rabbit genetics

Comparative evaluation of the osseointegration of titanium screws into cortical bone in female rabbits in different physiological situations

[1]Bianca Fernea, [1]Aurel Damian, [1]Cristian Martonos, [1]Dariana O. Bratfelan, [2]Aurelia Coroian, [1]Oana A. Pece

[1] Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; [2] Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania. Corresponding author: O. A. Pece, oanamastan@yahoo.com

Abstract. At 10 female rabbits, 5 nulliparous and 5 primiparous, titanium screws 5 mm long and 2 mm in diameter were inserted into the diaphysis of the femoral bone. After one month, the bone portions containing the screws were harvested, fixed in 10% formalin, decalcified with trichloroacetic acid, and embedded in paraffin. Sections with a thickness of 5µ were made, displayed on slides and stained with the Trichrome Goldner method. It was found that the osseointegration process proceeded normally in the two categories of animals, going through the same stages but with obvious differences between the two groups regarding the speed of development. After one month, the osseointegration process was obviously more advanced in nulliparous females compared to primiparous ones. It seems that going through a period of gestation and a period of lactation is very demanding on mothers who are deprived of a certain period of time with the impairment of the osseointegration process, which is obviously slower than in females who have not gone through such physiological periods.
Key Words: nulliparous females, osseointegration, primiparous females, rabbit.

Introduction. The differences in bone mass between males and females is known (Chrcanovic et al 2015), and this sexual dimorphism occurs during the growth period and is due to the increase in cortical thickness through bone deposition on the periosteal surface in males and mainly on the endocortical one in females (Kim et al 2003).

Carrying out a comparative study on male and female rabbits, Marcu et al (2020) concluded that osseointegration goes through the same stages in both sexes, but the speed at which it takes place is about 25% higher in males. The situation is more complex in the case of females who may find themselves in various demanding physiological situations that could influence the osseointegration process. One such situation is the state of gestation which is very demanding for the mother who has to provide the fetus with all the substances it needs. Moreover, this condition is accompanied by changes and increases in the level of sex hormones, and these imbalances affect some organs and influence the functioning of the immune system in the sense that there is a reduction in chemotaxis, phagocytic activity, T cell activity, antibody production (González-Jaranay et al 2017; Javed & Romanos 2018). Rabbit studies on the osseointegration of titanium implants have been the subject of several studies (Rațiu et al 2022; Duma et al 2023; Sabou et al 2023).

Also, some authors have reported the existence of a chronic maternal stress (Culhane et al 2001) that can be amplified by a nutritional deficit of the mother, which also affects the fetus (Wellinghausen 2001). The demand of the mother does not end with parturition because the state of gestation is immediately followed by a period of lactation. The lactation period, while a natural physiological process, imposes substantial demands on the doe, as she must supply her kits with all the essential nutrients required for postnatal development. Kits are known to prioritize their own needs, consuming the necessary resources without regard to the physiological strain placed on the mother. As a result, the doe, after undergoing the combined challenges of gestation and lactation, often experiences a temporary depletion of physiological reserves. These factors can influence various processes, including the speed of osseointegration, which may be slower in primiparous females compared to nulliparous ones. In this situation, the osseointegration process of some implants may suffer, at least in terms of the speed of development, compared to nulliparous females. The aim of this study was the comparative evaluation of the osseointegration process of titanium screws in nulliparous and primiparous females, one month after screw insertion.

Material and Method. This study was carried out between March and April 2021. The biological material used was represented by 10 domestic rabbits (*Oryctolagus cuniculus*), 5 nulliparous females and 5 primiparous females. The experiment had the approval of the bioethics committee, mentioned in the application form number 219 of 10.07.2020.

The materials used for testing were titanium screws with a length of 5 mm and a diameter of 2 mm. For their insertion in the diaphysis of the femoral bone, a hole was created with a 1.8 mm diameter drill. Animals were anesthetized by intramuscular administration of ketamine 40 mg kg^{-1} and xylazine 5 mg kg^{-1}. The intervention area was cleaned, then the skin and muscles were incised, highlighting the femur bone.

