Early effects of ovariectomy on bone microstructure, bone turnover markers and mechanical properties in rats

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Research Article

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Abstract

Background: Fragility fracture is one of the most serious consequences of female aging, which can increase the risk of death. Therefore, paying attention to the pathogenesis of PMOP is very important for elderly women.

Methods and materials: Forty 12-week-old female rats were divided into two groups including the ovariectomy group and the control group. Four rats in each group were selected at 1, 4, 8, 12 and 16 weeks after operation. Vertebral bodies and femurs were dissected completely for micro-CT scanning, biological modulus detection and histomorphological observation.

Results: In OVX group, BV/TV, Conn.D and Tb.N decreased significantly with time ($P<0.05$). The elastic modulus of femur in OVX group was lower than that in control group, but there was no significant difference between them ($P>0.05$). Over time, the TRAP, BGP, PINP and CTX-I in OVX group increased significantly ($P<0.05$). The micrographs of the OVX group showed sparse loss of the trabecular interconnectivity and widening intertrabecular spaces over time.

Conclusion: The bone turnover rate of OVX rats in the early stage of estrogen deficiency increased. The bone loss patterns of vertebral body and femur were different, and the changes of biomechanical properties weren't obvious.

Introduction

Mature bone requires continuous bone remodeling, which is the key mechanism to maintain normal bone mineral density(BMD). Bone formation is closely related to bone resorption and they are in a relatively balanced state in healthy individuals[1]. Postmenopausal osteoporosis (PMOP) is the most common primary osteoporosis in women. The main reason is that the sudden decline of estrogen level in the body leads to the imbalance of bone homeostasis in vivo, which leads to bone loss and even fragility fracture. Osteoporotic fragility fracture is one of the most serious consequences of female aging, which increases the risk of death in elderly women[2, 3]. Therefore, paying attention to the pathogenesis of PMOP and preventing brittle fracture as soon as possible is very important for maintaining the bone health of elderly women and reducing the social burden of aging population.

In addition to BMD, changes in bone microstructure and bone strength should also be considered when predicting the risk of fragility fracture. Micro-computed tomography(CT) can clearly describe the three-dimensional distribution and structural characteristics of bone, and accurately measure and monitor the changes of bone volume and bone microstructure[4–6]. Serum bone turnover markers (BTMS) can timely reflect the metabolic level of bone formation and bone resorption[7, 8], which is helpful to identify the high risk of fragility fracture.

At present, many scholars have discussed the characteristics of PMOP from the perspective of bone mass, bone microstructure or bone metabolic indexes. However there are few reports on the changes of

bone microstructure, bone strength and blood biochemistry over time, especially in the early stage. In this experiment, the ovariectomized rat model was used to simulate the postmenopausal state of women. We aimed to explore the early manifestations of estrogen deficiency in rats' axial bone and peripheral bone by Micro-CT combined with BTMS and mechanical properties.

Materials And Methods

Animals and experimental treatment

A total of forty female Sprague–Dawley rats (220–250g, 12 weeks) were purchased from the Beijing Weitonglihua Experimental Animal Technology Co., Ltd. They were randomly divided into two groups with 20 rats in each group. One group of rats underwent abdominal incision and bilateral ovariectomy (OVX), and then the incision was sutured. The other group was the control group which is only performed abdominal incision without ovariectomy, and then sutured the incision. The animals received ad libitum access to standard chow pellets and water throughout the experimental period.

At each time period of 1 week, 4 weeks, 8 weeks, 12 weeks and 16 weeks post-OVX surgery, four rats of each group weighted under anesthesia (3 ml of Urethane intraperitoneal injection, Shanghai Shanpu Chemical Co., Ltd), then were killed by over anesthesia to get the L4 and L5 vertebral bodies and bilateral femurs completely. And stored them in 70% ethanol solution respectively. Animal usage and all procedures were in strict compliance with the guidelines of the National Institutes of Health and approved by the Shandong First Medical University Institutional Animal Care and Use Committee. Our study is reported in accordance with ARRIVE guidelines.

