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Novel Method for Tooth Extraction in Rabbits: Advantages and Considerations

Erdal Ergünol[1], Remzi Orkun Akgün[2], Rabia Şemsi[3], Okan Ekim[4], Altay Uludamar[1], Aylin Sepici Dinçel[5]

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ABSTRACT

Background: Experimental studies are essential before clinical practice in human subjects. Rabbits are mostly the first choice for basic implant design studies due to their size, easy management, short life span and economical aspects of purchase and maintenance. However, no exact protocol related to tooth extraction can be found in the open literature. Our research explores various facets of dental implants through hands-on experimental surgical investigations.

Methods: A new approach to tooth extraction in adult New Zealand rabbits was evaluated. The extraction steps for the mandibular first premolar tooth under general anesthesia included a mouth opener usage, incision, mucoperiosteal flap removal, drilling, removal of bone in the mesial of the premolars, use of periodontal Gracey and surgical scraping curettes for tooth luxation, extraction of the luxated tooth, socket irrigation and primary suturing. Dental volumetric tomography images from three distinct angles: axial, coronal and sagittal of the extraction area were evaluated to check the correctness of the surgical procedure.

Result: Our technique has yielded a remarkable success rate for the first premolar tooth extraction. We determined that the routine application of elevators and forceps, which are typically employed in tooth extraction procedures, was insufficient. To establish an appropriate surgical setting, we introduced a mouth opener. Our findings indicate no discernible tooth remnants and the socket appeared fully vacated. The suggested experimental model suits bone graft/implant and other dental studies.

Key words: Alveolar bone healing, Rabbit, Tooth extraction.

INTRODUCTION

Animal models have long been integral to advancing medical and dental research. Tooth extraction in animal models, such as rabbits, plays a pivotal role in various experimental research areas, from dental implant studies to orthodontic research, because they gained prominence due to their dental anatomical similarities to humans. Mandibular first premolar tooth extraction in rabbits is essential for investigating various dental and oral conditions, evaluating treatment methods and studying tissue regeneration. After tooth extraction, healing the alveolar socket is necessary for the subsequent treatment options.

Scientists need help in tooth extraction while working on small animals due to limited access to the oral cavity. Fracture of the tooth during extraction and severe damage in the alveolar bone are some of the potential obstacles (Easson, 2013).

These obstacles include limited graft placement space, surgical access and visibility issues, graft stability and retention, host response, healing dynamics, biocompatibility, tissue compatibility and the risk of infection and complications. One of the main difficulties is that rabbits have smaller jaws and fragile bone structures. This discrepancy could lead to insufficient coverage, integration, or complications within the extraction socket. For these reasons, additional materials must be accessible for educational science research data. Integrating the principles and concepts of educational sciences into experimental research within medicine, dentistry and veterinary medicine offers a promising solution to bridge the gap between theoretical knowledge and practical application. This approach can bolster the significance of experimental models and promote effective teaching, learning and research practices across multiple disciplines.

[1]Alter Group, Innovation, Education, Consultation and Organisation Company, Istanbul, Türkiye.
[2]Department of Basic Sciences, Faculty of Dentistry, University of Çankırı Karatekin, Çankırı, Türkiye.
[3]Department of Medical Biochemistry, Institute of Health Sciences, University of Gazi, Ankara Türkiye.
[4]Department of Anatomy, Faculty of Veterinary Medicine, University of Ankara, Ankara, Türkiye.
[5]Department of Medical Biochemistry, Faculty of Medicine, University of Gazi, Ankara, Türkiye.

Corresponding Author: Rabia Şemsi, Department of Medical Biochemistry, Institute of Health Sciences, University of Gazi, Ankara Türkiye. Email: rabiasemsi2010@gmail.com
ORCIDs: https://orcid.org/0000-0002-7044-7524, https://orcid.org/0000-0001-6648-7059, https://orcid.org/0000-0002-8477-5537, https://orcid.org/0000-0002-3322-4161, https://orcid.org/0000-0002-2814-620X, https://orcid.org/0000-0001-5847-0556.

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Students and researchers can experience enhanced educational outcomes by utilizing educational research and practices, resulting in more effective utilization of experimental models in various scientific fields.

Experiments on laboratory animals provide the tools to explore and learn the fundamentals and skills of science students in all branches of medicine and related areas. The future use of laboratory animals to educate and train veterinarians will likely continue to evolve as technological advances are applied to experimental design and educational systems. Our current knowledge and experience of dental implants would certainly only be comprehensive and practical with experimental surgical research. According to international standards, rabbits represent an essential species for testing implants in bone.

