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EVALUATION OF THE OSTEOGENESIS PROCESS AFTER SHEEP DENTAL EXTRACTION WITH THE PURPOSE OF CREATING AN IMPLANT BED FOR TESTING MEDICAL DEVICES

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Abstract

The essential conditions for a successful osseointegration of dental implants are bone support and the bone-implant contact area. The aim of this study was to evaluate an innovative treatment in sheep periimplantitis, carried out in three stages: preparation of the implant bed, fixation of the implants and induction of periimplantitis. In phase I was verified the process of osteogenesis following the premolars extraction.
Ten sheep were prepared by putting them on a diet before surgery and by giving them antibiotics. Under inhalatory anesthesia, a total of 8 premolars/animal were extracted by dislocating each one, followed by rotational movements in the perpendicular axis and straight traction to the outside. A hemostatic sponge was inserted into the tooth alveoli and the gum was sutured. After extraction, the sheep received anti-inflammatory and antibiotic treatment. During the monitoring period, the local and general clinical conditions were monitored, and bone regeneration was assessed by Computer Tomography.
Clinically, the evolution was favorable, with no signs of discomfort, with gum scarring in 7 days and weight gain of 24-41%. Imagistic, a degree of bone atrophy within physiological limits could be observed, and the density of the neoformation bone tissue showed an optimal regeneration.
Through the approached technique we provided a favorable environment for performing the next stages of the study, the osteogenesis process being validated both clinically and paraclinical. We also propose this method for flocks of sheep with dental diseases.

Keywords: *osteogenesis, sheep, tooth extraction.*

INTRODUCTION

Orthopedic and dental implants are in a continuous development with the aim of creating an optimal interaction between the body and the implanted material. Reaching this goal was achievable by changing the surface of the implants, the topography or the coating with different biomaterials. An ideal implantable material is biocompatible in terms of chemical composition, has an excellent ability to resist corrosion and wear in the physiological environment and has an elasticity similar to bone that could prevent bone resorption periimplant. The safety of an implant is certified by its ability to minimize adverse tissue reactions, which is very important in long-term clinical situations. In order to determine whether a new material complies with biocompatibility and mechanical stability requirements, it must be subjected to rigorous *in vitro* and *in vivo* testing prior to clinical use (Pearce, 2007). The results of *in vitro* examinations are not always easy to extrapolate in *in vivo* tests, as no cell culture system is able to accurately reproduce physiological conditions (Aguirre et al, 2021). For this reason, animal models are the ideal solution for assessing, over a long period of time, the biocompatibility, tissue response and mechanical function of an orthopedic or dental medical device (Davies, 2006). Depending on the proposed objectives, when choosing a particular animal model, certain factors must be considered, namely: availability, cost of acquisition and maintenance, maneuverability. In the field of orthopedic research, animal models must have a good tolerance for surgery and the micro and the macrostructure of the bone must be as close as possible to the human one when it is desired to translate the obtained results (Muschler, 2010).

Factors worth considering when choosing an animal model for use in orthopedic research include blood reserves that support bone healing, sexual dimorphism, skeletal immaturity, or the influence of sex hormones (Kim, 2003; Meyer, 2001).

International standards have established the main species of animals suitable for testing orthopedic and dental devices which are represented by dogs, small ruminants, rabbits, pigs and rodents (ISO 10993-6:2016). Non-human primates are closest to human bone structure and dogs have been widely used in orthopedic and dental studies, but for ethical reasons, rodents, rabbits, pigs, and sheep are preferred. The latter has become increasingly popular in recent years as an animal model used in bone research due to their superior vertebrate quality and non-pet status. Sheep are readily available animal models that are inexpensive to maintain and have a high resistance to pain and respond very well to surgical procedures (Vlaminck, 2008).

In terms of macrostructure, adult sheep offer the advantage of having a human-like body weight, with skeletal support of suitable size for the placement of implants or human prostheses, which is not possible in the case of smaller animal species such as rabbits or rodents.