Micro-CT testing

Micro-computed tomograms (micro-CTs) of the harvested left femoral and L5 vertebral bones were acquired at 55 kVp, 145 μ A for 221ms, using Scanco μ CT80 scanner (Scanco medical AG, Switzerland). All trabecular bones of the left femoral shaft and L5 vertebral bones were analyzed as the region of interest(ROI). The bone volume/total volume (BV/TV, unit %), bone trabecular connection density (Conn.D, unit $1/\text{mm}^3$), trabecular bone number (Tb.N, unit $1/\text{mm}$),trabecular bone thickness(Tb.Th, unit mm) and trabecular bone separation (Tb.Sp, unit mm) were evaluated based on the micro-CT scan results. See Figure 1.

Biomechanical evaluation

The attached tissues of the right-femur and L4 vertebral bones specimens of rats were removed. Then cut the vertebral facet joints, upper and lower endplates with serrated surgical scissors to obtain lumbar vertebra samples similar to a cylinder. The compressive stress modulus was tested by Instron testing machine. For right-femur, the samples were placed on the center of the lower pressing plate with the patella facing upward. For vertebral bone, axially placed on the center of the lower pressing plate. The

compression test was performed until the pressure load drops by 40% and recorded the elastic modulus (E, unit MPa).

Serum biochemical analysis

Five milliliter whole blood was collected from the heart in terminal anesthesia (each animal) through cardiac puncture. Centrifuged for 10 min at 3000 r.p.m. for serum separation. The tartrate resistant acid phosphatase (TRAP), type I collagen carboxy terminal peptide (CTX-I), osteocalcin (BGP), type I procollagen amino terminal propeptide (PINP), interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α) were determined by ELISA kits, using an autoanalyzer.

Histomorphometric evaluations

The harvested bones including lumbar vertebra (L5) and left-femur were dissected to get free from soft tissues. And fixed in 10% neutral buffered formalin solution, then decalcified and dehydrated. Embedded in paraffin, and finally, 5 μ m thick sections were prepared and stained with hematoxylin and eosin (H&E). The histological sections used a microscope (20X) equipped with a camera to observe the morphological structure of trabeculae bone.

Statistical analysis

All statistical analyses were performed by SPSS software, Version 22.0.

One-way ANOVA with subsequent LSD post-hoc test, independent t-test or rank sum test were used to compare the quantitative data between the groups.

Results

At each time point of the experiment, there was no significant difference or abnormality in the appearance of rats in OVX group and control group, including hair color, claw shape and tail length.

Rats body weight

Over time, the body weight of rats in both groups increased significantly ($P < 0.05$). At 4-16 weeks post-OVX surgery, the weight of rats in OVX group was significantly higher than that in control group ($P < 0.05$). The results are shown in Table 1.

Micro-CT findings

Over time, BV/TV, Conn.D and Tb.N of vertebral body in OVX group decreased significantly, while Tb.Sp increased significantly ($P < 0.05$). There was no significant difference in Tb.Th of OVX group and all indexes of the control group ($P < 0.05$). OVX group BV/TV and Tb.Th was significantly lower than that in the control group at 4-16 weeks post-OVX surgery ($P < 0.05$). At 12-16 weeks post-OVX surgery, Conn. D

and Tb.N decreased significantly compared with the control group, while Tb.Sp increased significantly ($P \leq 0.05$). The results are shown in table 2-1.

Over time, BV/TV, Conn.D and Tb.N of femur in OVX group decreased significantly, while Tb.Sp increased significantly ($P \leq 0.05$). There was no significant difference in Tb.Th of OVX group and all indexes of the control group ($P \leq 0.05$). At 4-16 weeks post-OVX surgery, BV/TV, Conn.D and Tb.N were significantly lower than that in the control group ($P \leq 0.05$), Tb.Sp was significantly higher than that in the control group ($P \leq 0.05$). There was no significant difference in Tb.Th between the groups at each time point ($P \leq 0.05$). The results are shown in table 2-2.

Biomechanical performance

Over time, there was no significant difference in vertebral elastic modulus in OVX group ($P \leq 0.05$), and the elastic modulus of femur increased significantly ($P \leq 0.05$). The elastic modulus of vertebral body in the control group increased significantly ($P \leq 0.05$), but there was no significant difference in the elastic modulus of femur ($P \leq 0.05$). In terms of biomechanical analysis of femoral, the OVX rats demonstrated lower elastic modulus when compared with the control at 1-16 weeks after operation, but there was no significant difference between the two groups ($P \leq 0.05$). The results are shown in Table 3.

