

## **A histological evaluation of bone calluses in the treatment of tibia fractures in sheep with the use of a semicircular fixator**

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**ABSTRACT:** This study discusses the results of a histological examination of bone calluses in the treatment of tibia fractures in sheep with the involvement of a semi-circular fixator. In all sheep, callus samples revealed the presence of well-developed, compact bone tissue in the area of fracture healing. It was found that the use of a semi-circular fixator promoted bone growth, and that it is an effective method for tibia fracture treatment in sheep.

**Keywords:** external fixator; bone fracture; osteosynthesis; sheep

The restoration of bone continuity and bone union are complex processes, and their success is determined by the effectiveness of osteosynthesis. A correctly osteosynthesized fracture leads to the development of several overlapping phases in the healing process. The organization of the hematoma, fibroplasia, chondroplasia and osteoplasia has been determined within the space of several dozen micrometers. The newly-developed tissue initially forms homogenous cell clusters that gradually undergo metaplasia and transformation into osseous trabeculae and osseous lamellae. Various stabilization techniques are applied in the treatment of bone fractures in animals (Brinker and Verstraete, 1985). The aim of most osteosynthesis techniques is to provide a biological method for fracture treatment and to lower the incidence of the related complications. Only an estimated 5% of bone fractures are not united (Frost, 1989). In most cases, the selection of an adequate osteosynthesis technique and meeting osteosynthesis requirements lead to healing and the formation of healthy bone tissue (Jalynski et al., 2004). Disturbances in bone union pose a serious therapeutic problem. Existing treatment methods have been improved and new stabilization techniques have been developed to address this issue. One such solution is the use of a semi-circular external fixator, developed by

Adamiak (2010), for the osteosynthesis of long bone fractures. The results of clinical trials investigating the effectiveness of the semi-circular fixator in the treatment of tibia and ulna fractures were discussed by Adamiak (2010) in the *Veterinary Record*.

### **MATERIAL AND METHODS**

The surgical treatment of tibia bone fractures (osteosynthesis) was performed in eleven sheep aged 10 to 22 months with body weight from 36 to 51 kg. The operative technique and the fixator model have been described by Adamiak (2010). The affected limb was periodically examined radiologically over the course of treatment. Radiological evaluations showed signs of bone union between days 52 and 68 after surgery. During the period of limb stabilization with the proposed fixator, Kirschner pins had to be replaced in nine cases due to osteolysis at the place of bone-pin contact. In view of the loosening of the pins and serous drainage, the treated sheep were divided into two groups. The first group comprised nine animals showing complications related to pin insertion, and the other group consisted of two sheep with no complications.

The sheep were sacrificed (for financial reasons) in a period of six to 18 months after fixator removal.

Table 1. Histopathological changes observed after the treatment of tibia fractures in sheep with the use of a semi-circular fixator

Group	sheep No.	Histopathological changes at the place of bone fracture and stabilization						
		fibroplasia	chondroplasia	osteoplasia	inflammatory cell infiltration	necrotic foci	compact bone tissue	new trabeculae
I	1	+			+		++	++
	2	++		+			++	++
	3	++	+	++				
	4	++	+		++	+	+	+
	6	+	+	+	+	+	+	+
	7	+	+	+			+	+
	11	+		+	+		++	+
	5/8	++		+	+			
II	9						++	++
	10	+		+	+		++	+

+ = moderate change, ++ = marked change

Callus and bone samples were obtained from the operated limbs for histological analyses. The samples were fixed in 10% buffered formalin with pH of 7.4 and were decalcified by electrolysis in Romeis fluid and embedded in paraffin. Decalcified samples were sectioned using a Reichert microtome. The sections were stained with hematoxylin and eosin and subjected to a microscopic evaluation.

## RESULTS

The samples collected from the first group of animals showed a thick layer of compact bone tis-

sue with a normal trabecular and intertrabecular structure, well-developed blood vessels and bone marrow (Figure 1). Focal remodelling of compact bone tissue into thin, regularly arranged trabeculae of various shapes, surrounded by large quantities of bone marrow, was observed. Small necrotic foci and bone density reductions were also noted in the layer of compact bone tissue. Selected changes were marked by elevated hematoxylin absorption and a different trabecular structure with mostly parallel or multi-directional arrangement of the trabeculae. Connective tissue proliferation (fibroplasia) was also observed with the presence of phagocytes, single osteoblasts and osteoclasts (Figure 2).