The dental anatomy of sheep significantly differs from that of humans. The incisors are separated from the rest of the teeth (premolars and molars) by an edentulous area of 3-5 cm. The premolars are relatively small, have a long and prominent hypsodont crown compared to the small mesial and distal root. They have the best accessibility when it is desired to extract them for testing dental implants. In the premolar area of the sheep's mandible, thin apical bone plates are present along with a well-developed neurovascular canal. As the mandible matures, these plaques will become thicker, providing solid support for the implants to be tested (May, 1964).

The aim of the study was to evaluate an innovative treatment in periimplantitis in sheep, carried out in three stages: preparation of the implant bed (I), fixation of the implants (II) and induction of periimplantitis (III), and in phase I of the process of osteogenesis following the extraction of the first 2 premolars was verified.

MATERIALS AND METHODS

All procedures during the study were performed at Băneasa Animal Facility (BAF), Preclinical Testing Unit, Cantacuzino National Medico-Military Institute for Research and Development (CI). Animal studies have been approved by the CI Ethics Committee and authorized by the competent authority, in accordance with the provisions of EU Directive 63/2010 on the rules for the care, use and protection of animals used for scientific purposes. BAF is also an authorized unit under current legislation as a user of animals used for scientific purposes.

Ten 4-year-old Ţigae sheep were included with an average weight of 45 kg at the beginning of the study. The animals came from a farm in Hungary and on arrival at the BAF, they were weighed and clinically examined by a veterinarian, who monitored their general condition and identified possible oral abnormalities. Throughout the quarantine period, the sheep were scored weekly for welfare, sheltered together, receiving water and fodder ad libitum.

To achieve the objectives of the study, the sheep were subjected to the procedure of extraction of the first 2 premolars, from each arch, maxilla and mandible, left-right, so that at the end of the procedure a total of 8 teeth were extracted. 12 hours before, the animals were weighed, their blood was collected for biochemical examination (pre-anesthetic profile), put on a total diet of water and food and received a preventive dose of antibiotic (Enrofloxacin FP 10%, Farmavet Group, Romania). Anesthesia was aimed at premedicating animals with Dexmedetomidine (Dexodomitor, 0.5 mg/ml, Orion Pharma Finland) and Ketamine (Ketabel 100 mg/ml, Bela-Pharma, Germany) IM, followed by administration of Propofol (Propofol Fresenius, 10 mg/ml, Fresenius Kabi, Germany) IV and intubation. Maintenance of anesthesia was performed with Isoflurane (Anesteran, Rompharm Company, Romania) 3%. The sheep were placed in lateral recumbency on the surgical table and the oral cavity was disinfected on the outside with Betadine 3% (Figure 1).

Figure 1: Anesthetized sheep, ready for tooth extraction

From each half arch, the premolars were extracted by dislocating each with a dental elevator. By rotational movements in the axis of the tooth and traction perpendicular to the outside, with the pliers (Figure 2), the periodontal ligament was cut and thus the tooth was extracted. (Figure 3).

Figure 2: Dental instruments used for premolars extraction

Figure 3: The tooths after extraction

A hemostatic sponge was inserted into the tooth socket (Figure 4) and the gum was sutured with 3/0 resorbable thread (Megasorb, Vetro Design, Romania) over it.

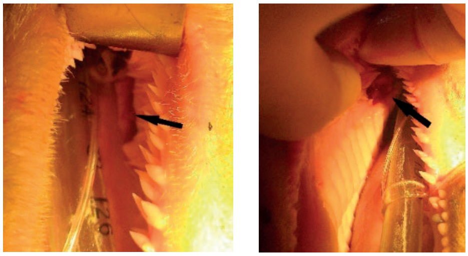

Figure 4: Tooth alveoli after the extraction

After extraction, the animals were infused with 0.9% saline and received anti-inflammatory treatment (Meloxicam 0.5 mg / kg – Melovem 5 mg/ml, Dopharma Romania) for 3 days and antibiotic (Enrofloxacin 2.5 mg/kg) for 5 days.
During the monitoring period (3 months), the way of animals feeding was followed but also the body weight and the local clinical condition. The hepatic and renal profiles were monitored paraclinical, and bone regeneration was evaluated by computed tomography (CT) examination.