Serum biochemical parameters

Over time, the levels of TRAP, CTX-I, BGP and PINP increased significantly in OVX group ($P \leq 0.05$). IL-6 and TNF- α also increased, but there was no statistical significance ($P \leq 0.05$). In the control group, only TRAP increased significantly ($P \leq 0.05$). At 16 weeks after operation, TNF- α of the OVX group was significantly higher than the control group ($P \leq 0.05$). The results are shown in Table 4.

Histopathological findings

All H&E-stained sections from two groups at different time were comparatively evaluated histologically (Fig.2). Normal compactness of the vertebral body and femoral trabeculae were found in the normal animals. The lumbar vertebral and femoral head micrographs of the OVX

group showed sparse loss of the trabecular interconnectivity and thinning of the trabeculae, resulting in widened intertrabecular spaces over time.

Discussion

With the prolongation of life span, the incidence rate of PMOP and fragility fracture is increasing year by year. According to Shang Q et al. [9] showed that fragility fractures are related to up 50% of the disability rate and 20% of the mortality rate. The medical expenses related to fragility fractures in China are expected to reach 163 billion RMB in 2050. The skeletal muscle, endocrine and digestive systems of rats are similar to those of humans in function and structure [10]. Ovariectomized rats have been proved to be one of the best models to simulate the clinical situation of postmenopausal human bone tissue. It is

confirmed by tissue morphology that the osteoporosis model can be induced 3 months after ovariectomy [11]. In the experiment of Xu H et al. [12] showed that the weight of OVX rats increased significantly compared with the sham group at 5 weeks after operation. Our results are similar with them that the weight of OVX rats increased by 38% at 4 weeks after operation. Emami AJ et al. [13] mentioned that 1-2 months old is adolescent mice, 3 months old is about 20-25 years old, and reproductive development has matured. The research of Shi J et al. [14] showed that the L5 and L6 vertebrae of 3-6 months old mice changed by only about 0.20mm, and the femoral length increased by only 0.5-0.7mm with the change of stress and gender. In other words, the bone growth of rats is almost completed after puberty, and the length of bone will not change much. 12-week-old rats were selected for ovariectomy in our experiment, which can effectively avoid the significant effect of adolescent growth hormone on bone, and estrogen was set as a single variable.

Bone is a composite biomaterial composed of 35% - 45% mineral crystals, 40% organic matrix and 15-25% water [15]. It has been reported that the bone loss of human postmenopausal axial bone is more obvious than that of peripheral bone [16]. In our experiment, the vertebral trabecular parameters BV/TV and Conn.D decreased by 26.8% and 39.1% respectively at 16 weeks after operation, while the femoral BV/TV and Conn.D decreased by 58.3% and 71.5% respectively. The degree of femoral bone loss was more obvious than that of vertebral body. Similarly, Wu ZX et al. [17] reported that the changes in microstructure and biomechanical parameters of femur 12 months after ovariectomy in sheep were also more obvious than those of spine.

Tb.N, Tb.Th and Tb.Sp are main index to evaluate the spatial morphological structure of trabecular bone [6-7]. The study [18,19] showed that lumbar bone loss is mainly caused by bone trabecular thinning after OVX, and the bone loss of tibia and femur is mainly caused by the disappearance of bone trabecula. We observed a similar phenomenon that vertebral body Tb.Sp(5.2%) of OVX rats was mainly caused by Tb.N reduction (2.9%) rather than trabecular thinning (0.0%) at 1 week after operation. It was mainly caused by thinning of bone trabecula at 4-8 weeks after operation, and was mainly related to Tb.N decline at 12-16 weeks after operation. The relationship between femoral Tb.Sp of OVX rats and Tb.N is closer than that of Tb.Th at 1-16 weeks after operation. All values were expressed as a percentage of the absolute value of 1 minus OVX group / control group. O'Sullivan LM et al. [4] found that bone mass lost rapidly after estrogen deficiency, and then reached the plateau stage, and then returned to a more balanced remodeling process. The homeostasis of bone reconstruction may be due to the compensatory thickening of bone trabecula after the 14 weeks of estrogen deficiency. In our study, the Tb.Th of vertebral body and femur of two groups did not change significantly with time and estrogen within 16 weeks after operation, which was similar to the experimental results of Luu An et al. [20], and no bone trabecular thickening was observed.

