [33]. In an attempt to improve

Table 3
sMOAKS scores per group and observer.

	Observer 1		Observer 2		Average	Percentage
	1	2	1	2		
Group 1 (*ACL transection*)						
PFJ	21.50	22.25	19.75	14.75	19.56	23.2%
FTLJ	22.75	17.75	21.75	19.50	20.44	28.3%
FTMJ	22.00	23.00	19.50	21.00	21.38	29.6%
Total group 1					61.38	26.2%
Group 2 (*meniscal tear*)						
PFJ	20.00	22.75	19.00	14.00	18.94	22.5%
FTLJ	18.00	17.75	19.75	17.25	18.19	25.2%
FTMJ	20.00	20.50	19.00	19.25	19.69	27.3%
Total group 2					56.82	24.2%
Group 3 (*ACLt + medial meniscectomy*)						
PFJ	23.25	17.00	18.25	11.50	17.50	21.3%
FTLJ	17.50	21.50	15.00	20.00	18.50	25.6%
FTLJ	22.50	23.75	21.50	20.25	22.00	30.5%
Total group 3					58.00	24.7%
Group 4 (*medial meniscectomy*)						
PFJ	18.00	12.40	12.40	9.00	12.95	15.7%
FTLJ	16.00	12.40	13.80	13.60	13.95	19.3%
FTMJ	22.00	19.40	20.40	18.60	20.10	21.9%
Total group 4					47.00	20.0%

Table 4
Percentage of contribution to the global score of the characteristics evaluated in sMOAKS.

	PTS (X)	PPT (%)	AAS ($X \pm SD$)	PAC (% $\pm SD$)
Cartilage	72	30	57.6 ± *7*	80 ± *9.7*
BML/cyst	108	46	1.08 ± *0.08*	<1 ± *0.07*
Osteophytes	48	20	9.12 ± *0.97*	19 ± *2.02*
Synovitis	3	2	0.03 ± *0.08*	<1 ± *0.01*
Effusion	3	2	0.02 ± *0.06*	1 ± *0.01*

PTS: possible total score. PPT: possible percentage of total. AAS: actual average score. PAC: percent average contribution. (X): average. SD: standard deviation. (%): percentage.

Table 5
Results from the histologic evaluation with MANKIN and OARSI Scales (mean group values ± SD).

	Mankin		OARSI	
	Femur ($X \pm SD$)	Tibia ($X \pm SD$)	Femur ($X \pm SD$)	Tibia ($X \pm SD$)
Group 1 *ACL transection*	*3.4 ± 0.9*	*3.0 ± 0.6*	*1.2 ± 0.5*	*0.8 ± 0.3*
Group 2 *Meniscal tear*	*8.6 ± 1.4*	*8.6 ± 1.3*	*3.8 ± 0.9*	*2.6 ± 0.9*
Group 3 *ACLt + medial meniscectomy*	*11 ± 4.2*	*11.8 ± 3.8*	*4 ± 1.4*	*4.6 ± 1.1*
Group 4 *Medial meniscectomy*	*9.5 ± 2.5*	*12.2 ± 3.6*	*3.5 ± 1.2*	*4.2 ± 1.4*

