INTRODUCTION
Meniscal injuries are common among physically active humans. Due to the low regenerative capacity of the meniscus, repair of meniscal lesions is rarely possible. Hence, partial meniscectomy is the most frequent treatment, which often leads to premature osteoarthritis. To avoid this, replacement of the injured part of the meniscus would be desirable. This study investigates the behavior of an innovative silk scaffold for partial meniscal replacement in an in vivo sheep model.

METHODS
The scaffolds were supplied by Orthox Ltd. (Abingdon, UK). They were derived from silk fibers and processed into an open porous matrix (pores >100 μm). Thirty-four female sheep (4 ± 1 years, 89 ± 13 kg) underwent a medial arthroscopy at the right stifle joint. Animals of group 1 (n=8) served as sham-operated controls without intervention at the meniscus. In group 2 (n=8), 3 (n=9) and 4 (n=9) a partial meniscectomy at the anterior horn of the medial meniscus was carried out. The defect in group 2 animals remained untreated, whereas a scaffold was sutured into the defect of group 3 and 4 animals (Fig. 1). All unoperated left knees served as additional internal controls.

Animals of group 3 were sacrificed after 3 months, animals of group 1, 2, and 4 after 6 months. Upon opening of the knee joints gross inspection of the genual region was made with particular attention to possible inflammatory changes, fibrotic reactions and to the condition of the scaffold and the articular cartilage. To assess the mechanical quality of the articular cartilage indentation tests were performed to determine its equilibrium modulus (Eeq) at three measuring points (MP) per plateau (Fig. 2). Eeq of the meniscal tissue that was harvested by creating the defect and of the scaffolds before and after 3 and 6 months implantation were determined by a stress-relaxation test under confined compression at 20 % strain. Afterwards the plateaus, femoral condyles, menisci and synovial membranes were fixed in formaldehyde for histology. The data were analyzed by a Wilcoxon and Mann-Whitney-U test.

RESULTS
A) GENERAL RESULTS: All sheep were free of lameness within 4 days postoperatively. Neither the surrounding tissue nor the synovial membrane or the meniscus showed macroscopic signs of inflammation. This observation was confirmed by histological sections of these tissues (Fig. 3). In histological sections of the scaffolds, amorphous material, some fibroblast-like cell clusters and connective tissue formation was visible inside the pores of the scaffold (Fig. 4).

The initial Eeq of the meniscal tissue was higher than the modulus of the scaffolds before implantation. Due to occasional shape alterations not all implanted scaffolds could be tested. After 3 months implantation time the scaffold’s modulus increased significantly (Fig. 5).

B) 3 MONTHS RESULTS: In 6 animals the scaffold filled the defect completely. Macroscopically, the cartilage of the right stifle joint did not evince more degeneration compared to the unoperated side. The indentation test of the tibial plateaus showed no significant differences between the Eeq of the operated and unoperated joints (Tab. 1).

Table 1: Median (±IQR) of Em in MPa at tibial plateau (n=6)

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<thead>
<tr>
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<th>MP 1</th>
<th>MP 2</th>
<th>MP 3</th>
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<tr>
<td>Unoperated</td>
<td>1.14 ± 0.21</td>
<td>0.76 ± 0.35</td>
<td>0.85 ± 1.61</td>
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<tr>
<td>Operated</td>
<td>0.98 ± 0.48</td>
<td>0.48 ± 0.58</td>
<td>0.86 ± 0.45</td>
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C) 6 MONTHS RESULTS: The scaffold filled the meniscal defect in 6 animals, but in 3 cases a little gap at the posterior edge appeared. Unoperated as well as operated joints showed a similar degree of cartilage degeneration macroscopically. The Eeq at MP 1 was significantly reduced in the empty defect group compared to the sham-operated group. The cartilage of the scaffold-implanted group, however, showed no reduction of the Eeq at this MP (Fig. 6).

Fig. 5: Eeq of meniscus (n=19), scaffold (pre-op, n=12), scaffold after 3 (n=6) and 6 months implantation (n=3); * outlier; (Mann-Whitney-U: * p<0.05, ** p<0.01; Wilcoxon: $ p<0.05)

Fig. 6: Eeq of articular cartilage of sham-operated (n=8), empty defect (n=8) and scaffold group (n=6); * outlier, • extreme value; (Mann-Whitney-U: * p<0.05)

DISCUSSION
The presented silk scaffold could withstand the loads, occurring during the 3 and 6 months implantation period. It showed good biocompatibility and promising results concerning cartilage protection compared to partial meniscectomy. Furthermore, the mechanical properties started to approach the properties of meniscal tissue.

SIGNIFICANCE
The findings of the present short-term study indicate that the new silk scaffold might be a successful treatment strategy for meniscal lesions. However, the surgical fixation technique still needs to be improved and the long-term performance should be further investigated.

ACKNOWLEDGEMENT
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