

The combined therapy of teriparatide and raloxifene improves osseointegration of dental implants in the osteoporotic rabbit model

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Abstract

Purpose: The study aimed to evaluate the efficacy of combined therapy of teriparatide and raloxifene on the osseointegration of titanium dental implants in a rabbit model of osteoporotic bone.

Materials and Methods: Fifty-three female rabbits were randomly divided into six groups. The sham ovariectomy group (control) consisted of animals that received no medication. Animals in the ovariectomy group (OVX) underwent ovariectomy and received no medication. The combined group consisted of ovariectomized animals receiving combined teriparatide (10mg/kg) for 12 weeks and raloxifene (10mg/kg) for 12 weeks of therapy. Ovariectomized animals in the sequential group (SEQ) received teriparatide (10mg/kg) for the first 6 weeks and raloxifene therapy (10mg/kg) for the following 6 weeks sequentially. The parathormone (PTH) and raloxifene (RAL) groups consisted of ovariectomized animals receiving only teriparatide (10mg/kg) for 12 weeks and raloxifene (10mg/kg) for 12 weeks, respectively. Dental implants (Bilimplant®, Turkey) were placed in the proximal metaphysis of both tibias in all rabbits. Histomorphometric and micro-CT studies were performed on the specimens obtained from the right tibia bone. Removal torque (RTQ) and implant stability tests (ISQ) were performed on the specimens obtained from the left tibia bone. The results were compared and evaluated statistically.

Results: RTQ analysis revealed a statistically significant difference between the mean values of the combined group (93.01 ± 27.19 Ncm) and the OVX group (49.6 ± 12.5 Ncm) ($p=0.015$). The highest mean T0 (implantation day) value was obtained in the control group (67.1 ± 3.4) and the lowest mean value was obtained in the OVX group (61.4 ± 3.8). The highest T1 mean (three months after implantation) was obtained by the combined group (76.6 ± 3.8) and the lowest mean by the OVX group (68.9 ± 6.2). As a result of histomorphometric analyses, it was found that the mean percent bone-implant contact (BIC%) value of the combined group (51.2%) was significantly higher than that of the OVX group (28.6%) ($p=0.006$). In the micro-CT

31 examinations, it was found that the mean (BIC%) value of the combined group (41.1%) was
32 significantly higher than that of the OVX group (24.1%) ($p < 0.001$).

33 **Conclusion:** According to the results of the current study, combined therapy of teriparatide and
34 raloxifene improves bone-implant contact and osseointegration of titanium dental implants in
35 osteoporotic bone compared with sequential or sole therapy with these agents.

36 **Keywords:** Dental implants, Teriparatide, Raloxifene, Osseointegration, Osteoporosis

37 **Introduction**

38 Dental implants have changed and evolved in recent years and have become one of the
39 indispensable treatment materials in today's dental practice (1). The long-term success of dental
40 implants depends on many factors. The level of training and experience of the physician
41 performing the procedure, the amount and density of bone in the edentulous region, and the
42 general health of the patient are among the most important factors. The surgeon may not be able
43 to control some factors, such as bone quality. To achieve better clinical results, it is critical to
44 determine bone quality prior to implant placement. The quality of the bone plays an important
45 role in the primary stabilization of dental implants, which is crucial for the success of the
46 implant (2). One of the diseases that causes poor bone quality is osteoporosis. Osteoporosis is
47 a disease characterized by low bone density and increased bone fragility (3). Disease-related
48 loss of mass in trabecular and cortical bone, in general, has become an increasingly important
49 public health problem as the human lifespan has lengthened (4). Therefore, the disease is
50 important in dental implantology (5). Osteoporosis may cause bone loss surrounding the dental
51 implant, which can lead to dental implant failure (6, 7). Disruption of systemic bone metabolism
52 may be a risk factor affecting osseointegration. Little is known about the relationship between
53 osteoporosis disorder and dental implant therapy (8).

54 The goals of osteoporosis treatment are to prevent fractures and skeletal deformities, increase
55 bone mineral density (BMD), and thus improve quality of life. Numerous drugs that increase
56 bone formation (parathormone) and decrease bone resorption (bisphosphonates, denosumab,
57 raloxifene) are used in the current treatment strategies of osteoporosis (9). Drugs used in
58 antiresorptive therapies can increase BMD only to some extent by reducing the number of
59 osteoclasts and preventing new bone synthesis by osteoblasts (10). Selective estrogen receptor
60 modulators (SERMs) are drugs that can both mimic and block the action of estrogen in various
61 tissues and are used to treat osteoporosis. Raloxifene, one of the most used SERM drugs, may

62 decrease bone resorption rate, increase BMD, and decrease the risk of fractures. (11).
63 Teriparatide is a form of PTH consisting of the first 34 amino acids, which is the bioactive part
64 of the hormone. Intermittent intake of teriparatide produces an osteoanabolic effect by
65 stimulating osteoblasts in the treatment of osteoporosis (12). It has been reported that some
66 antiresorptive drugs (bisphosphonates, denosumab), when used alone, can cause adverse side
67 effects during procedures such as tooth extraction and dental implant placement (13). Therefore,
68 combined, or sequential drug therapy is becoming increasingly important in the treatment of
69 osteoporosis. It is believed that reducing bone resorption by antiresorptive drugs and increasing
70 BMD by concomitant anabolic drugs may be effective in the treatment of osteoporosis.
71 Although there is no significant difference between the anabolic effects of taking teriparatide
72 and raloxifene alone, combined or sequentially, studies have shown that bone resorption
73 decreases significantly with combined use (14). Based on this information, we believe that
74 managing osteoporosis with an effective medical treatment will improve the osseointegration
75 capacity of dental implants. In this study, it was hypothesized that osseointegration of dental
76 implants in patient with low bone density would be increased by the combined use of
77 osteoanabolic and antiresorptive drugs. To our knowledge, there is no study in the literature
78 investigating the effects of the combined use of teriparatide and raloxifene on the
79 osseointegration of dental implants.