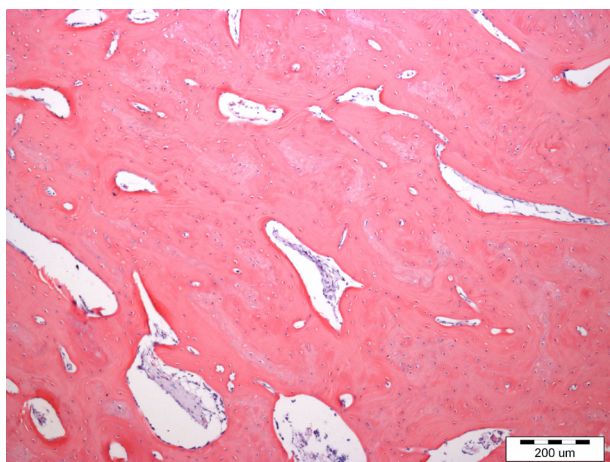


Figure 1. Thick layer of compact bone tissue with a normal trabecular and intertrabecular structure

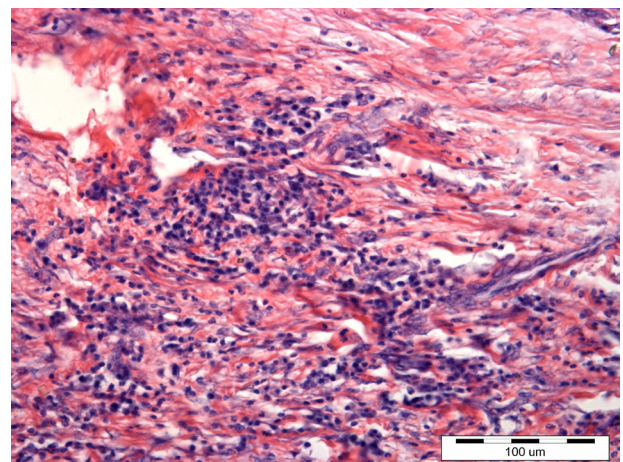


Figure 2. Sample from first group. Phagocytes, single osteoblasts and osteoclasts are observed

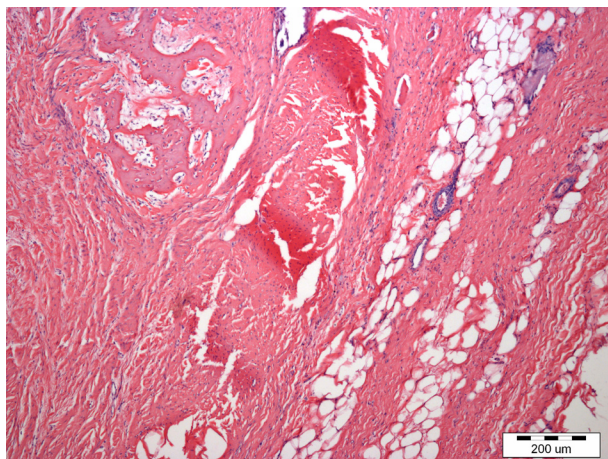


Figure 3. Osteoplasia and bone remodelling in callus sample from the second group

Connective tissue had a focal arrangement in the form of thin fibres with weakly developed trabeculae surrounded by osteogenic cells.

The samples obtained from the second group of animals were characterized by the presence of well-developed compact bone tissue with normal trabeculae and intertrabecular substance. The layer of spongy bone tissue had regular structure comprising relatively thick trabeculae and well preserved bone marrow. Very large quantities of loose connective tissue and collagen fibres with multi-directional arrangement were found in the area of bone fractures. Osteoplasia was observed in the connective tissue, and the formed trabeculae of varied thickness were surrounded by osteoblasts. Intense focal remodelling of bone tissue was noted (Figure 3). Foci of intense and chaotic osteoplasia were also observed. The observed histological changes in the bone tissue of the analyzed sheep are presented in detail in Table 1.

## DISCUSSION

Examinations of bone calluses in clinical animals, i.e., animals which are not used solely for the needs of an experiment and are sacrificed within a given time frame, are rarely performed. All sheep treated with the involvement of a semi-circular fixator developed by the author survived for six to 18 months after the removal of the fixator (subject to intended use). Therefore, the results of histopathological analyses of bone callus samples from such animals constitute valuable contributions to the research field.

Bone union was observed in all treated animals. The results of histopathological examinations confirmed full healing of tibial bones. The time of treatment with the use of the semi-circular fixator did not differ from other osteosynthesis techniques using external fixators (Anderson and St Jean, 1996; Aithal et al., 2004). A common disadvantage of all external fixators is frequent loosening of pins and serous drainage at the place of contact with the skin. The clinical suitability of an external fixator is determined by its structure and the option of quick and easy replacement of the loosened pins. The discussed semi-circular fixator meets the above requirements. In contrast to other devices of the same type, the studied fixator supports the replacement of Kirschner pins and the insertion of pins into the bone in the same plane but at different angles.

The evaluation of bone callus samples from both animal groups showed a thick layer of compact bone tissue with a regular trabecular and intertrabecular structure, well-developed blood vessels and bone marrow. In the group of animals with loosened pins, small necrotic foci were additionally observed in the direct proximity of bone tissue with Kirschner pins. This is indicative of complications involving the loss of bone stabilization and serous drainage.

The results of histopathological analyses of bone calluses in the treatment of tibia fractures suggest that the semi-circular fixator offers an effective method for restoring “mechanical silence” in the fracture site which is indispensable for bone healing.

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