In addition to bone microstructure, bone cortex plays a decisive role in bone biomechanical ability and fracture risk [20-21]. Anumula S et al. [22] found that ovariectomy did not affect the mechanical properties of bone cortical materials. Similarly, Giavaresi G et al. [23] found no significant effect of ovariectomy on elastic modulus in 10-month-old rats. In our experiment, the elastic modulus of vertebral

body and femur of the two groups did not decrease but increased, and there was no significant difference between the groups from 1 to 16 weeks. Studies [24-25] have shown that inflammatory cytokines play an important role in bone remodeling, and inflammatory markers in serum of the elderly are positively correlated with fracture risk. The International Osteoporosis Foundation and the International Federation of Clinical Chemistry recommend that all studies should at least include serum PINP (bone formation) and CTX-I (bone resorption) as references [26]. The level of human BTMS reaches the lowest around the age of 35, and increases rapidly and significantly during menopause [27].

PMOP is characterized by a high bone turnover rate [28], which is opposite to the low bone turnover rate of senile osteoporosis. Our experiment found TRAP, CTX-I, BGP and PINP in the serum of OVX rats changed significantly over time, indicating that estrogen deficiency led to the acceleration of bone formation and bone resorption, and the increase of bone turnover rate. In addition, we also observed the level of TNF- α of OVX group was significantly higher than that of the control group at 16 weeks after operation, which can earlier reflect the microenvironment state after estrogen deficiency.

Some limitations of this study need to be mentioned.

Firstly, CTX-I is the product of type I collagen decomposition, which has a strong circadian rhythm and needs to be collected in the morning [29]. However the blood collection in this study is mainly at night, and the final result value may be affected to some extent. Secondly, although the bone changes after ovariectomy are consistent with the manifestations of osteoporosis, there is no evidence by detecting the level of estrogen in blood. Thirdly, due to the limitation of equipment, the stress of femur is not measured according to the "three-point bending" mode in most studies, but the results of elastic modulus should still have a certain reference value. Laib A et al. [18] mentioned that the proximal tibia of ovariectomized rats lost bone trabeculae rapidly within the first week after operation. And the bone quality still decreased further after one week, but the bone loss rate decreased. In this study, the bone microstructure of rats before OVX was not evaluated, and the related changes of bone within 1 week after OVX were not observed.

Conclusion

In conclusion, the bone turnover rate of OVX rats in the early stage of estrogen deficiency increased. The bone loss patterns of vertebral body and femur were different, and the changes of biomechanical properties were not obvious.

Abbreviations

BMD bone mineral density

PMOP Postmenopausal osteoporosis

BTMS bone turnover markers

OVX ovariectomy

ROI the region of interest

BV/TV bone volume/total volume

Conn.D bone trabecular connection density

Tb.N trabecular bone number

Tb.Th trabecular bone thickness

Tb.Sp trabecular bone separation

L lumbar vertebra

Declarations

Ethics approval and consent to participate: The study was approved by the Institutional Animal Care and Use Committee of Shandong First Medical University and carried out in accordance with the guidelines for the use of laboratory animals of the National Institutes of Health. This study was reported in accordance with ARRIVE guidelines.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions:

Xingman Guo: Writing-Original draft preparation, Methodology, Data curation

Xiyue Yu: Visualization, Software

Qianqian Yao: Formal analysis, Visualization

Jian Qin: Supervision, Resources, Writing- Reviewing and Editing

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Tables

Table 1. Weight (g) results of two groups in different periods

	Time	OVX Group n=4	Control Group n=4	P Value
Weight(g)	1 week	273.33±15.27	279.67±4.50	0.742
	4 weeks	384.50±26.76	317.00±25.91	0.001
	8 weeks	409.50±15.58	332.75±8.65	0.001
	12 weeks	446.75±22.44	353.50±35.40	0.001
	16 weeks	472.00±29.41	361.50±24.40	0.001
	P Value	0.001	0.004	

Data are presented as mean±SD. The body weight of rats in both groups increased significantly ($P \leq 0.05$).