80 The aim of the study was to evaluate the efficacy of combined therapy of teriparatide and
81 raloxifene on the osseointegration of titanium dental implants in a rabbit model of osteoporotic
82 bone.

83 **Material and methods**

84 ***Animals***

85 Sixty adult female New Zealand rabbits (*Oryctolagus cuniculus* L) aged eight months (3.0-3.5
86 kg body weight) were used for this study. All animal experiments were approved by the
87 Experimental Animal Ethics Committee of Erciyes University and were performed according
88 to the Guidelines for the Care and Handling of Experimental Animals of the Medical Research
89 Centre of Erciyes University, Kayseri, Turkey (17/078). The study protocol has compliance
90 with the ARRIVE guidelines.

91 ***Group desig***

The rabbits were randomly divided into six groups. The first group was a sham ovariectomy group (control), consisting of animals without drugs. The second group was the ovariectomy group (OVX). Animals in this group underwent ovariectomy and received no medication. The third group was the combined group, which consisted of ovariectomized animals receiving combined teriparatide and raloxifene therapy. The fourth group was the sequential group (SEQ), which consisted of ovariectomized animals receiving teriparatide and raloxifene, respectively. The fifth and sixth groups were the parathormone (PTH) and raloxifene (RAL) groups, which consisted of ovariectomized animals receiving only teriparatide and raloxifene, respectively (Figure 1).

Experimental design

Fifty animals underwent bilateral ovariectomy under general anesthesia. The remaining ten animals underwent sham ovariectomy and served as a control group. Anesthesia was induced with an intramuscular injection of 50 mg/kg ketamine HCL (Ketalar, Pfizer, Turkey) and 25 mg/kg xylazine (Rompun, Bayer, Germany) and maintained with an additional dose of 0.2 mL ketamine. This process was repeated if necessary, following the same protocol as previous studies (11, 12).

To prevent infections in the animals, 50 mg/kg cefazolin (Cefamezin® IM Vial, Eczacıbaşı, Turkey) was injected for 3 days before and after surgery. 1 mg/kg diclofenac (Diclofen®, Turkey) was injected intramuscularly into the animals to relieve pain caused by surgery. Two weeks after ovariectomy, 1 mg/kg of methylprednisolone (Prednol-1 40 Mg 1 ampule) was administered intramuscularly for 4 weeks to accelerate osteoporosis in all animals except the control group. It is now known that the combined use of the ovariectomy procedure and steroid administration is an acceptable method of inducing osteoporosis in rabbits (15, 16). At 8 weeks after ovariectomy, all animals underwent implant surgery under general anesthesia. To achieve standardization, the placement of dental implants was performed by the same researcher. A tissue level titanium dental implant measuring 4,1x6 mm in diameter and length was used as the implant material (Bilimplant®, Turkey). Of the 120 dental implants used, 60 were placed in the left tibial bone of the rabbits and 60 in the right tibial bone of the rabbits. The healing abutments of the implants were inserted using a ratchet with a torque force of 10 Ncm. Before placement of the implant healing abutments, primary stability was measured with the ISQ device (Osstell AB, Gothenburg, Sweden). Histomorphometric and micro- CT studies were performed on the specimens obtained from the right tibia bone. Removal torque (RTQ) and

implant stability tests (ISQ) were performed on the specimens obtained from the left tibia bone. Medication was started after the surgical procedure completed in each group.

Medication

After ovariectomy and dental implant surgery, medical treatment was started in the PTH, RAL, combined, and SEQ groups. In the PTH group, teriparatide (Forsteo®, teriparatide 250 mg/ml, 3 ml injectable, Lilly, Turkey) was administered at a subcutaneous dose of 10 mg per kg, 5 times per week for 12 weeks. Rabbits in the RAL group received only raloxifene (Evista, Lilly) orally at a dose of 10 mg per kg daily for 12 weeks. In the combined group, teriparatide was co-administered 5 times per week subcutaneously at a dose of 10 mg per kg and raloxifene orally at a dose of 10 mg per kg daily for 12 weeks. Rabbits in the SEQ group received teriparatide at a subcutaneous dose of 10 mg per kg, 5 times per week for the first 6 weeks, and raloxifene orally at a dose of 10 mg per kg daily for the following 6 weeks, respectively. At the end of drug therapy, all animals were sacrificed for further test and analyses.

Biomechanical tests

Measurement of the implant stability quotient (ISQ)

Resonance frequency analysis (RFA) was performed using Osstell ISQ® (Osstell; Integration Diagnostics, Goteborg, Sweden). Results are shown both graphically on the instrument display and as numerical ISQ values. It was used to measure and evaluate the primary (T0) and secondary (T1) stability of the implants immediately and 12 weeks after implantation. Measurements were taken from four different directions, and all values obtained from these measurements were calculated as mean values.

Removal torque test (RTQ)

Tibia specimens were wrapped in a cloth and soaked in physiological saline before being stored at -20 °C until testing. The specimens were then thawed at room temperature on the day of the mechanical test. A gradually increasing unscrewing torque was applied to the implants, and the highest torque value required for loosening the implants was recorded with a digital torque meter (MARK-10 MTT01-12, NY, ABD).

Micro-computed tomography (micro-CT) analyses

In the study, a micro-CT device (Bruker SkyScan-1272® Kontich, Belgium) from Hacettepe University Advanced Technologies Application and Research Centre was used to scan the

154 samples. The following characteristics of the micro-CT device were used in the study: X-ray
155 source; 20-100 kV, 10 W ($<5\ \mu\text{m}$ spot size at 4W), X-ray camera; 16 MP, 4904×3280 px or
156 11 MP, 4032×2668 , pixel size at maximum magnification; the expression $<0.35\ \mu\text{m}$ for the 16
157 MP camera is $<0.45\ \mu\text{m}$ for the 11 MP camera. The dental implant and bone contact area were
158 examined in 2-3 dimensions, and measurements were taken in the 2-mm area around the
159 implant.