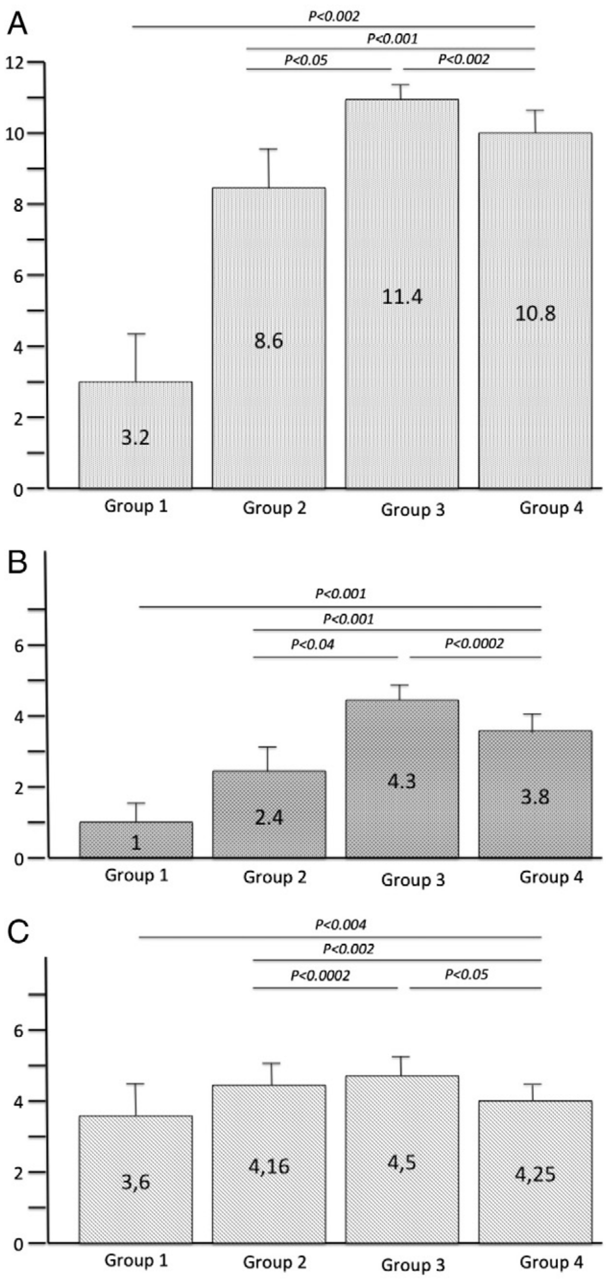

Figure 3. A) Evaluation of femoro-tibial articular cartilage with Mankin Scale per Groups. B) Evaluation of femoro-tibial articular cartilage with OARSI Scale per Groups. C) Evaluation of femoro-tibial articular cartilage with sMOAKS Scale per Groups. ANOVA values presented as averages and standard deviation per groups.

the understanding of the mechanisms of OA, ACL transection became one of the most common procedures to induce OA in animals with well documented results. However, the development of degenerative changes in the ovine knee after ACL transection remains still controversial [33,34] and most of the research done in sheep OA have focused on the effect of different meniscal injuries. There is; however, an absence of studies comparing the effect of the transection of the ACL, isolated or in combination with different meniscal injuries, in the same group of sheep [35]. The results of this comparative study, using the sMOAKS score, indicate that the isolated transection of the ACL in the ovine knee induces a higher degree of degeneration than meniscectomy or any other combination, eight weeks after surgery, at the expense of an increased presence of osteophytes. Although the differences observed between groups were small, these were statistically significant, with the biggest difference between the ACL transection group and the group with a complete medial meniscectomy. We also believe that the EDL tendon in the ovine stifle acts as the ACL, thus, preventing the anterior displacement of the tibia.

Future studies could possibly focus on the MR evaluation of the combined lesion of the ACL and EDL tendons which may even induce a higher amount of degeneration. Although there is an absence of a control group and/or radiographs, MRs done at baseline, all knees were carefully inspected during surgery and none of them presented macroscopic abnormalities. In addition, while the ACL transected group presented the highest amount of degeneration eight weeks after surgery, we cannot predict if these changes are maintained over time. Another concern is the time needed to observe degenerative changes after the surgical induction of OA. Different studies have described OA findings ranging from two weeks to 12 months after surgery. However, the majority of the studies used histological methods to evaluate the degenerative changes focusing on cartilage damage and none of them utilized MR to detect early degenerative changes.

Some investigators have observed that articular cartilage lesions precede sub-chondral lesions [36,37] while others have reported contradictory findings [38–40]. Changes in sub-chondral bone composition are important in the progression of OA. These are degenerative lesions consisting of edema, bone marrow necrosis, fibrosis, and trabecular abnormalities [15,41], which have been often detected in conjunction with neighboring cartilage damage and seem to have correlation with progressive cartilage damage [11,42]. Lacourt et al. [43] observed a correlation between sub-chondral bone changes and cartilage lesions, suggesting that the sub-chondral bone is involved and plays a role in the pathogenesis of naturally occurring repetitive impact OA in trauma-induced equine osteoarthritis. Although bone sclerosis and sub-chondral bone abnormalities are well documented in OA, we could not detect variations in the sub-chondral bone structure in the early stage of the disease in this experimental model. However, this study was not designed with that intention and; therefore, it does not evaluate the sequence of events of OA. To what extent the etiology of OA could influence the sequence of the degenerative process and the progression of the disease have not yet been addressed.