After making the insertion hole, the screws were installed with a special device for self-tapping screws. To close the lesion, muscle suture followed by skin suture was performed. Postoperative treatment consisted of subcutaneous administration of enrofloxacin 20 mg kg^{-1} and meloxicam 1 mg kg^{-1}. After one month the animals were euthanized and the portion of bone containing the screw was harvested for histological investigations.

The harvested parts were fixed in 10% formalin for 7 days, decalcified in trichloroacetic acid and embedded in paraffin. Sections with a thickness of 5 µm were cut, which were stained by the Goldner trichrome method. Sections were examined with an Olympus BX4 microscope, and images captured with an Olympus E-330 digital camera.

Results and Discussion. The overall evaluation of the intervention area shows that it is free of inflammatory processes, so we can say that the reparative processes have evolved as much in the case of the tissue as the hard ones. Proliferated structures can be accurately traced over the entire surface of the interface, from periosteum to endosteum, so that we can appreciate bone proliferation for each area from surface to depth.

In nulliparous females, reparative processes are present over the entire surface of the interface, but with differences from one area to another. Their intensity and the stage they reached one month after the insertion of the screws are directly related to the position relative to the two starting points of the repair processes, endosteum and periosteum respectively (Figure 1).

In the periosteal area, the newly proliferated bone forms a well-represented layer, but it consists of young (primary) bone, with numerous areolae. A certain number of bony lamellae outlines are also present, some even tending to be concentrically arranged around some areolae. As we progress deeper into the interface, the layer of newly proliferated bone tissue thins visibly and appears discontinuous and even absent on certain surfaces at the interface. Moreover, in the depth of these areas, the remaining bone appears visibly modified, but there are still some relatively discrete points of bone restoration in it.

The most newly proliferated bone is in the endosteal area. It is still young primary bone but in a slightly more advanced remodeling stage than in the other areas, including the periosteal one. Here the tendency to form bony lamellae is more visible, so that they even tend to organize themselves concentrically around the vascularized areolae. These formations are starting points for future osteons.