160 Before scanning the samples, the micro-CT device was set up with a 0.11mm aluminum (Al)-
161 copper (Cu) filter, a 0.4° rotation state, a 13-micron pixel size, 2K resolution, and 360°
162 acquisition. All samples were scanned with those parameters at the same time. NRecon v.1.6.3
163 software (Bruker® micro-CT, Belgium) was used to convert data obtained from samples
164 scanned with micro-CT, and CTAn v.1.12 program (Bruker® micro-CT, Belgium) was used
165 for analysis. All data sets were segmented and standardized with a user-defined global
166 threshold. A fixed threshold range of 115 to 255 was applied to the image of all implants and a
167 threshold range of 35 to 255 was applied to the image of all bone tissue (CTAn v.1.12 software,
168 Bruker® micro-CT, Belgium).

169 In micro-CT, the percentage of bone-implant contact (BIC%), percentage of bone volume (BV
170 /TV%), and percentage of total porosity (Po (tot)%) at the examination site were measured. As
171 a result of these micro-CT measurements, information was obtained about the osseointegration
172 of the implant and the trabecular microarchitecture around the implant.

173 *Histomorphometric analyses*

174 All implants placed in the right tibial metaphysis of each experimental animal were resected
175 with the surrounding bone for evaluation and brought to the laboratory in 4% neutral buffered
176 formalin. The samples were dehydrated in an ethyl alcohol solution for one day. The dehydrated
177 samples were placed under a vacuum for 24 hours and infiltrated with a mixture of methyl
178 methacrylate resin and alcohol. The samples were then embedded under vacuum in plastic
179 molds containing methyl methacrylate. These cans containing the samples were polymerized
180 under light at 40°C for 8 hours at a wavelength of 450 nm. The fully cured blocks were removed
181 from the transparent boxes to prepare the first cut and prepare the parallel surface. The flat
182 underside was glued to a Plexiglas slide under vacuum with Technovit 7210 VLC (Kulzer &
183 CO. GmbH, Friedrichsdorf, Germany). The 300-350 μm thick cuts were obtained with a
184 diamond saw (Exakt 300 CL, Exakt Apparatebau, Norderstedt, Germany) connected to a
185 precision cutter. These sections were thinned to a thickness of 40 μm using abrasives attached

to the microablation system (Exakt 400 CS, Exakt Apparatebau, Norderstedt, Germany). The deparaffinized and rehydrated sections were stained with toluidine blue and covered with a coverslip for histological and histomorphometric evaluations. Stained sections were examined with a Leica DMRB (Germany) light microscope. Sections of all specimens in the groups were digitally scanned and recorded using digital specimen scanning software (Microvisioneer, Germany). The obtained digital sections were analyzed by Sedene-Pathcore (Canada). Measurements were made in actual length units of one millimeter [mm] and one micrometer [μm]. Measurements of the bone-implant contact (BIC) percentage were made at the same site of the bone where the implant was placed using the Sedeen Digital Cross-Section program. The following formula was used for this purpose:

$$\text{BIC} = \frac{\text{Bone-implant contact length}}{\text{The peripheral length of the entire implant}} \times 100$$

The results of each group were statistically analysed.

Statistical analysis

Statistical analysis was performed using Turcosa® Analytical Software (Kayseri, Turkey). A Shapiro-Wilk test was performed to check the normal distribution of the data. One-way test ANOVA was used for multiple comparisons of the normally distributed data, and Kruskal Wallis test was used for non-normally distributed data. As a result, p-values below 0.05 were considered statistically significant.

Results

At the end of the study, seven rabbits died due to infection and were excluded from the study. The study was completed on 53 rabbits and 106 dental implants. The experimental animals had 53 dental implants in their right tibial bones and 53 dental implants in their left tibial bones.

Implant stability quotient (ISQ) results

The highest mean T0 value was obtained in the control group (67.1 ± 3.4) and the lowest mean value in the OVX group (61.4 ± 3.8) (Table 1). The values of the other groups were close to each other. The differences between the T0 values of each group are shown in Table 2.

There was a statistically significant increase in T1 values in all groups compared to T0 values ($p < 0.05$). The highest mean T1 value was obtained from the combined group (76.6 ± 3.8) and

the lowest mean T1 value was obtained from the OVX group (68.9 ± 6.2) (Table 1). The differences in T1 values between groups are shown in Table 2.

Removal torque test results

The mean value of the combined group (93.01 ± 27.19 Ncm) was found to be the highest. The lowest mean value was obtained from the OVX group (49.6 ± 12.5 Ncm) (Figure 2). The difference between the combined group and the OVX group proved to be statistically significant ($p=0.015$). The means of the other groups were close, but there were no statistically significant differences ($p>0.05$) (Table 3).

Histomorphometric results

The highest mean BIC% value was obtained from the combined group ($51.2 \pm 12.9\%$), followed by the control group ($48.9 \pm 12.06\%$). The lowest mean BIC% value was obtained from the OVX group ($28.6 \pm 7.7\%$). Statistical analysis between groups revealed a statistically significant difference between the combined and OVX groups and between the control and OVX groups ($p=0.006$) (Table 4) (Figure 3).

Micro-CT results

The results of the analysis showed that the mean (BIC%) value of the OVX group was the lowest ($24.14 \pm 3.5\%$), and the mean (BIC%) value of the combined group was measured as the highest ($41.1 \pm 10.06\%$) (Figure 4). The difference between the two groups proved to be statistically significant ($p<0.001$) (Table 5). The difference between the control group and the OVX group also proved to be statistically significant ($p<0.001$) (Table 5).