Table 2-1. Results and comparison of micro-CT microstructure indexes of vertebral bodies between the two groups

	Time	OVX Group n=4	Control Group n=4	P Value
BV/TV %	1 week	0.41±0.03	0.42±0.08	0.628
	4 weeks	0.34±0.04	0.46±0.03	0.001
	8 weeks	0.34±0.03	0.44±0.05	0.005
	12 weeks	0.30±0.03	0.45±0.03	0.001
	16 weeks	0.30±0.02	0.47±0.04	0.001
	P Value	0.003	0.832	
Conn.D 1/mm ³	1 week	110.90±18.05	115.99±16.36	0.625
	4 weeks	90.63±12.66	90.87±22.21	0.982
	8 weeks	96.99±9.13	84.43±13.40	0.232
	12 weeks	73.71±5.41	100.08±15.51	0.016
	16 weeks	67.52±8.99	97.93±16.27	0.006
	P Value	0.001	0.155	
Tb.N 1/mm	1 week	4.55±0.38	4.69±0.36	0.586
	4 weeks	4.23±0.18	4.64±0.61	0.117
	8 weeks	4.39±0.27	4.78±0.46	0.138
	12 weeks	3.88±0.42	5.02±0.19	0.001
	16 weeks	3.69±0.20	5.09±0.29	0.001
	P Value	0.008	0.470	
Tb.Th mm	1 week	0.08±0.00	0.08±0.01	0.452
	4 weeks	0.08±0.00	0.09±0.00	0.006
	8 weeks	0.07±0.00	0.08±0.00	0.019
	12 weeks	0.07±0.00	0.08±0.00	0.032
	16 weeks	0.07±0.00	0.08±0.00	0.017
	P Value	0.281	0.687	
Tb.Sp mm	1 week	0.20±0.02	0.19±0.02	0.826
	4 weeks	0.21±0.01	0.19±0.03	0.204
	8 weeks	0.20±0.01	0.18±0.01	0.251
	12 weeks	0.23±0.03	0.17±0.00	0.001

16 weeks	0.25±0.01	0.17±0.01	∅0.001
<i>P</i> Value	0.018	0.339	

Table 2-2. Results and comparison of micro-CT microstructure indexes of femur between the two groups

	Time	OVX Group n=4	Control Group n=4	P Value
BV/TV %	1 week	0.12±0.03	0.12±0.05	0.738
	4 weeks	0.07±0.02	0.16±0.04	0.001
	8 weeks	0.06±0.01	0.18±0.03	0.001
	12 weeks	0.05±0.01	0.13±0.05	0.001
	16 weeks	0.05±0.01	0.13±0.01	0.006
	P Value	0.002	0.206	
Conn. D 1/mm ³	1 week	49.36±16.36	47.14±24.78	0.842
	4 weeks	25.17±8.45	76.88±21.43	0.001
	8 weeks	23.13±7.29	63.82±2.21	0.001
	12 weeks	14.29±6.86	51.47±29.70	0.002
	16 weeks	14.04±1.88	48.01±4.16	0.004
	P Value	0.001	0.216	
Tb.N 1/mm	1 week	2.74±0.39	2.36±0.43	0.176
	4 weeks	1.28±0.19	3.29±0.51	0.001
	8 weeks	0.82±0.21	3.09±0.21	0.001
	12 weeks	0.96±0.24	2.56±0.78	0.001
	16 weeks	1.09±0.17	2.59±0.22	0.001
	P Value	0.001	0.074	
Tb.Th mm	1 week	0.06±0.00	0.07±0.00	0.899
	4 weeks	0.06±0.01	0.06±0.00	0.083
	8 weeks	0.07±0.00	0.07±0.00	0.564
	12 weeks	0.07±0.00	0.07±0.00	0.831
	16 weeks	0.07±0.00	0.06±0.00	0.528
	P Value	0.313	0.312	
Tb.Sp mm	1 week	0.36±0.06	0.44±0.09	0.492
	4 weeks	0.81±0.12	0.30±0.05	0.001
	8 weeks	1.31±0.34	0.32±0.03	0.001
	12 weeks	1.10±0.25	0.41±0.12	0.001

16 weeks	0.97±0.16	0.39±0.03	0.001
<i>P</i> Value	0.001	0.100	

Data are presented as mean±SD. BV/TV-the bone volume/total volume; Conn.D-bone trabecular connection density; Tb.N-trabecular bone number; Tb.Th-trabecular bone thickness; Tb.Sp-trabecular bone separation.

