INTRODUCTION:
Delayed and non-unions are still severe complications in fracture healing. Possible causes are periosteal damage, impaired blood supply and insufficient stability. Experimental models for induction of a delayed or non-union comprise segmental bone defects, periosteal destruction or devascularisation in small or large animals. To our knowledge, there is no mechanically induced critical bone healing model in large animals, which is well standardized and characterized. Moreover, the long-term healing outcome has not been adequately documented. Therefore, the aim of this study was to compare the healing course and outcome of a mechanically induced critical healing in a sheep osteotomy model in comparison to a standard healing situation by biomechanical and histological evaluation.

METHODS:
A mid-shaft tibial osteotomy was performed in 72 sheep and stabilized with either a rigid external fixator (group I) leading to standard healing or mechanically critical one (group II). The sheep were sacrificed at 2, 3, 6 and 9 weeks post (n=8, each). To distinguish between the development of a delayed or a non-union, one further group treated with the critical fixator was sacrificed 6 months after surgery (n=8). The study was approved by the local legal representative (LAGeSo, Berlin: GI0224/01, GI0172/04). Following sacrifice, biomechanical testing of the healed and contralateral tibiae was performed. For histological analysis, sections of the callus region were stained with Movat Pentachrome and the callus tissue differentiation was evaluated by histomorphometry. Statistical comparisons between the groups were performed using the Mann-Whitney U test (SPSS 15.0). A p-value of less than 0.05 was taken as a significant difference.

RESULTS:
Biomechanical testing after 9 weeks demonstrated a significantly lower torsional stiffness (p = 0.006) of the 4 tested tibiae stabilized with the critical fixator (group II) compared to the 8 tested tibiae of the standard healing group (group I) (27 [12-61] % vs. 98 [75-115] %). The remaining specimens of group II had negligible mechanical properties and were therefore not tested. At 6 months, the biomechanically tested tibiae of group II (5 out of 8) exhibited a considerable increase in torsional stiffness (102 [92-104] %), which was comparable to the tibiae of group I tested at 9 weeks. Histological evaluation of group I specimens 2 weeks postop demonstrated periosteal woven bone formation at the cortical bone fragments adjacent to the gap. In group II, the bone fragments were highly displaced. Periosteal woven bone formation was also present, but at a great distance from the gap which was mainly filled with hematoma. At 3 weeks in group I, the periosteal callus had increased in size. In group II, the callus formed periosteally reached the gap with cartilage formation on top. The gap still contained large remnants of the fracture hematoma (Tab. 1). At 6 weeks, the periosteal callus bridged the gap in most specimens of group I. In group II, periosteal woven bone formation proceeded to the center of the displaced fragments with large amounts of hyaline cartilage on the ossification fronts. In contrast, the formerly formed periosteal callus on the outer surfaces of the highly displaced cortical fragments had already been resorbed. In the center of the gap, a pseudo-joint space was present which was lined by a thin layer of cells. At 9 weeks, complete bridging of the intercortical zone was present in group I and callus resorption and cortical remodeling occurred. In group II, woven bone formation proceeded, but instead of a bony bridge the gap contained fibrocartilage and little remnants of the fracture hematoma. In some animals, the cortical ends had been resorbed leading to a rounded appearance of the bone edges. At 9 weeks, the pseudo-joint space in the gap was still present. At 6 months, the healing outcome was quite different between the animals. In 5 out of 8 animals, large amounts of compact bone were present resulting in a union of the two central opposite cortical bone fragments. In contrast, in the other 3 animals the osteotomy gap was enlarged and filled with fibrous tissue. No bony bridging was detected, but resorption of the cortical bone fragments proceeded (Fig. 1).

DISCUSSION:
This study compared the healing course and outcome of a tibial osteotomy after rigid and mechanically critical fixation. After 9 weeks, the healing outcome of the critical fixator group remained unclear. Therefore, the 6 months time point was chosen to distinguish between the development of a delayed or a non-union. After 6 months, 5 sheep treated with the critical fixator showed a torsional stiffness comparable to the standard healing group after 9 weeks. Furthermore, histologically complete bridging of the gap was visible. However, the remaining 3 animals had developed a non-union. Interestingly, new bone formation and bone resorption seemed to depend on the localization of the cortical bone fragments and the local biomechanical conditions. If a bony union was possible, woven bone formation continued during the healing course leading to an atypical, but mechanically stiff union in the center of the dislocated fragments. But, if a union of the displaced bone fragments was impossible, resorption processes of the callus and cortical bone fragments prevailed. In this study, mechanical instability was employed to induce a critically delayed healing model in sheep. In some cases, this approach even initiated a non-union. Furthermore, this study illustrates the immense capability of the bone to adapt to the prevailing mechanical conditions.

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