The mean values of (BV/TV%) were examined, and the highest value was obtained from the control group ($44.4 \pm 10.6\%$), and the lowest value was from the OVX group ($17.3 \pm 5.2\%$) (Figure 4). The mean (BV/TV%) value of the control group was compared with each of the values of the OVX, SEQ, PTH, and RAL groups, and the results were found to be statistically significant ($p<0.001$) (Table 5). In addition, there was a statistically significant difference between the combined group and the OVX group ($p=0.019$) (Table 5).

In the Po(tot)% analyses, the mean of the control group ($54.02 \pm 10.5\%$) was the lowest and the mean of the OVX group ($82.3 \pm 5.3\%$) was the highest (Figure 4). The mean Po(tot)% value of the control group was compared with the means of the other groups separately, and the results were found to be statistically significant ($p<0.05$) (Table 5). A statistically significant

difference was found between the combined and OVX groups ($p=0,041$) (Table 5). Micro-CT images obtained from the groups are shown in Figure 5.

Discussion

The study aimed to investigate the effect of combined use of the osteoanabolic and antiresorptive agents on the osseointegration of dental implants in an osteoporosis-generated animal model. The results of the study showed that combined therapy with teriparatide and raloxifene significantly improved implant stability and osseointegration in osteoporotic bone.

One of the diseases causing low bone quality and quantity is osteoporosis. Osteoporosis-related changes in the jaws are not different from those in other bones in the body (17). Various pharmaceutical agents are used as a treatment option to improve the osseointegration of dental implants in patients with low bone density. These medications are categorized as antiresorptive agents and osteoanabolic agents (18). After taking antiresorptive drugs, BMD can be increased only to a certain extent by reducing the number of osteoclasts and thus the inability of osteoblasts to synthesize new bone. In these cases, it can be assumed that the treatment protocol with antiresorptive drugs will interfere with the osseointegration process. In addition, some antiresorptive drugs, such as bisphosphonates and denosumab, are known to cause undesirable side effects in dental implant surgery (19). Therefore, we preferred to use teriparatide as an osteoanabolic drug and raloxifene as an antiresorptive drug instead of other antiresorptive drugs in the osteoporotic bone model to increase the stability of the dental implant.

Numerous experimental studies have been conducted in the literature to investigate the efficacy of various osteoporosis medications on the osseointegration of dental implants (1, 20-22). Park et al. investigated the effects of raloxifene administration on the osseointegration of dental implants and reported that raloxifene therapy increased the amount of newly formed bone around the implant as shown by histomorphometry and microtomography results (21). In another experimental study by Ramalho-Ferreira et al., alendronate and raloxifene were administered to osteoporotic rats to investigate bone healing capacity at the bone-implant interface. The authors found that raloxifene therapy had positive effects on osseointegration in terms of histologic and biochemical outcomes in their study (23). In this study, no statistically significant differences were found between the biomechanical results of the RAL group and the other groups. The histomorphometric and microtomography findings of the study were also parallel to the biomechanical results (1, 23). In our opinion, these results indicate that treatment with raloxifene alone was not sufficient to improve osseointegration in the present study. In one

of the studies investigating the effects of teriparatide treatment in an osteoporotic animal model, Almagro et al. found that intermittent teriparatide treatment had beneficial effects on osseointegration of dental implants in rabbits (20). According to the histomorphometric and micro-CT results of the present study, the values of the PTH group approached but did not exceed those of the control group. Moreover, the difference between these two groups was not statistically significant. It can be speculated that the reason why the micro-CT and histomorphometry values of the PTH and control groups were close to each other was the osteoanabolic effect of teriparatide therapy. Although PTH treatment was found to have favorable effects on dental implant osseointegration, it was thought that better results may be reached by combining it with an antiresorptive drug, as in the treatment of osteoporosis.

According to the osteoporosis therapy strategy, the addition of antiresorptive drugs to ongoing teriparatide therapy usually produces good results. Some studies indicate that BMD levels decrease rapidly after the end of teriparatide treatment in patients not taking antiresorptive drugs (24-26). In an experimental study, alendronate and raloxifene were administered sequentially to ovariectomized rabbits. The results showed that sequential treatment may be effective in the treatment of osteoporosis (11). The results of this study show that sequential treatment on the osteoporotic bone model has positive effects on osseointegration of dental implants but is not as effective as combined treatment. In our opinion, the reason for this is the synergistic effect that results from the simultaneous use of osseoanabolic and antiresorptive drugs with different modes of action in the treatment of osteoporosis. In this way, the bone structure is strengthened and its osseointegration capacity increases.

Several studies in the literature have shown that the combination of an anabolic and an antiresorptive agent improves bone quality and strength after osteoporosis. In a study by Cosman et al., BMD increased more in the combination group (teriparatide and bisphosphonate) than in the monotherapy group (teriparatide or bisphosphonate) in postmenopausal women with osteoporosis (27). Deal et al. conducted a double-blind, placebo-controlled trial in 137 patients with postmenopausal osteoporosis. Patients were randomized to receive either teriparatide alone or teriparatide and raloxifene concomitantly. The authors emphasized that combined therapy with raloxifene may enhance the bone-forming effect of teriparatide (14). To our knowledge, there is no study investigating the effects of raloxifene and teriparatide on osseointegration of dental implants when used sequentially or in combination. The main hypothesis of the combined use of these two drugs is to maintain and promote new bone formation. In the present study, our results supported this hypothesis, and it was observed that

osseointegration and bone formation around the implant increased significantly, especially after combined therapy. In healthy bone, the bone remodeling process is subject to a continuous and balanced formation and resorption mechanism. However, in the case of osteoporosis, the balance of this resorption and formation mechanism is in favor of resorption. In this study, one of the main reasons for the more successful results of combination therapy compared with administration of these drugs alone or sequentially may be explained by a realignment of the mechanism of bone remodeling in osteoporotic bone by increasing bone production and slowing bone resorption. According to a standard study protocol, an osteoporosis model was first created in experimental animals, and dental implants were applied to the osteoporotic bone. Patients with osteoporosis are typically encountered in clinical practice after the disease has been diagnosed and medication started. The main objective of this study is to determine an appropriate medical treatment method that improves the osseointegration capacity of bone when its microarchitecture is compromised due to osteoporosis. When osteoporosis patients have an effective medical treatment after the disease is diagnosed, the bone's ability to osseointegrate will improve, and the dental implant procedure will be successful. The results of our study support this situation. At the same time, it is believed that doctors could modify the ongoing medical treatment method for osteoporosis patients to improve the osseointegration capacity of those patients who will have dental implant treatment.

