Introduction: Bone healing involves a cascade of events that is initiated by the formation of a hematoma and an inflammatory response. During this initial phase, immune cells at the fracture site release cytokines leading to the recruitment of effector cells. It was shown that the fracture hematoma (FH) is essential for the initiation of the healing cascade [1], but the cellular composition of the hematoma remains largely unknown [2]. The aim of this study was to characterize and quantify the immune cell population present in the ovine FH. It was hypothesized that scarless regeneration of bone may be related to the cell composition of the hematoma.

Materials and Methods: A mid-shaft osteotomy of both tibiae was performed in skeletally mature female sheep (n=6) and stabilized with an external fixator. In addition, a soft tissue trauma was induced in the M. gracilis of the hind limb. The study was approved by the local legal representative (LAGeSo, Berlin: G0368/05).

The hematomas were harvested 1h and 4h postoperatively. Preoperatively, peripheral blood was taken as a reference. The cells were prepared for FACS-analysis and labeled with CD45-PE, CD5-FITC, CD2-sekPE, CD8-PE, CD4-AF647, CD21-FITC, CD14-FITC, WC1-FITC, CD25-FITC (Acris, Serotec, Dianova). The FACS-analysis was performed with a BD FACS-Calibur system and the FlowJo software. Percentage values were calculated and given as median and 25/75 percentiles. For statistic evaluation, the Wilcoxon test was used (SPSS 14). A p-value of less than 0.05 was taken as a statistically significant.

Results: Distribution of immune cell subsets in FH: The percentage composition of leukocytes (CD45+) in the FH 1h and 4h postoperatively differs (Table 1). The percentage of CD4+ cells increased in the FH compared to peripheral blood while that of CD8+ cells decreased (Table 2). In all investigated animals, the fraction of the CD8 cells positive for the CD25 marker showed a marked difference for the 1h and 4h time points with a lower ratio in the STH than in the FH (p=0.063).

Differences in the cellular composition of the FH and soft tissue hematoma (STH): A significantly lower percentage (p=0.031) of granulocytes was present in the FH compared to the STH 1h post operatively. The CD21+ cells showed an increase from 1h to 4h in the FH, whilst in the STH the CD21+ cell population declined. In the 4h hematomas, a distinctly higher percentage of CD21+ cells (p=0.063) was found in the FH compared to STH. CD5 positive T-cells showed a higher percentage in the FH 1h and 4h postoperatively compared to STH. Furthermore, a considerably higher percentage of the CD4+ T-cell subset was found in the FH compared to STH 1h postoperatively (p=0.063). The CD4/CD8 ratio showed a marked difference for the 1h and 4h time points with a lower ratio in the STH (p=0.063).

Discussion: In this study, the immune cell population in the ovine FH was characterized and quantified within the first four hours after osteotomy. 1h after injury, we found differences in the cellular composition compared to peripheral blood taken preoperatively with a decreased percentage of the CD5+ T-cells and B-cells and an increased fraction of macrophages/monocytes. However, after 4h these differences were reduced.

To evaluate whether the cellular composition might play a role in the capacity of bone to heal without scar tissue formation a comparative analysis of the cells present in the initial FH and STH was performed. In the FH, there were a significantly lower percentage of granulocytes and a higher percentage of lymphocytes compared to the STH. Furthermore, the composition of these lymphocytes differed as well. The B-cell percentage in the FH exceeded that of the STH. This might be explained by the close proximity of the FH to the bone marrow where B-cells are formed. Differences between FH and STH were also found in the T-cell population. The T-helper cell population was larger in the FH than in the STH. This was confirmed by differences in the CD4/CD8 ratio. Differences in the T-helper cell percentage between FH and STH may be explained by a differential expression of cytokines present responsible for migration of cells to the site of injury.

The distinct cellular composition of the initial fracture hematoma may contribute to bones capacity to heal without scar formation.