The Mankin and OARSI histopathological scoring methods allowed for useful comparison with the sMOAKS scale in this animal model of early OA. However, most of the histopathological scores are plagued by issues and questions of sampling scale and are deficient in only evaluating cartilage and no other OA-affected joint tissues. Therefore, it still remains unclear whether histology fully captures the pathological process of OA, and there is little or no information on the correlation of histopathology changes and clinical signs of disease as joint pain [33]. The absence of overall correlations in this study might be related to the fact that sMOAKS evaluates and quantifies the damage of different joint structures in the femoro-tibial and patellofemoral compartments and the Mankin–OARSI scales only evaluate the damage of the articular cartilage in the femoro-tibial compartments. However, it was in that particular scenario (the evaluation of articular cartilage in the femoro-tibial compartments), when a high correlation was observed in between the three scales.

Several possible limitations in the study should be considered. Whereas this in vivo design was optimal for delineating the pathologic changes associated with the lesion of different intraarticular structures, the model may not accurately mimic the naturally disease state. Also the length, timing, and nature of the study does not allow for modeling or assessing the full spectrum of changes associated with OA. Although we did not have a control group in order to evaluate the effect of an isolated arthrotomy the knees were carefully inspected at the time of surgery and none of them presented gross structural abnormalities. In addition, MR observers reported some difficulties when evaluating some of the images as the slices presented a certain degree of obliquity. In those instances the evaluation of the different features was performed in more than one slice. Lastly, although non-parametric statistical analyses would have been a better choice, given the nature of the variables, most of our results presented statistically significant *p values.*

In summary, sMOAKS is a reliable semi-quantitative MR scale to evaluate and quantify the effect of different induction lesions in the ovine knee and presents a high correlation with the Mankin and OARSI scales in the evaluation of the femoro-tibial articular cartilage. Although ACLt induced the highest degree of degeneration at the expense of an increase presence of osteophytes, most of these scores were in the lower quartile of their possible range. While articular cartilage lesions and osteophytes were the predominant affected structures, bone marrow lesions and cyst, were poorly identified. We conclude from this study that although minor differences were observed between the different surgical procedures for the induction of OA in the ovine knee, the isolated transection of the ACL proved to be the intervention that produced the highest amount of degeneration eight weeks postoperatively.

Conflict of interest

The authors of this work report no conflict of interest.

Author contributions

As corresponding author, I affirm that each author has approved this manuscript. Furthermore, each author listed has contributed to the conception and design of the study, acquisition and interpretation of data and the intellectual content of the manuscript agrees with all interpretations and conclusions.

Acknowledgments

Dr. Moya-Angeler and Dr. Forriol received a research grant from Mapfre Foundation (FM2010/00614). This study was conducted under agreement with the respective Universities employing the researchers. Special thanks also to Arianna Gianakos (Research Assistant at the Hospital for Special Surgery) for the grammatical suggestions.