The study has some limitations. The first limitation of the study is that dental implants were inserted in the legs of rabbits and were unaffected by pathogenic microorganisms in the oral cavity. In the future, a further study can be performed with another animal model that dental implants will be placed in the oral cavity. The second limitation of the study is that only BIC% measurements were performed during the histomorphometric analysis. However, in addition to histomorphometric analysis, the study included radiographic (micro-CT) and biomechanical analysis (RTQ and ISQ tests).

In conclusion, combined therapy of teriparatide and raloxifene significantly improves bone-implant contact and osseointegration of titanium dental implants in osteoporotic bone compared with sequential or alone therapy with these agents. However, the results obtained in this study should be supported by further experimental and clinical studies.

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Conflict of Interests

The authors declare that there is no conflict of interest

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Figure legends

Figure 1. The group design of the study is briefly shown in the figure

Figure 2. Removal torque values according to groups

Figure 3. Ground section of the dental implants with the surrounding bone taken from the control (A), combined (B), OVX (C), sequential (D), PTH (E), and RAL (F) groups. In the control and combined groups, bone penetrates the area between the threads to the implant surface. Cortical bone porosity can be seen in the OVX group (toluidine blue, images magnification×10).

Figure 4. The mean values of the micro-CT parameters by groups

Figure 5. Images of coronal (X-Z) sections from micro-CT scans of the control (A), combined (B), sequential (D), RAL (D), PTH (E), and OVX (F) (NRecon 1.6.3 software, Bruker-micro-CT, Belgium). The colored areas shown in red around the dental implant represent the bone.