RESULTS AND DISCUSSIONS

Clinically, the animals performed favorably. Immediately after recovery from anesthesia, resuming the physiological process of chewing. The chewing of the fodder, both pellets and of the green mass, did not show any sign of oral discomfort. Bodyweight monitored on days 0, 45 and 90 showed exponential increases of up to 18% compared to day 0 (Figure 5). After extraction, the dental alveoli began the healing process, after the first 7 days, so that at the clinical examination on day 14, the dental alveoli, both the maxillary and the mandibular ones were in different stages of physiological refilling (Figure 6).

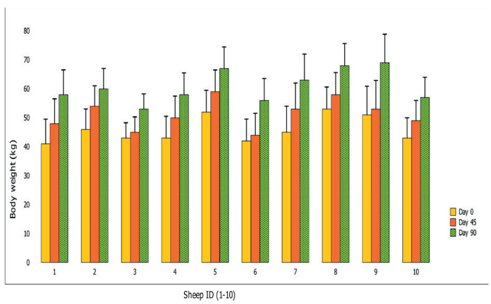

Figure 5: The average body weight/sheep

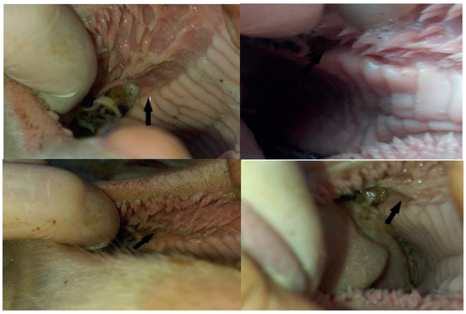

Figure 6: Dental alveola in different stages of healing (day 14)

The biochemical examination, performed on an Idexx VetTest 8008 device, on days 0, 45 and 90 followed any changes due to the anesthesia-induced on the day of extraction but also before performing the Computer Tomography examination. A slight increase in urea and ALT could be observed, on day 45, because of anesthesia from day 0, values that resolved on their own until the next operation, without additional drug support (Tabel 1).

Tabel 1: Biochemic profile of the sheep on day 0, 45 and 90

		Urea	Crea	ALT	ALK P	PT
D0	Sheep 1	6,1	62	37	77	81
D45		12,1	44	26	79	86
D90		6,8	53	31	48	66
D0	Sheep 2	5	53	26	79	86
D45		9,6	53	30	61	84
D90		3,9	53	29	16	38
D0	Sheep 3	6,8	62	10	169	75
D45		10,4	71	38	181	77
D90		6,8	71	19	91	79
D0	Sheep 4	6,8	53	28	161	74
D45		11,1	62	33	186	71
D90		5,4	44	30	74	57
D0	Sheep 5	6,8	44	41	116	83
D45		9,6	53	52	171	85
D90		6,1	53	20	78	93
D0	Sheep 6	6,8	53	22	134	80
D45		10	62	49	55	86
D90		3,2	35	20	78	93
D0	Sheep 7	7,9	53	30	150	80
D45		12,5	53	38	103	87
D90		7,5	62	34	107	75
D0	Sheep 8	6,4	53	39	132	80
D45		10	44	48	81	84
D90		4,6	44	34	57	69
D0	Sheep 9	6,8	44	32	76	80
D45		9,6	44	48	88	85
D90		3,9	35	39	37	57
D0	Sheep 10	6,8	35	31	258	77
D45		10,4	44	42	345	84
D90		6,1	62	39	207	76

Imaging examination - 90 days after the extraction, the tomography of the head was obtained under general anesthesia. Each sheep was positioned in ventrodorsal recumbency on the table of the CT scanner, and the position of the head was adjusted for symmetry. CT images, obtained with a conventional CT scanner (Philips, USA) of the 4Vet imaging center, Bucharest, Romania, showed a degree of bone atrophy within physiological limits. The density of neoformation bone tissue indicated an optimal regeneration, ensuring a uniform bone bed. Only in two cases were observed small pieces of the roots which were sequestered inside the alveolar bone (Figure 7).