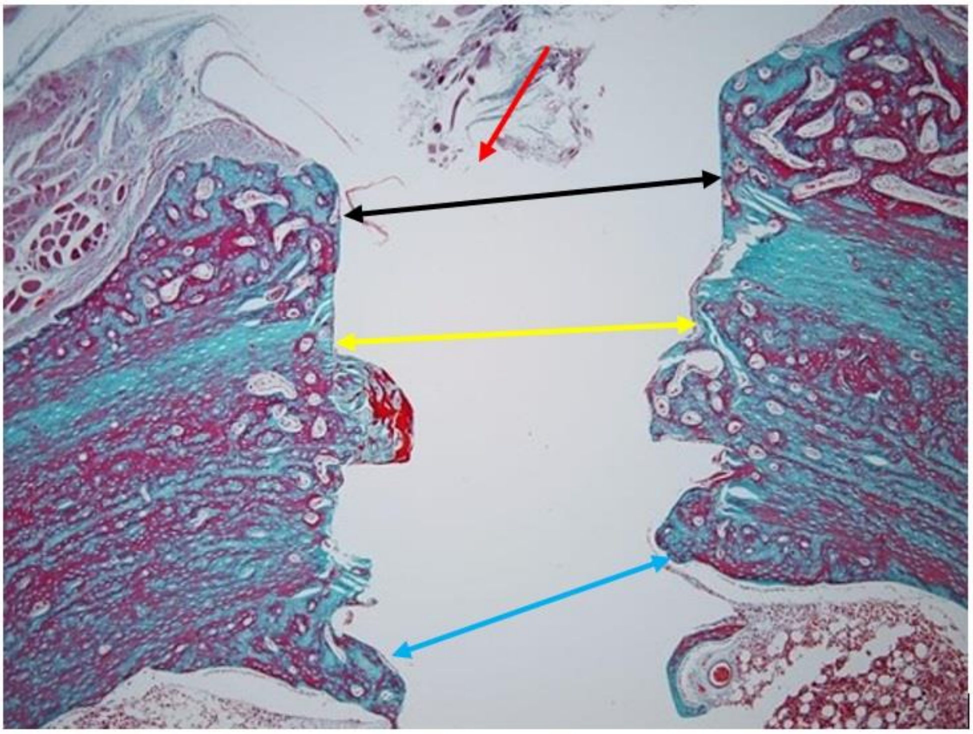

Figure 1. Nulliparous female interface: red arrow – implant site; black arrow – periosteal area; yellow arrow – osteal area; blue arrow – endosteal area (Tricom Goldner, ob. 4X).

As we proceed to the depth of the interface, the layer of newly proliferated bone tissue becomes thinner and thinner until it disappears. The osteal area (middle) of the interface is not yet covered by newly proliferated bone tissue but only by augmentation material, quantitatively different from one portion to another. The endosteal zone of the interface contains the largest amount of newly proliferated bone, here it fills the trench between the implant turns and extends over the turns as a continuous and relatively well-represented layer. Moreover, this newly proliferated bone is relatively well anchored to the deep bone structures. As a stage of proliferation and remodeling, it shows differences from one area to another. It is a young bone tissue that in some places presents relatively numerous and highly polymorphic areolae, and in other places the areolae are mostly significantly smaller in size. This proves that this bone is in full proliferation process, and from the structural point of view, it is primary bone. A very discrete tendency to form a very small number of bone lamellae outlines is also observed (Figure 2).

In primiparous females the periosteal area of the interface appears covered by a thin and fragile layer of newly proliferated bone tissue. Beneath this thin layer, there is a relatively deep area in which bone affected by the operative trauma is present, which has not yet recovered. In this bone there are numerous polymorphic spaces in both shape and size, devoid of content.

Females have a lower bone volume than males and as a result are expected to experience bone loss to a greater extent (Kim et al 2003). The role of estrogens in the differentiation of osteoblasts from osteoprogenitor cells is known (Riggs 2000).

The decrease in the level of estrogens, above a certain limit in females in different physiological or pathological states, causes a significant decrease in bone mass (Bonnick 2000). Inadequate estrogen level is not the only risk factor, there are several risk factors within a population. They cannot be separated to quantify the specific effect of an individual risk factor, and there is even the possibility that some risk factors are more harmful together than when taken individually (Klokkevold & Han 2007).

Compromise beyond a certain limit of bone metabolism can be a risk factor affecting osseointegration. The rabbit is the most used experimental animal, being preferred to large animals because it reaches skeletal maturity at a relatively young age

(8-11 months), and compared to small animals it undergoes secondary osteonal remodeling (Wancket 2015).