Even though the primary purpose of dentistry is to preserve teeth, tooth extraction for various reasons may be in the interest of the individual presenting with a clinical problem. Additionally, tooth extraction is the most commonly performed oral surgical procedure in humans (Batenburg *et al.*, 2000) and domestic animals (Gaughan, 1998; Bellows, 2004a). There is a need for a better understanding and approach to the complexities of dental implant applications, particularly in effectively utilizing animal models and translating experimental findings into practical clinical solutions. Our research seeks to address this gap by harnessing the benefits of animal models, specifically rabbits, in dental implant research.

Our study is centered on delving into dental implants' comprehensive and practical aspects through experimental surgical research. We aim to offer valuable insights into implant biocompatibility, toxicity evaluation and material design enhancement by leveraging rabbits, widely acknowledged as a critical species for testing bone implants.

In dental research, it is crucial to utilize instruments and surgical methods that cater to the unique anatomical features of each species' teeth and soft tissues. While there may be similarities in tooth extraction techniques and equipment among various species, it is essential to acknowledge the differences in dental anatomy that necessitate specific considerations. One such example is the distinct dental structure of rabbits, which calls for specialized approaches. Compared to other commonly studied species, rabbits possess exceptional dental anatomy. Their teeth are open-rooted and continue to grow continuously throughout their lifespan, requiring specialized techniques for tooth extraction. Due to these anatomical differences, rabbits require specific instruments and surgical approaches (Dwayne *et al.,* 2017).

Therefore, rabbits occupy a unique position in the literature due to their exceptional dental anatomy, emphasizing the significance of species-specific considerations in dental research and practice. To get the best results, it is necessary to choose the most appropriate technique, which requires a comprehensive assessment. While making the decision, the experimental animal, surgical experience, knowledge of the anatomy of the oral structures and different extraction techniques and the availability of surgical equipment should be considered (David *et al.*, 2018; Ryan *et al.*, 2019).

This article explains a novel surgical technique to overcome all the limitations and difficulties of confined access cavities while extracting the mandibular first premolar in rabbits. The surgical protocol for rabbits' mandibular first premolar extraction offers distinct advantages over existing models by providing ease of application, speed of operation, reduced pain and tissue invasion and optimized assessment of bone healing outcomes. These advantages contribute to the protocol's potential to become a valuable tool for researchers investigating grafting materials and bone healing in dental and biomedical research.

MATERIALS AND METHODS

Animal preparation

Experimental animals, adult New Zealand Rabbits, were used for the bilateral tooth extraction experimental model in compliance with G.U.ET-21.010 University Animal Experiments Local Ethics Committee Regulations and entitled "The Preparation of Premolar Tooth Extraction Educational Material for Experimental Studies on New Zealand Rabbit (*Oryctolagus cuniculus* L.)". All animals were anesthetized using xylazine HCl (5 mg/kg, i.m.) and ketamine (35 mg/kg, i.m.) (Özbek *et al.*, 2012). Although various mouth opener devices are available in the markets, the Turkish Patent Office granted a specially designed month opener as the Utility Model Patent 2021-008511 as "Auxiliary Oral Support For Veterinary Applications". It mainly fits rabbits' anatomical structure and oral cavity to make the surgeons work more comfortably. The subject invention relates to an oral support structure placed on the occlusal surfaces of mandibular and maxillary molars on one side of the mouth for use in veterinary applications, requiring an animal's mouth to be kept stable and secure in the open position for extended periods (Fig 1).

Surgical procedures for tooth extraction

The mouth is opened with the help of a gauze pad or rope from the lingual of the lower and upper jaw incisive teeth. In the continuation of this process, the structure whose design and patent application belong to us, which is held with the help of a clamp, is applied. Thus, it is placed between the lower and upper molars and premolars on the opposite side of the working area where the application is made.

For the part not to dislodge and block the throat area, the rope passed through the hole on the design product is clamped outside the mouth (Fig 2).

The first extraction step began with an incision of 1 cm made from the mesial aspect of the lower first premolar towards the buccal aspect (the scalpel tip number 15 was used) (Fig 3a, b). The mucoperiosteal flap was raised from

the buccal and lingual aspects with the periost elevator. Then, the neck and alveolar bone of the tooth were exposed (Fig 3c). Last, the mesial alveolar wall of the premolar tooth was removed with a micromotor+contra-angle+tungsten carbide 016 diameter drill.