References

[1] Poole R, Blake S, Buschmann M, Goldring S, Laverty S, Lockwood S, et al. Recommendations for the use of preclinical models in the study and treatment of osteoarthritis. Osteoarthritis Cartilage 2010;18(Suppl. 3):S10–6.
[2] Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000; 133:635–46.
[3] Felson DT. Risk factors for osteoarthritis: understanding joint vulnerability. Clin Orthop Rel Res 2004;427(Suppl):16–21.
[4] Pritzker KP. Animal models for osteoarthritis: processes, problems and prospects. Ann Rheum Dis 1994;53:406–20.
[5] Little C, Smith M. Animal models of osteoarthritis. Curr Rheumatol Rev 2008;4: 175–82.
[6] Smith M, Little C. Experimental models of osteoarthritis. In: Moskowitz R, Altman R, Hochberg M, Buckwalter J, Goldberg V, editors. Osteoarthritis: diagnosis and medical/surgical management. Philadelphia: Lipincott Williams & Wilkins; 2007. p. 107–25.
[7] Eckstein F, Mosher T, Hunter D. Imaging of knee osteoarthritis: data beyond the beauty. Curr Opin Rheumatol 2007;19:435–43.
[8] Hunter DJ, Guermazi A, Lo GH, Grainger AJ, Conaghan PG, Boudreau RM, et al. Evolution of semi-quantitative whole joint assessment of knee OA: MOAKS (MRI Osteoarthritis Knee Score). Osteoarthritis Cartilage 2011;19:990–1002.
[9] Hunter DJ, Zhang W, Conaghan PG, Hirko K, Menashe L, Reichmann WM, et al. Responsiveness and reliability of MRI in knee osteoarthritis: a meta-analysis of published evidence. Osteoarthritis Cartilage 2011;19:589–605.
[10] Hunter DJ, Lo GH, Gale D, Grainger AJ, Guermazi A, Conaghan PG. The reliability of a new scoring system for knee osteoarthritis MRI and the validity of bone marrow lesion assessment: BLOKS (Boston Leeds Osteoarthritis Knee Score). Ann Rheum Dis 2008;67:206–11.
[11] Hunter DJ, Conaghan PG. Imaging outcomes and their role in outcomes in osteoarthritis and rheumatoid arthritis. Curr Opin Rheumatol 2006;18:157–62.
[12] Ho C, Cervilla V, Kjellin I, Haghigi P, Amiel D, Trudell D, et al. Magnetic resonance imaging in assessing cartilage changes in experimental osteoarthrosis of the knee. Invest Radiol 1992;27:84–90.
[13] Kornaat PR, Ceulemans RY, Kroon HM, Riyazi N, Kloppenburg M, Carter WO, et al. MRI assessment of knee osteoarthritis: knee osteoarthritis scoring system (KOSS) inter-observer and intraobserver reproducibility of a compartment-based scoring system. Skeletal Radiol 2005;34:95–102.
[14] Peterfy CG, Guermazi A, Zaim S, Tirman PF, Miaux Y, White D, et al. Whole-organ magnetic resonance imaging score (WORMS) of the knee in osteoarthritis. Osteoarthritis Cartilage 2004;12:177–90.
[15] Taljanovic MS, Graham AR, Benjamin JB, Gmitro AF, Krupinski EA, Schwartz SA, et al. Bone marrow edema pattern in advanced hip osteoarthritis: quantitative assessment with magnetic resonance imaging and correlation with clinical examination, radiographic findings, and histopathology. Skeletal Radiol 2008;37:423–31.
[16] Gregory MH, Capito N, Kuroki K, Stoker AM, Cook JL, Sherman SL. A review of translational animal models for knee osteoarthritis. Arthritis 2012;2012:764621.
[17] Cake MA, Read MA, Corfield G, Daniel A, Burkhardt D, Smith MM, et al. Comparison of gait and pathology outcomes of three meniscal procedures for induction of knee osteoarthritis in sheep. Osteoarthritis Cartilage 2013;21:226–36.
[18] Hunt P, Scheffler SU, Unterhauser FN, Weiler A. A model of soft-tissue graft anterior cruciate ligament reconstruction in sheep. Arch Orthop Trauma Surg 2005;125:238–48.
[19] Parker D, Hwa S-Y, Sambrook P, Ghosh P. Estrogen replacement therapy mitigates the loss of joint cartilage proteoglycans and bone mineral density induced by ovariectomy and osteoarthritis. APLAR J Rheumatol 2003;6:116–7.
[20] Oakley SP, Lassere MN, Portek I, Szomor Z, Ghosh P, Kirkham BW, et al. Biomechanical, histologic and macroscopic assessment of articular cartilage in a sheep model of osteoarthritis. Osteoarthritis Cartilage 2004;12:667–79.
[21] Cake MA, Read RA, Appleyard RC, Hwa SY, Ghosh P. The nitric oxide donor glyceryl trinitrate increases subchondral bone sclerosis and cartilage degeneration following ovine meniscectomy. Osteoarthritis Cartilage 2004;12:974–81.