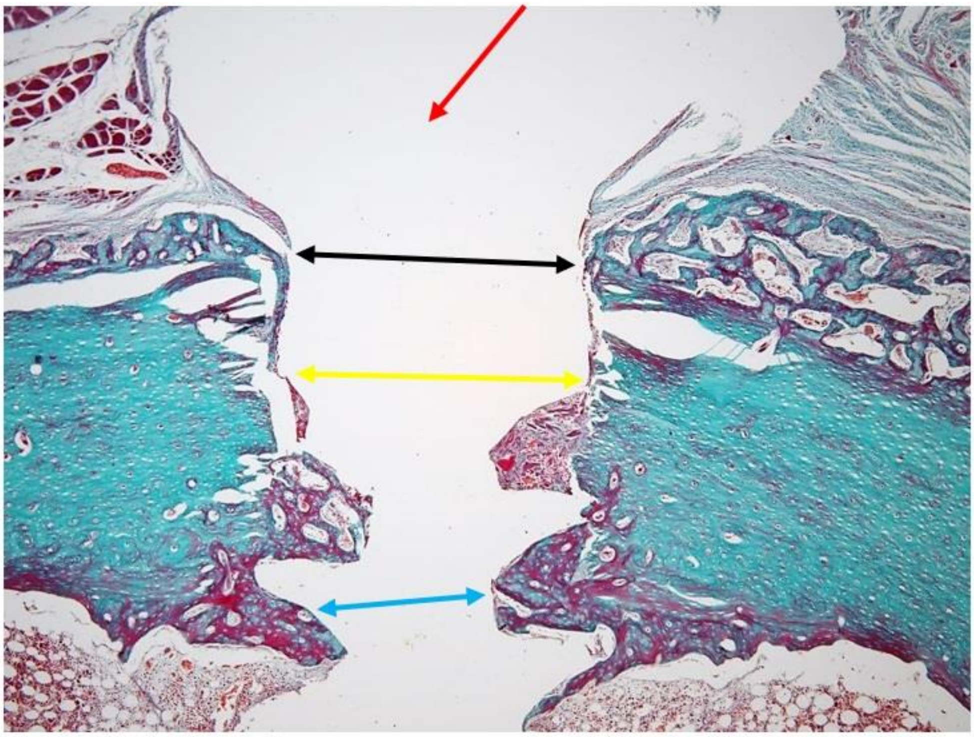

Figure 2. Primiparous female interface: red arrow – implant site; black arrow – periosteal area; yellow arrow – osteal area; blue arrow – endosteal area (Trichrome Goldner, ob. 4X).

After inserting a screw into bone, the bone interface decreases in strength over a fairly long period, and some authors believe that the critical point is reached somewhere between 3 and 6 weeks post-implantation (Lazzara et al 1996). There are authors who claim that the bone interface is weak compared to that existing at the time of screw insertion, even up to 3 months (Balshi et al 2005).

As an experimental period, we followed the one presented by Lazzara et al (1996), namely 4 weeks post-implantation. Also, our results are comparable to those reported by Lazzara et al (1996) and Balshi et al (2005), the bone-implant interface is weak in terms of mechanical resistance in both groups of animals studied.

It should be noted that between the two experimental groups there are certain differences in the sense that the interface is weaker in primiparous females compared to nulliparous ones. This proves that primiparous females are more vulnerable, weaker, for a period of time after parturition and lactation, they need a certain period of time to return to the situation existing before the establishment of gestation. It should be noted, however, that although there are differences between females in the two different physiological situations, osseointegration evolves normally in both groups, going through the same stages, and only the speed with which they take place is lower in primiparous females compared to nulliparous ones. In other words, osseointegration takes place comparably in the two categories of animals studied in terms of the stages it goes through, only that in primiparous females it takes place more slowly, an aspect that must be taken into account when using orthopedic screws in this category of animals.

Conclusions. In the case of nulliparous females, the presence of reparative processes was observed on the entire surface of the interface, with differences from one area to another. The periosteal area of the interface in the case of primiparous females appears covered with a thin, fragile layer of newly proliferated bone tissue, thus also in this case the stage of proliferation and remodeling can be observed with differences from one area to another. The ossification of titanium screws into cortical bone in female rabbits follows the same steps regardless of the physiological situation in which they are located, but the speed of this process is influenced by the physiological situation in which the females are located. The period of gestation and lactation are very demanding and after completing these stages, the females are deprived of a period of time, an aspect that influences the osseointegration of titanium implants in this category of animals.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Balshi S. F., Allen F. D., Wolfinger G. J., Balshi T. J., 2005 A resonance frequency analysis assessment of maxillary and mandibular immediately loaded implants. The International Journal of Oral and Maxillofacial Implants 20(4):584-594.

Bonnick S. L., 2000 Monitoring osteoporosis therapy with bone densitometry: a vital tool or regression toward mediocrity? Journal of Clinical Endocrinology and Metabolism 85(10):3493-3495.

Chrcanovic B. R., Albrektsson T., Wennerberg A., 2015 Dental implants inserted in male versus female patients: a systematic review and meta-analysis. Journal of Oral Rehabilitation 42(9):709-722.