After this stage, great care was taken not to break the tooth from the cervical region. The routinely used dentistry surgical procedures (elevators and dental forceps) were one of the causes of fractures. Instead, periodontal Gracey curettes and surgical scraping curettes for tooth luxation should be preferred.

The tooth luxation must be ensured by applying force from the buccodistal of the first premolar tooth to the mesial occlusal and lingual. The procedure needs to be done slowly and patiently and might take minutes. After the luxation of the premolar tooth was achieved, it was intervened by entering from the mesial side. The area was aspirated continuously to prevent bleeding. Finally, the tooth was extracted by applying force to the bottom of the tooth with a periodontal curette (Gracey) in the direction of the socket entrance. The socket was closed with 5-0 nylon thread sutures using non-traumatic needles. Rabbits were

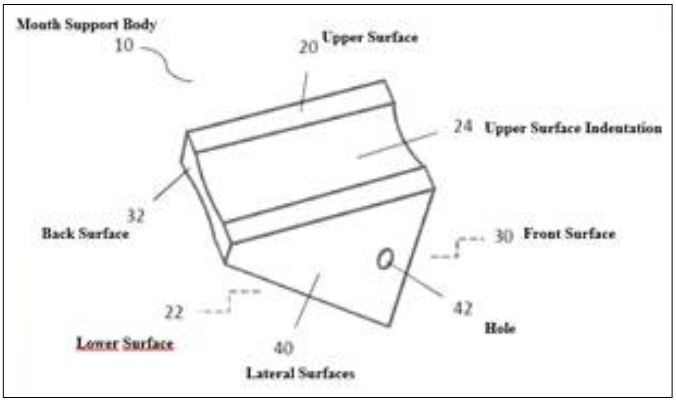


Fig 1: Representative perspective view of the oral support structuring in veterinary practice.

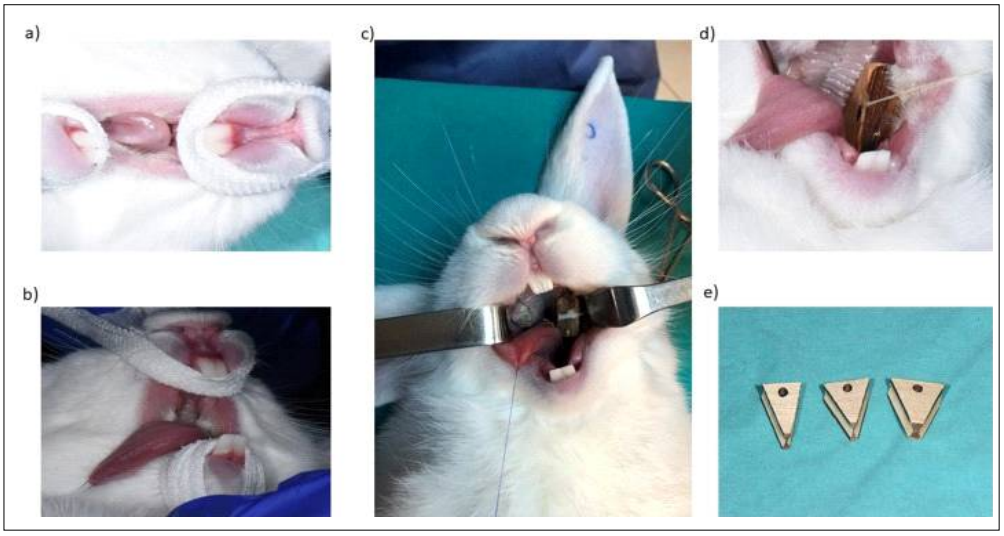


Fig 2: Preparing the area for operation by mouth opener.

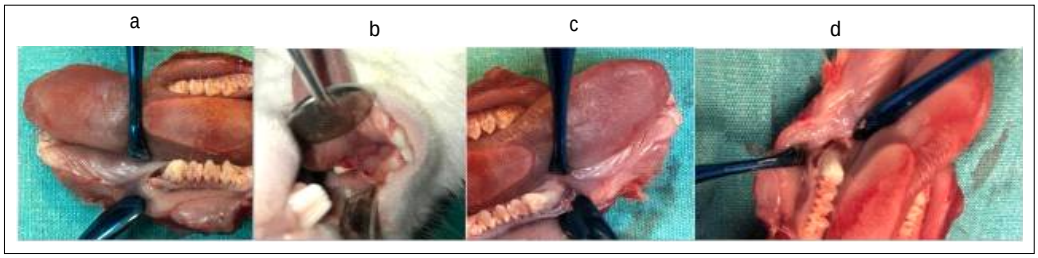


Fig 3: (a) and (b) incision procedure, (c) and (d) illustration of removal of mucoperiosteal flap.

euthanized 15 days after the operation. Then, their lower jaws were removed from the body for further analysis.