[22] Appleyard RC, Burkhardt D, Ghosh P, Read R, Cake M, Swain MV, et al. Topographical analysis of the structural, biochemical and dynamic biomechanical properties of cartilage in an ovine model of osteoarthritis. Osteoarthritis Cartilage 2003;11:65–77.
[23] Funakoshi Y, Hariu M, Tapper JE, Marchuk LL, Shrive NG, Kanaya F, et al. Periarticular ligament changes following ACL/MCL transection in an ovine stifle joint model of osteoarthritis. J Orthop Res 2007;25:997–1006.
[24] Aigner T1, Cook JL, Gerwin N, Glasson SS, Laverty S, Little CB, et al. Histopathology atlas of animal model systems — overview of guiding principles. Osteoarthritis Cartilage 2010;18(Suppl. 3):S2–6.
[25] Allen MJ, Houlton JE, Adams SB, Rushton N. The surgical anatomy of the stifle joint in sheep. Vet Surg 1998;27:596–605.
[26] Little CB, Smith MM, Cake MA, Read RA, Murphy MJ, Barry FP. The OARSI histopathology initiative — recommendations for histological assessments of osteoarthritis in sheep and goats. Osteoarthritis Cartilage 2010;18(Suppl. 3):S80–92.
[27] Mankin HJ, Dorfman H, Lippiell L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53-A:523–37.
[28] Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, et al. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29.
[29] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159–74.
[30] Recht MP, Goodwin DW, Winalski CS, White LM. MRI of articular cartilage: revisiting current status and future directions. Am J Roentgenol 2005;185:899–914.
[31] Osterhoff G, Löffler S, Steinke H, Feja C, Josten C, Hepp P. Comparative anatomical measurements of osseous structures in the ovine and human knee. Knee 2011;18:98–103.
[32] Mosher TJ, Walker EA, Petscavage-Thomas J, Guermazi A. Osteoarthritis year 2013 in review: imaging. Osteoarthritis Cartilage 2013;21:1425–35.
[33] Louboutin H, Debarge R, Richou J, Selmi TAS, Donell ST, Neyret P, et al. Osteoarthritis in patients with anterior cruciate ligament rupture: a review of risk factors. Knee 2009;9:239–44.
[34] Intema F, Hazewinkel HA, Gouwens D, Bijlsma JW, Weinans H, Lafeber FP, et al. In early OA, thinning of the subchondral plate is directly related to cartilage damage: results from a canine ACLT–meniscectomy model. Osteoarthritis Cartilage 2010;18:691–8.
[35] Rorvik AM, Teige J. Unstable stifles without clinical or radiographic osteoarthritis in young goats: an experimental study. Acta Vet Scand 1996;13:265–72.
[36] Burr DB. Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthrosis osteoarthritis cartilage 12(Suppl A):S20–30; 2004.
[37] Panula HE, Nieminen J, Parkkinen JJ, Arnala I, Kroger H, Alhava E. Subchondral bone remodeling increases in early experimental osteoarthrosis in young beagle dogs. Acta Orthop Scand 1998;69:627–32.
[38] Calvo E, Palacios I, Delgado E, Ruiz-Cabello J, Hernández P, Sánchez-Pernaute O, et al. High-resolution MRI detects cartilage swelling at the early stages of experimental osteoarthritis. Osteoarthritis Cartilage 2001;9:463–72.
[39] Botter SM, van Osch GJ, Waarsing JH, van der Linden JC, Verhaar JA, Pols HA, et al. Cartilage damage pattern in relation to subchondral plate thickness in a collagenase-induced model of osteoarthritis. Osteoarthritis Cartilage 2008;16:506–14.
[40] Sniekers YH, Intema F, Lafeber FP, van Osch GJ, van Leeuwen JP, Weinans H, et al. A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models. BMC Musculoskelet Disord 2008;9:20.
[41] Zanetti M, Bruder E, Romero J, Hodler J. Bone marrow edema pattern in osteoarthritic knees: correlation between MR imaging and histologic findings. Radiology 2000; 215:835–40.
[42] Felson DT, McLaughlin S, Goggins J, LaValley MP, Gale ME, Totterman S, et al. Bone marrow edema and its relation to progression of knee osteoarthritis. Ann Intern Med 2003;139:330–6.
[43] Lacourt M, Gao C, Li A, Girard C, Beauchamp G, Henderson JE, et al. Relationship between cartilage and subchondral bone lesions in repetitive impact trauma-induced equine osteoarthritis. Osteoarthritis Cartilage 2012;20:572–83.