Figures

Figure 1

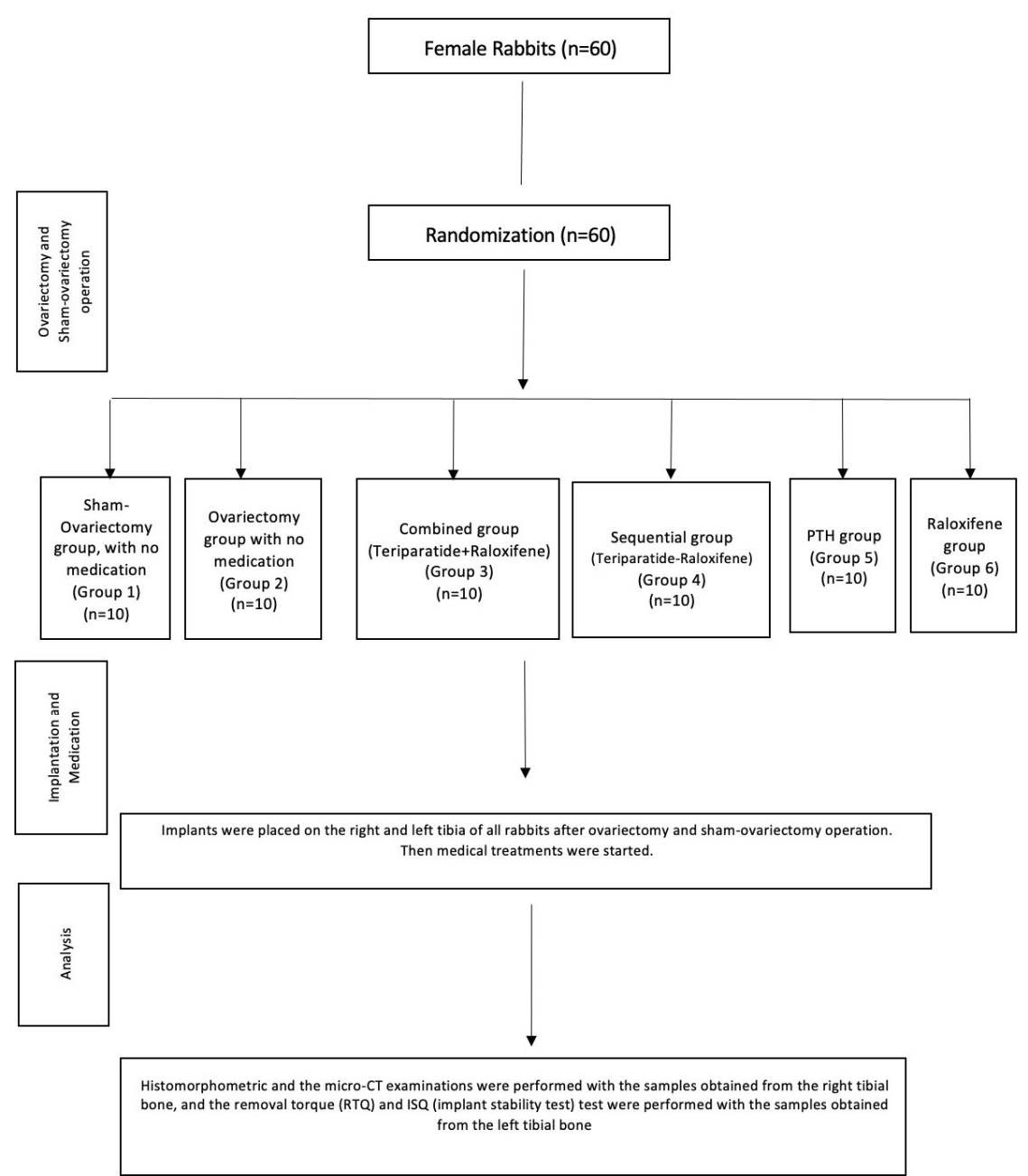


Figure 2

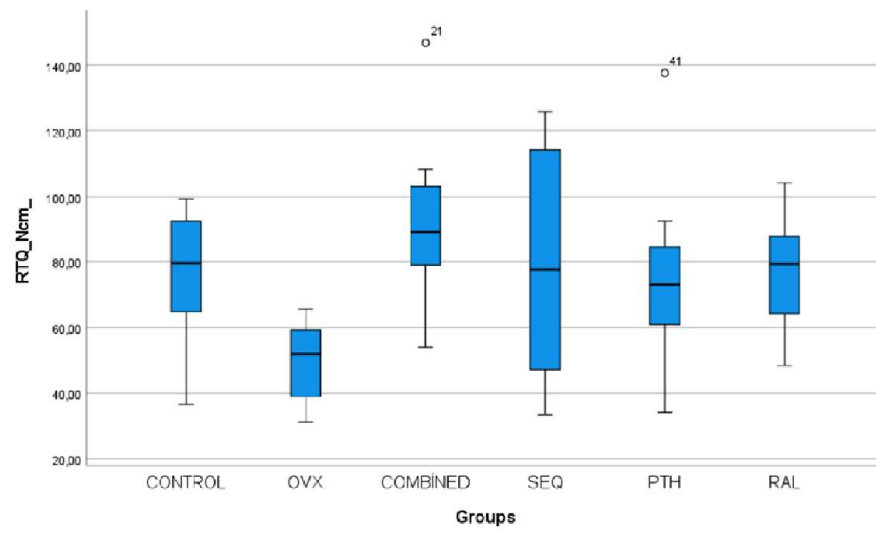


Figure 3

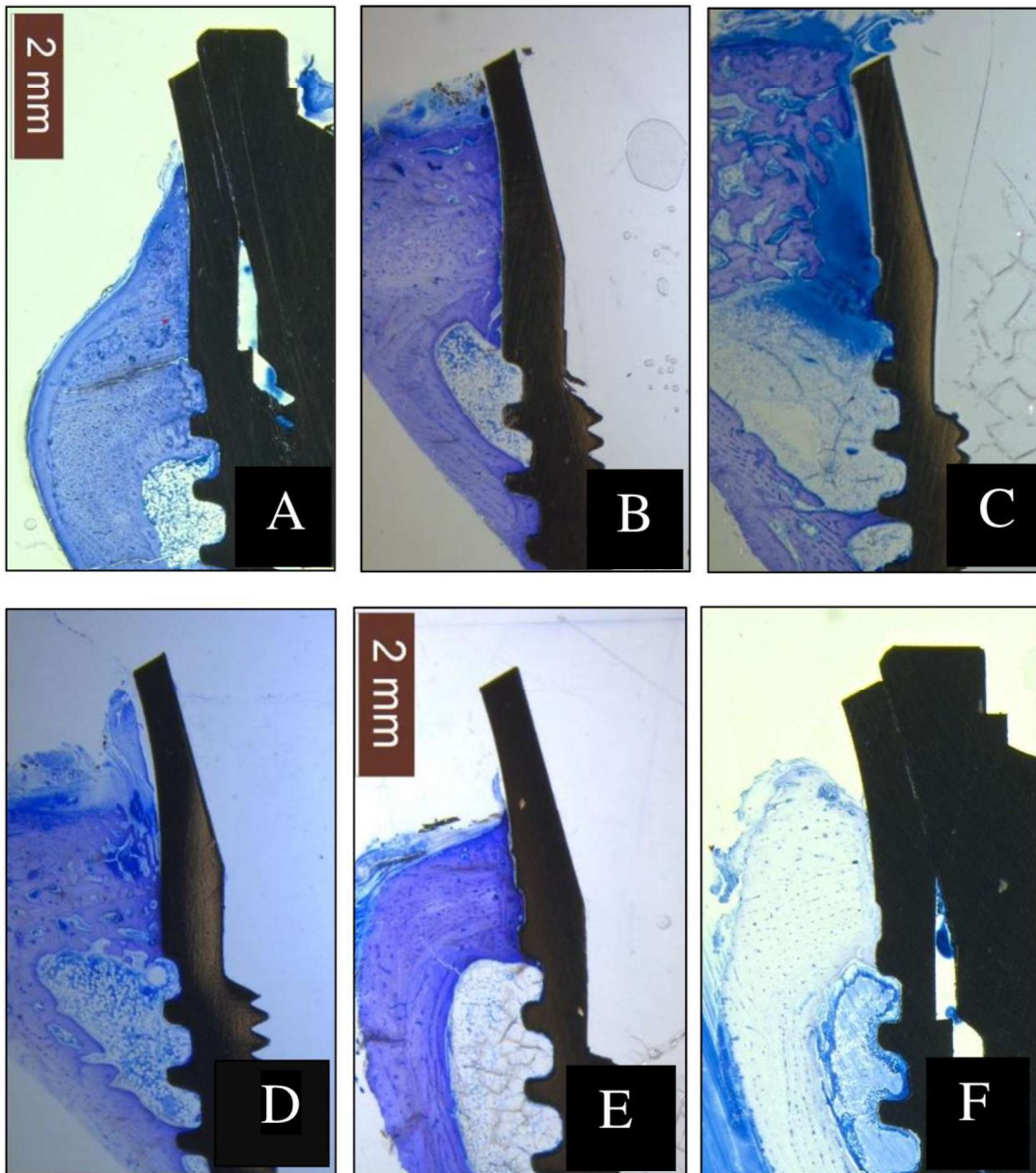


Figure 4

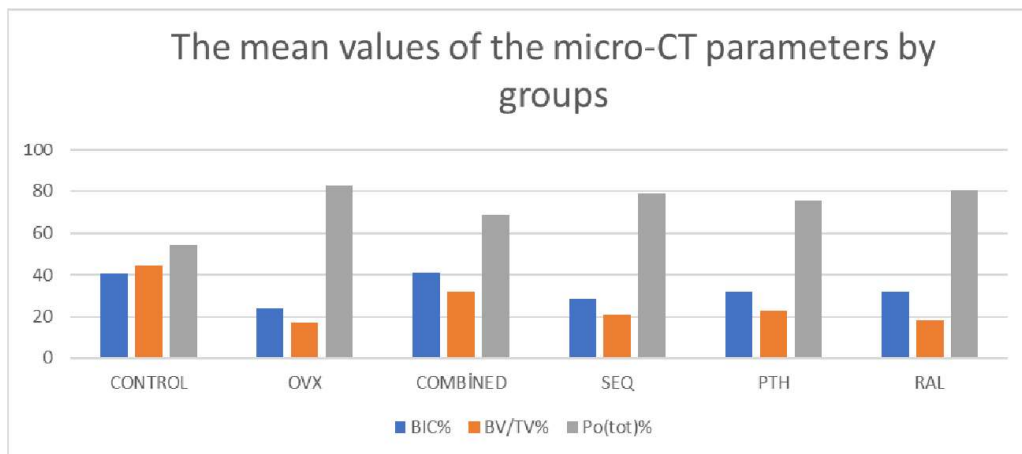