Moreover, in this study, cone beam computed tomography images of the molar tooth socket in rabbits were also evaluated after tooth extraction. The surgical procedures for tooth extraction steps was presented in a brief flowchart in Fig 4.

RESULTS AND DISCUSSION

This article focuses specifically on a novel surgical method discovered for mandibular first premolar tooth extraction in rabbits. Tooth extraction is one of the most challenging surgical procedures among surgical practices. After failing regular surgical procedures for the various extractions, we extracted the mandibular first premolars without fractures. Our team decided to explore a new technique to overcome tooth fracture problems, which will negatively affect a successful study outcome. By sharing our novel surgical tooth extraction technique, we encourage current and future scientists to work on rabbits' tooth extraction sockets to overcome the conceivable difficulties. With this method, 90% success has been achieved in removing the lower first premolar teeth. The routine use of elevators and forceps in daily practice has been observed as inappropriate. Insertion of the mouth opener was the first step in implementing a proper surgical field. As shown in Fig 1, an oral support placed on the occlusal surfaces of the mandibular and maxillary molars on one side of the mouth requires the animal's mouth to be held in the open position stably and securely for a long time. The body of the support includes upper and lower surfaces of different heights, preferably having concave recesses along its length. It is configured to have a hole that passes through the lateral surfaces with a drawstring and is equipped with a non-slip layer on the body if needed. It can be produced from wood, rubber, silicone, or plastic for practical, safe, low-cost and serial use.

Each experimental model has its advantages and disadvantages. Finding the optimal experimental model and animal species is a significant issue for researchers. Animal models provide essential data on evaluating toxicity and biocompatibility and improving implant material design in dental implant applications. Numerous studies emphasize the importance of rabbits as a model for dental research, especially in tooth extraction and implant studies (Dwayne *et al.*, 2017).

The New Zealand Rabbit (*Oryctolagus cuniculus* L.) is a laboratory animal often preferred by researchers. In terms of its anatomical features, it stands out as an animal model to be used in implant dentistry research. According to international standards concerning species convenient for testing implants in bone, rabbits symbolize a significant species (Manso *et al.*, 2011; Stübinger and Dard, 2013). Economic, scientific, practical and ethical considerations make the rabbit an ideal model for pre-use new implant and grafting materials.

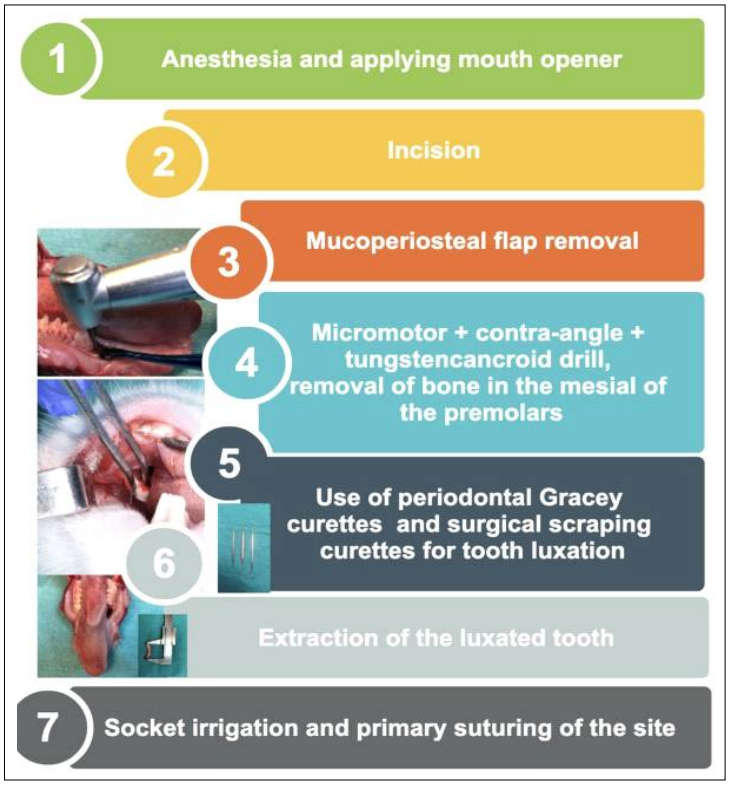


Fig 4: The flowchart of the brief steps of the surgical procedure is as follows.

