Skeletally immature sheep as an animal model for tendon to bone healing in children: faster healing and enhanced biomechanics following anterior cruciate ligament reconstruction

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Introduction: Postoperative mobilization, physical therapy, range of motion and return to functional activity are largely dictated by the need to protect the healing of the tendon graft in the bone tunnel. A broad scope of studies and experiences regarding most aspects of ACL reconstruction in adults is available but only limited data exist for the tendon graft to bone healing process in children. This is surprising, as intraligamentous ruptures of the ACL are diagnosed with increasing frequency. We aimed to investigate the basic mechanisms of the graft tissue incorporation into the bone tunnel and the physis tissue as well as to determine the biomechanical behaviour of the ACL reconstructed knee. We hypothesized that the graft incorporation into the bone tunnel is faster and more complete when compared to adult sheep models of ACL reconstruction.

Materials and Methods: All 32 animals underwent a fully transphyseal reconstruction of their right ACL. The left knee served as a control. Four groups of eight animals each were sacrificed at 3, 6, 12 and 24 weeks following surgery. Two animals of each group were used for the histological and 6 animals for the biomechanical analysis. The graft was fixed using the Endobutton proximally and the Suture Washer device distally. After sacrifice, the tunnels dedicated for the histological workup were overdrilled and embedded in Technovit 9100 New. The histological workup consisted of a Toluidine blue staining and an in situ hybridisation for collagen III mRNA. The biomechanical evaluation consisted of a load to failure testing of the intact (left) and ACL reconstructed (right) knees. For statistical analysis, a two-way ANOVA was used and differences were considered to be significant at a probability level of p ≤ 0.05.

Results: All animals demonstrated a quick return to full mobilization within 6 weeks. The gross inspection of the ACL reconstructed knees after sacrifice demonstrated intact knee joints with no meniscal lesion and no signs of osteoarthritis. All grafts were in place and covered by a synovial sheath, even as early as 3 weeks following surgery. The graft remodelling process within the tunnel followed the stages of necrosis, inflammation and reorganisation of the matrix. The remodelling of the tunnel wall started at the fixation site and proceeded towards the articular tunnel entrance. Sharpey like fibers could be demonstrated as early as 3 weeks following surgery. The physis tissue protruded into the tunnel lumen in the early postoperative stages and was in line with the tunnel wall in the later stages of the tendon graft to bone healing process. Many collagen III mRNA positive cells were present in the fibrovascular interface at three and six weeks, whereas in the later phase only scattered positive cells were demonstrable.

The intact contralateral knees (FATC) demonstrated a mean load to failure of 759.17 ± 114.11 N. The operated knees (FGTC) immediately following surgery failed at 124.76 ± 31.9 N (16,4% of the intact ACLs). At 24 weeks, the FGTC gained further stability, resulting in a load to failure of 522.85 ± 113.85 N, (69% of the intact ACLs, p = 0.02).

Discussion: To get more insights into the healing processes after ACL reconstruction in children, we used skeletally immature sheep as a large animal model. We found a quick remodelling of the tendon graft within the tunnel and an early formation of anchoring, Sharpey-like fibers as early as 3 weeks following surgery. We also found a complex response of the growth plate tissue at the passing site of the tendon graft. Interestingly, two comparable studies using adult sheep demonstrated Sharpey like fibers not before 12 weeks and found much weaker failure loads and stiffness values within the first six months following surgery.

Our data indicate that the present high rate of reruptures and persisting instabilities encountered with ACL reconstruction in children is not due to inferior healing when compared to adults. Quite the contrary, the tendon graft to bone healing process is very sound, as the remodelling of the graft, the anchoring of the graft to to tunnel wall as well as the biomechanical data are superior when compared to adults. This shows the urgent necessity for a child-specific rehabilitation protocol following ACL reconstruction.

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