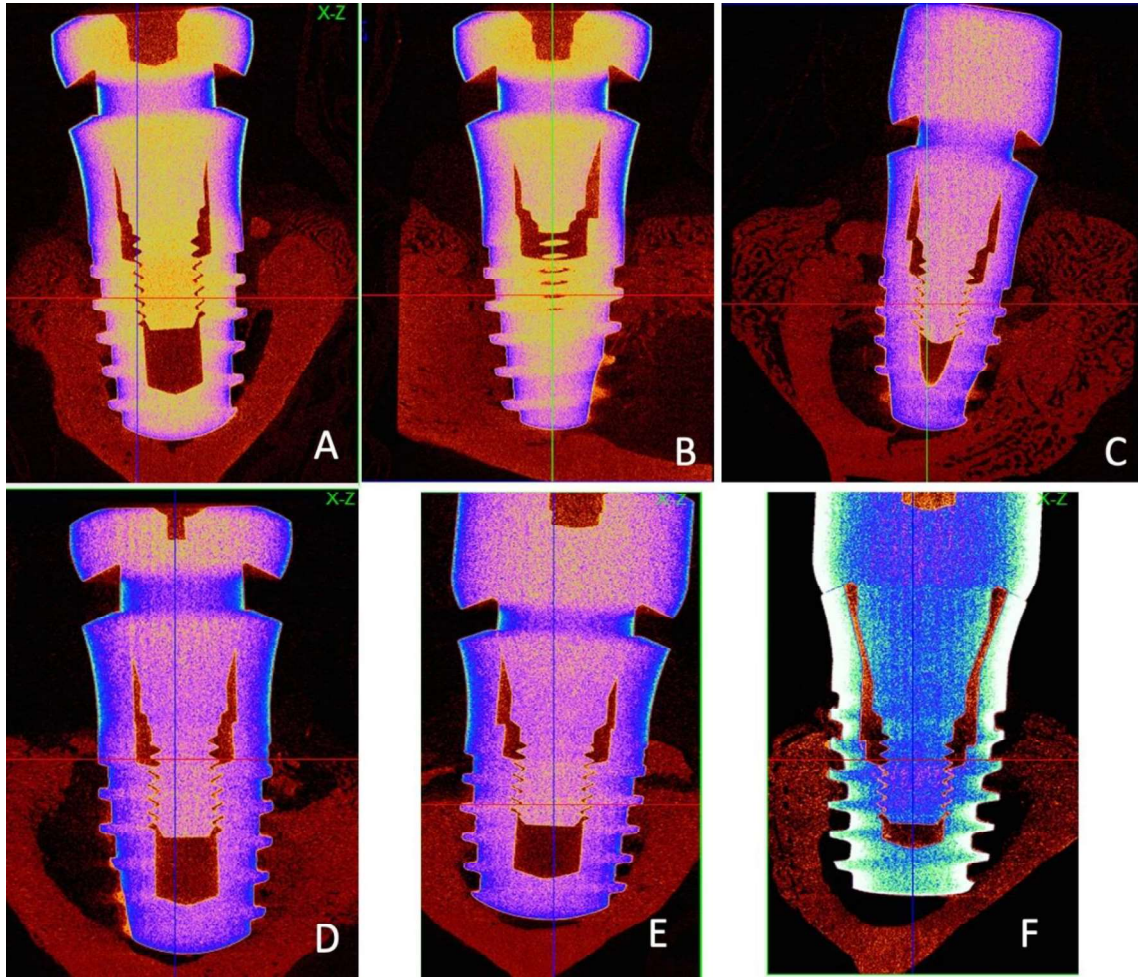


Figure 5



Tables

Table 1. The mean ISQ values at T0 (day of implantation) and T1 (three months after implantation), T1-T0 differences between groups.