Some studies in which experimental tooth extraction was performed in rabbits (Manso *et al.*, 2011; Kamal *et al.*, 2017; Pei *et al.*, 2015) require updated methods or brief guidelines. After tooth extraction, the alveolar bone changes in quality and quantity (Amler *et al.*, 1960). Mainly, bone tissue loss is frequently observed. Different materials, such as dental implants, bone grafts, or physical barriers, prevent bone damage after tooth extraction (Manso *et al.*, 2011, Rekhapathak *et al.*, 2021). In addition, few studies have shown periodontal disease induction in rabbits (Balcı Yüce 2017) and the effects of graft materials on bone healing in rabbit mandibular defects were investigated.

The premolars and molars of rabbit teeth are anatomically identical; they are cylindrical and have a natural curve and the occlusal surface of the mandibular teeth curves lingually. Although it was stated that the first molar tooth was extracted (Manso *et al.*, 2011) in a study, it was clear that the extracted tooth was not the first molar but the first premolar. Researchers have created precise protocols and tailored equipment to address the unique dental anatomy of rabbits, ensuring accurate and efficient tooth extraction procedures. In addition, investigations into the biomechanics of rabbit teeth and their response to dental interventions provide valuable insights into dental research. The most crucial difference between our study and this study was that we did not perform a skin incision. Thus, the risk of infection was further reduced in our study.

We extracted the first mandibular premolars *via* regular surgical procedures for the first groups and only one complete extraction was achieved. A total of 11 extractions ended up with teeth fractures. Fragile tooth configuration was perceived as a principal reason for the fractures. The anatomical shapes of the teeth are narrow in the mid-third. Therefore, most fractures were seen around this area (Flowchart 6). In the tomography images obtained in our study, it was observed that using the alveolar cavity in experimental studies was quite convenient. The tomography images were evaluated in three planes: axial, coronal and sagittal; no tooth residues were observed and the tooth socket was utterly empty.

In various experimental studies, it was stated that the incisive tooth of rabbits was extracted (Pei *et al.*, 2015; Lalani *et al.*, 2005; Marei *et al.*, 2005). Although extraoral access reduces surgical difficulty, the bottom of the incisors have significant curvature and prevent their extraction. In our study, mouth openers specially designed according to the occlusion surface of a molar tooth significantly reduced the difficulty of surgical intervention. The device helped provide adequate exposure and did not occlude the surgical field during the procedure. In addition, the bottom curvature of the premolar tooth is less inclined than the incisor, which provides ease of extraction.

CONCLUSION

This study described a new surgical approach for first premolar tooth extraction in rabbits. The success of animal surgical procedures is vital to prioritize the animals' well-being and efficiently conduct experiments, collect data and optimize the research timeline. Thorough surgical planning and execution are essential in experimental studies as they directly affect the reliability and validity of research results. Using proper surgical techniques, researchers can reduce the risk of complications and ensure that animals are healthy enough to continue with experiments, resulting in more precise and consistent data. Therefore, prioritizing the highest surgical standards is crucial to upholding the ethical treatment of animals and maintaining the integrity of experimental data. The tooth socket of the first premolar of the rabbit is a reliable and feasible area for surgical procedures to study bone healing, which will directly represent ossification instead of commenting on healing indirectly by endochondral ossification.

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Author contributions

Erdal Ergünol

Conception and design of study/review/case series, Acquisition of data: laboratory or clinical/literature search, Analysis and interpretation of data collected, Final approval and guarantor of the manuscript.

Remzi orkun akgün

Acquisition of data: laboratory or clinical/literature search.

Rabia semsi

Acquisition of data: laboratory or clinical/literature search, Drafting of article and/or critical revision. final approval and guarantor of manuscript.

Okan ekim

Acquisition of data: laboratory or clinical/literature search.

Altay uludamar

Analysis and interpretation of data collected, drafting of article and/or critical revision, final approval and guarantor of the manuscript.

Aylin sepici dinçel

Conception and design of study/review/case series, drafting of article and/or critical revision, final approval and guarantor of manuscript.

Ethics statement

The study was approved by the Gazi University Animal Experiments Local Ethics Committee Regulations and is entitled. Approval number: G.U.E.T-21.010.

Data availability

Not applicable, available upon request.

Conflict of interest

The authors declared no competing interests.

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