Figure 7: CT images which show a good bone regeneration

The osseointegration was originally defined as a direct structural and functional link between the living bone and the surface of an implant. It is now said that an implant is considered osseointegrated when there is no relative progressive movement between it and the bone with which it is in direct contact. Although the term "osseointegration' was originally used to refer to titanium metal implants, the concept is currently applied to all biomaterials that have the ability to osseointegrate. (Marquezan et al, 2012).

Primary stability in implant placement is one of the most critical factors determining the outcome of implant therapy. The key factors in improving the primary stability of the implant are bone density (Trisi et al, 2011), surgical protocol, (Turkyilmaz, 2008), and implant type (Dos Santos, 2011). Primary stability is ensured by the mechanical friction between the outer surface of the implant and the osteotomy walls of the implant.

Sheep have a healing ability comparable to that of humans, making them potentially interesting for studying bone remodeling (Martini, 2001). It is generally accepted that the chances of successful osseointegration of an implant into the oral area increase when a non-stress-free healing period can be provided, but the preparation of the implant bed is just as important as the implant itself. When choosing the technique of premolar extraction, it must take into account that sheep are ruminants, and their mandibular structures are constantly exposed to strong compressive forces, shear and continuous muscle activity (May, 1964). These elements can influence the process of tissue regeneration, through excess alveolar bone lesions, the bone support becomes improper to place an implant (Barzilay, 1993). Clinical, radiological, and CT parameters are generally used to assess the dentoalveolar reconstruction (Schouten, 2009) and the healing progress is assessed based on bone changes obtained from imaging examination. Although two-dimensional evaluation of dentoalveolar bone remodeling can be done by normal radiographs, such an analysis is insufficient to appreciate subtle changes in bone density (Kim et al, 2008). CT seems to be the best imagistic method for the morphological and qualitative analysis of bone and an additional advantage of CT measurement is that bone density can also be calculated (Brett, 2015). In our rough evaluation of the CT-based 3D reconstruction of the sheep's jaws, 90 days after extraction, the dental alveoli showed a large amount of bone augmentation and uniformity of the alveolar ridge.

Sheep suffer seasonal bone loss. While in mature sheep, the trabecular bone is denser and stronger than in humans, immature sheep have a weaker, lower-density trabecular bone, which is very flexible due to its high collagen content. For the research of implant-type medical devices, it is recommended to choose adult sheep to eliminate the risk of implant failure (Bonucci, 2014).

From a veterinary perspective, surgery, such as dental extractions, is necessary in case of dental abnormalities or premature loss of incisor teeth. These are major problems because the affected sheep cannot bite the pasture resulting in malnutrition, poor production, and weight loss as is the case with premolar and molar teeth whose excessive growth, wear or absence causes problems with chewing fibrous feed and subsequent weight loss.

Effectively functioning teeth are fundamental for optimal sheep production. Dental disorders are relatively common and can affect individual sheep or multiple sheep within a flock. A careful examination of the incisor teeth is a straightforward and essential part of any veterinary examination of sheep and provides valuable information on age and dental abnormalities. Examination of the cheek teeth is more difficult but should be attempted in any animal or flock with a history of weight loss, cud-staining, unusual mastication, or jaw swelling. By the method approached in this study, we observed an increase in weight gain and an obvious general good condition, so we can recommend intervening by tooth extraction when dental abnormalities are encountered that endanger the welfare of the animals and harm the breeders.

CONCLUSIONS

The extraction of the first 2 premolar teeth, left-right, top and bottom provided the uniform and compact bone support necessary for the next stages of the study. Osteointegration could be demonstrated both by clinical examination and by assessment of bone structure on CT. We also propose the technique applied for sheep herds in which there are dental diseases; it can serve as a starting point for the further development of comprehensive, valid, and practicable protocols for sheep welfare.

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