Table 3. Results and comparison of elastic modulus (MPa) of vertebral body and femur of two groups of rats

	Time	OVX Group n=4	Control Group n=4	<i>P</i> Value
elastic modulus (MPa) of vertebral body	1 week	6.60±0.77	5.78±2.85	0.769
	4 weeks	9.88±4.92	10.04±1.86	0.954
	8 weeks	10.17±1.97	10.06±1.62	0.968
	12 weeks	11.11±7.17	13.19±3.52	0.454
	16 weeks	12.12±5.59	12.78±3.60	0.812
	<i>P</i> Value	0.557	0.015	
elastic modulus (MPa) of femur	1 week	5.22±0.89	8.47±1.25	0.286
	4 weeks	14.72±1.75	17.00±6.04	0.416
	8 weeks	12.72±1.79	14.72±3.44	0.473
	12 weeks	14.93±1.94	16.79±2.22	0.507
	16 weeks	16.39±4.52	19.30±7.87	0.301
	<i>P</i> Value	0.001	0.116	

Data are presented as mean±SD.

Table 4. Results and comparison of BTMS and inflammatory markers between the two groups

	Time	OVX Group (n=4)	Control Group (n=4)	P Value
TRAP	1 week	3.70±0.53	3.37±0.24	0.702
	4 weeks	2.99±0.42	3.45±0.67	0.491
	8 weeks	2.86±1.56	3.62±1.48	0.266
	12 weeks	1.87±0.32	2.83±1.02	0.158
	16 weeks	6.52±0.49	6.89±0.97	0.581
	P Value	0.001	0.001	
CTX-I	1 week	0.53±0.14	0.72±0.26	0.351
	4 weeks	0.56±0.14	0.50±0.17	0.744
	8 weeks	0.39±0.25	0.58±0.36	0.243
	12 weeks	0.39±0.08	0.45±0.20	0.721
	16 weeks	0.95±0.26	0.76±0.23	0.237
	P Value	0.011	0.441	
BGP	1 week	12.99±1.71	12.97±2.24	0.996
	4 weeks	11.01±0.50	10.36±2.04	0.786
	8 weeks	6.20±3.16	10.03±3.93	0.119
	12 weeks	7.50±1.56	10.76±6.96	0.181
	16 weeks	12.19±2.94	11.39±2.89	0.738
	P Value	0.007	0.939	
PINP	1 week	11.90±1.15	13.57±5.12	0.554
	4 weeks	9.64±1.30	8.22±2.57	0.518
	8 weeks	6.11±3.66	8.84±5.02	0.218
	12 weeks	6.96±1.84	8.89±3.40	0.379
	16 weeks	14.88±2.01	13.35±2.92	0.488
	P Value	0.001	0.222	
IL-6	1 week	12.79±1.08	13.66±4.30	0.881
	4 weeks	11.93±1.76	12.76±1.27	0.853
	8 weeks	11.65±1.30	10.86±2.90	0.863
	12 weeks	13.21±2.37	13.35±3.57	0.977

	16 weeks	23.17±17.06	14.35±5.39	0.059
	<i>P</i> Value	0.284	0.734	
TNF- α	1 week	24.61±3.57	28.02±7.60	0.725
	4 weeks	25.72±3.65	24.76±2.83	0.898
	8 weeks	24.17±2.47	22.61±5.92	0.835
	12 weeks	25.42±2.54	24.67±5.63	0.920
	16 weeks	45.59±28.06	28.41±8.60	0.011
	<i>P</i> Value	0.172	0.704	

Data are presented as mean±SD. TRAP-the tartrate resistant acid phosphatase; CTX-I-type I collagen carboxy terminal peptide; BGP-osteocalcin; PINP-type I procollagen amino terminal protopeptide; IL-6-interleukin-6; TNF- α -tumor necrosis factor α .

Figures

Figure 1

Figure A-D shows the micro-CT cross-sectional imaging of L5 vertebral body (Fig. A and B) and left femoral shaft (Fig. C and D) in OVX group respectively. Analyzed the bone trabecular indexes in the region

of interest(green circle). Figures A and C show 1 week after operation, and figures B and D show 16 weeks after operation.

Figure 2

L5 vertebral body (Fig. A and B) and left femoral head (Fig. C and D) of OVX group rats were histologically evaluated at 1 week (Fig. A and C) and 16 weeks (Fig. B and D). The micrographs of OVX rats showed a typical osteopenia with loss of trabecular bone numbers and interconnectivity with the passage of time (arrow heads). H&E staining.