Culhane J. F., Rauh V., McCollum K. F., Hogan V. K., Agnew K., Wadhwa P. D., 2001 Maternal stress is associated with bacterial vaginosis in human pregnancy. Maternal and Child Health Journal 5(2):127-134.

Duma V., Gal A. F., Rus V., Matei-Latiu M. C., Rațiu C., Alexandru B. C., Latiu C., Martonos C., Oana L. I., 2023 Comparative assessment of contact osteogenesis at the titanium implant-bone junction in male rabbits with dissimilar femoral defects. International Journal of Morphology 41(5):1317-1322.

González-Jaranay M., Téllez L., Roa-López A., Gómez-Moreno G., Moreu G., 2017 Periodontal status during pregnancy and postpartum. PLoS ONE 12(5):e0178234.

Javed F., Romanos G. E., 2018 Success and survival of dental implants during pregnancy. In: Evidence-based implant dentistry and systemic conditions. John Wiley & Sons, Inc., pp. 65-66.

Kim B. T., Mosekilde L., Duan Y., Zhang X. Z., Tornvig L., Thomsen J. S., Seeman E., 2003 The structural and hormonal basis of sex differences in peak appendicular bone strength in rats. Journal of Bone and Mineral Research 18(1):150-155.

Klokkevold P. R., Han T. J., 2007 How do smoking, diabetes, and periodontitis affect outcomes of implant treatment? The International Journal of Oral and Maxillofacial Implants 22(Suppl):173-202.

Lazzara R., Siddiqui A. A., Binon P., Feldman S. A., Weiner R., Phillips R., Gonshor A., 1996 Retrospective multicenter analysis of 3i endosseous dental implants placed over a five-year period. Clinical Oral Implants Research 7(1):73-83.

Marcu T., Gal A., Martonos C., Rus V., Damian A., Miclăuș V., Mihai A. T., 2020 Comparative osteointegration testing of titanium implants in relation to gender after one month of implantation in rabbit femur. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine 77(2):131-137.

Rațiu C. A., Rațiu I. A., Miclăuș V., Pantor M., Rus V., Martonos C. O., Lacatus R., Purdoiu R. C., Gal A. F., 2022 The influence of haematogenous bone marrow on the early osseointegration of a titanium implant which penetrates the endosteum. International Journal of Morphology 40(1):188-193.

Riggs B. L., 2000 The mechanisms of estrogen regulation of bone resorption. The Journal of Clinical Investigation 106(10):1203-1204.

Sabou I., Gal A. F., Matei-Latiu M. C., Rus V., Miclăuș V., Raţiu C., Martonos C. O., Oros N., Oana L., 2023 Does the diameter of the titanium orthopaedic screw insertion hollow influence the mechanism of contact osteogenesis? A comparative assessment in the female rabbits' femoral bone. Revista Română de Medicină Veterinară 33(4): 27-32.

Wancket L. M., 2015 Animal models for evaluation of bone implants and devices: comparative bone structure and common model use. Veterinary Pathology 52(5): 842-850.

Wellinghausen N., 2001 Immunobiology of gestational zinc deficiency. The British Journal of Nutrition 85(2):81-86.

Received: 29 September 2024. Accepted: 27 November 2024. Published online: 29 December 2024.
Authors:
Bianca Fernea, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: bianca-nicolasa.fernea@student.usamv-cluj.ro
Aurel Damian, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: aurel.damian@usamvcluj.ro
Cristian Martonos, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: CMartonos@Rossvet.edu.kn
Dariana Olivia Bratfelan, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: darianabratfelan@yahoo.com
Aurelia Coroian, Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: aurelia.coroian@usamvcluj.ro
Oana Andreea Pece, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: oanamastan@yahoo.com

How to cite this article:
Fernea B., Damian A., Martonos C., Bratfelan D. O., Coroian A., Pece O. A., 2024 Comparative evaluation of the osseointegration of titanium screws into cortical bone in female rabbits in different physiological situations. Rabbit Gen 14(1):21-26.