Groups (n)	T0± SD	T1± SD	T1-T0 (Difference)	<i>P</i>
Control group (n=20)	67.1±3.4	73.9±4.9	6.7	<0,001*
OVX group (n=16)	61.4±3.8	68.9±6.2	7.5	<0,001*
Combined group (n=16)	62.9±2.8	76.6±3.8	13.7	<0,001*
SEQ group (n=18)	63.5±2.5	75.9±3.9	12.4	<0,001*
PTH group (n=18)	61.9±3.9	74.8±2.0	12.9	<0,001*
RAL group (n=18)	61.8±4.0	76±4.2	14.1	<0,001*

Abbreviations: n, Number of the subjects

*Statistical difference between groups

Table 2. Comparison of ISQ value differences at T0 (day of implantation) and T1 (three months after implantation) between groups

	ISQ value differences at T0	p	ISQ value differences at T1	p
Control group - OVX group	5.73*	<0,001	4.96*	0,013
Combined group - Control group	4.2	0,006	2.78	0,412
SEQ group – Control group	3.6	0,020	2.04	0,706
Control group - PTH group	5.2*	<0,001	0.9	0,982
RAL group - Control group	5.2*	<0,001	2.1	0,682
Combined group - OVX group	1.4	0,841	7.75*	<0,001
SEQ group - OVX group	2.06	0,522	7.006*	<0,001
PTH group - OVX group	0.5	0,998	5.95*	0,002
RAL group - OVX group	0.4	0,999	7.06*	<0,001
Combined group - SEQ group	0.5	0,996	0.7	0,996
Combined group - PTH group	0.9	0,967	1.7	0,839
Combined group - RAL group	1.01	0,958	0.68	0,997
SEQ group - PTH group	1.5	0,764	1.05	0,979
RAL group - SEQ group	1.6	0,736	0.05	0,999
RAL group - PTH group	0.5	0,999	1.11	0,974

Abbreviations: ISQ, Implant stability quotient

* Statistical difference between groups, $p < 0,05$

Table 3. The differences between the mean (RTQ) values of the groups

Groups	Value difference (Ncm)	p value
Control group- OVX group	24.6 Ncm	0.24
Combined group-Control group	16.7 Ncm	0.72
SEQ group-Control group	2.1 Ncm	1.00
Control group-PTH group	1.43 Ncm	1.00
RAL group-Control group	2.28 Ncm	1.00
Combined group-OVX group	43.38 Ncm	0.015*
SEQ group-OVX group	28.8 Ncm	0.19
PTH group-OVX group	25.2 Ncm	0.32
RAL group-OVX group	28.9 Ncm	0.19
Combined group-SEQ group	14.5 Ncm	0.8
Combined group-PTH group	18.1 Ncm	0.6
Combined group-RAL group	14.45 Ncm	0.8
SEQ group-PTH group	3.6 Ncm	1.00
RAL group-SEQ group	0.11 Ncm	1.00
RAL group-PTH group	3.7 Ncm	1.00

Abbreviations: RTQ, Removal torque test

* Statistically difference between groups

Table 4. The mean (BIC%) values from histomorphometry analysis and statistical evaluation of value differences between groups

	Control [Mean±SD (n)]	OVX [Mean±SD(n)]	Combined [Mean±SD (n)]	SEQ [Mean±SD(n)]	PTH [Mean±SD (n)]	RAL [Mean±SD(n)]
BIC%	48.9± 12.06 (10)	28.6± 7.7 (8)	51.2± 12.9 (8)	35.8± 9.6 (9)	42.5± 18.08 (9)	44.2± 8.4 (9)
Differences in the mean (BIC%) values						
Groups			Difference (%)	p value		
Control group-OVX group			20.3	0.01*		
Combined group-Control group			2.2	0.99		
SEQ group-Control group			13.08	0.19		
Control group-PTH group			6.44	0.85		
RAL group-Control group			4.6	0.95		
Combined group-OVX group			22.5	0.006*		
SEQ group- OVX group			7.2	0.81		
PTH group-OVX group			13.9	0.18		
RAL group-OVX group			15.6	0.09		
Combined group-SEQ group			15.3	0.11		
Combined group-PTH group			8.6	0.67		
Combined group-RAL group			6.9	0.84		
SEQ group-PTH group			6.63	0.85		
RAL group-SEQ group			8.3	0.68		
RAL group-PTH group			1.7	1		

Abbreviations: SD, Standard deviation; n, Number of subjects; BIC%, Percentage of bone-implant contact

* Statistical difference between groups

Table 5. The differences in the mean values of micro-CT parameters between groups

	BV/TV%	p	Po(tot)%	p	BIC%	p
Control group- OVX group	27.04**	<0,001	-28.3**	<0,001	16.63**	<0,001
Control group - Combined group	12.2	0,053	-14.8*	0,12	0.3	0,999
Control group – SEQ group	23.5**	<0,001	-24.7**	<0,001	12.2*	0,002
Control group - PTH group	21.5**	<0,001	-21.4**	<0,001	8.5	0,070
Control group - RAL group	25.8**	<0,001	-26.2**	<0,001	8.7	0,060
Combined group - OVX group	14.7*	0,019	-13.5*	0,041	16.9**	<0,001
SEQ group - OVX group	3.5	0,962	-3.6	0,958	4.4	0,737
PTH group - OVX group	5.4	0,793	-6.8	0,606	8.1	0,131
RAL group - OVX group	1.22	0,999	-2.1	0,996	7.9	0,149
Combined group - SEQ group	11.2	0,110	-9.8	0,215	12.5*	0,003
Combined group - PTH group	9.2	0,271	-6.6	0,637	8.85	0,078
Combined group - RAL group	13.5	0,031	-11.4	0,105	9.05	0,068
PTH group - SEQ group	1.9	0,997	3.2	0,970	3.7	0,833
SEQ group - RAL group	2.2	0,994	-1.5	0,999	3.5	0,863
PTH group - RAL group	4.2	0,907	-4.7	0,861	0.2	0,999

Abbreviations: (BV/TV%), Percent of bone volume; Po(tot)%, Percentage of total porosity; (BIC%), Percentage of bone-implant contact

* Statistical difference between groups, $p < 0.05$

** Statistical difference between groups, $p < 0.001$