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The variation of cancellous bones at lumbar vertebra, femoral neck, mandibular angle and rib in ovariectomized sheep

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ABSTRACT

This study aimed to compare the variation of cancellous bones at four skeletal sites: lumbar vertebra, femoral neck, mandibular angle and rib in ovariectomized sheep. Sixteen adult sheep were randomly divided into two groups: eight sheep were ovariectomized served as experimental group; the other eight untreated sheep were served as control group. Bone mineral density was assessed by dual-energy X-ray absorptiometry on lumbar vertebrae at baseline and twelve months after ovariectomy. After 12 months, lumbar vertebrae L3 and L4, femoral necks, mandibular angles and the fourth ribs were harvested for micro-CT scanning, histological analysis and biomechanical test. The results showed that bone mineral density of lumbar vertebra decreased significantly in twelfth month ($p < 0.05$). The results of micro-CT showed that the bone volume/total volume decreased by 45.6%, 36.1% 21.3% and 18.7% in lumbar vertebrae, femoral necks, mandibular angles and ribs in experimental group ($p < 0.05$) respectively. The trabecular number showed the same downtrend ($p < 0.05$). Histological analysis showed trabecular area/tissue area decreased by 32.1%, 23.2% and 20.7% in lumbar vertebrae, femoral necks and mandibular angles respectively ($p < 0.05$), but no significant difference in ribs. Specimens elastic modulus from lumbar vertebra, femoral neck and mandibular angle were 952 ± 76 MPa (628 ± 70 MPa), 961 ± 173 MPa (610 ± 72 MPa) and 595 ± 60 MPa (444 ± 31 MPa) in control group (experimental group) respectively. These datum indicated that the sensibility of cancellous bones to oestrogen deficiency in ovariectomized sheep was site-specific on a pattern as follows: lumbar vertebra, femoral neck, mandibular angle and rib.

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1. Introduction

Osteoporosis (OP) is a skeletal disorder which characterized by low bone mass and microarchitectural deterioration of bone tissue.[1] The main consequence of OP is disability and high healthcare cost resulting from fractures of lumbar vertebra, hip or rib fractures etc. It is estimated that more than 75 million people worldwide are affected by OP.[2] Because a previous fracture in addition to low bone mass will significantly increase the risk of developing a future fracture,[3] it is of great importance to early detect and evaluate the risk of postmenopausal OP to prevent the first fracture.

American endocrinologist Fuller Albright first reported the relation between systemic OP and postmenopausal women in 1940,[4] Oestrogen deficiency due to menopause or ovariectomy is the main cause of OP in postmenopausal women. However, bone health in human is also affected by other risk factors including age, height, gender, race, vitamin D or calcium intake, exercise, family history, skeletal sites and certain chronic medications.[5] It is difficult to balance these risk factors in human OP study, thus many ovariectomized (OVX) animal models have been used in OP study such as mice, rats, rabbits etc. Recently, there is an increasing interest in large animal models, one of which is sheep. Sheep have cortical and trabecular remodelling cycles, skeletal turnover kinetics similar to humans, spontaneously ovulate and their body size and weight are comparable to human beings.[6–8]

A study by Wu et al.[7] measured the variations of lumbar vertebrae and femurs in OVX sheep, and they demonstrated that OVX after 12 months or longer was necessary to induce a substantial reduction of bone density and mechanical competence in sheep. Veigel[8] also found significant microstructural changes of lumbar vertebra and mandible in OVX sheep, and they suggested these changes have a generalized nature. Besides external factors (season fluctuations,[9] diet[10]) that affect the variations of bones in OP, internal factors such as evolutionary origin,[10,11] osteogenesis,[12] histological characteristics[13] and mechanical environment[14] also play an important role in bone properties. These internal factors all relate to the difference of skeletal site. To clarify the relation of bone loss at different skeletal sites in postmenopausal OP has great significance to predict and evaluate the risk of fracture, especially for secondary fracture and concomitant fracture etc.

In this study, we utilized OVX sheep model to investigate the variation of cancellous bone parameters at four typical skeletal sites: lumbar vertebra, femoral neck, mandibular angle and rib by micro-CT scanning, histological analysis and biomechanical test, so as to evaluate the feasibility of these four skeletal sites as predictions of postmenopausal OP.

2. Materials and methods

2.1. Animal model

The animal protocol was approved by the Animal Care and Use Committee at the Fourth Military Medical University. All the experimental procedures were in strict accordance with the ethics guidelines established by the Fourth Military Medical University. Sixteen sheep (age, 4 ± 0.5 years old; mean weight, 45 ± 4.0 kg) were divided into two groups: eight sheep underwent OVX and served as experimental group (EG); the other eight untreated sheep were served as control group (CG). All sheep were housed indoor under identical conditions and sacrificed after 12 months. Lumbar vertebrae L3 and L4, bilateral mandibular angles, bilateral femoral necks and bilateral fourth ribs of each sheep were harvested. For each sheep, lumbar vertebra L3 and L4, as well as bilateral mandibular angles, bilateral femoral necks and bilateral fourth ribs, were randomly assigned to micro-CT analysis and biomechanical compression test respectively. The specimens evaluated by micro-CT were also used for histological analysis (Table 1).

2.2. BMD

Bone mineral density was measured using a dual energy X-ray absorption metre (Lunar Corp., Madison, WI, USA) on all the lumbar vertebra specimens at baseline (pre-operation) and in 12th month after OVX under general anaesthesia.

2.3. Micro-computed tomography

The specimens were scanned using micro-CT (Inveon, Siemens, Germany. 80 kV, 500 μA, 800 ms integration time). The two dimensional (2D) images were reconstructed for quantitative evaluation. The voxel size was 47 μm. The regions of interest (ROI) of trabecular bone in each specimen were selected, and bone volume/total volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) were evaluated. The ROI of lumbar vertebra L3 and L4 were cube-shaped as 8 mm × 8 mm × 5 mm in the

Table 1 – The number of specimens for micro-CT/histological analysis and biomechanical compression test.

	BMD	Micro-CT/Histological analysis	Biomechanical compression test
Lumbar vertebra	EG (16) + CG (16)	EG (8) + CG (8)	EG (8) + CG (8)
Femoral neck	–	EG (8) + CG (8)	EG (8) + CG (8)
Mandibular angle	–	EG (8) + CG (8)	EG (8) + CG (8)
Rib	–	EG (8) + CG (8)	–

The symbol"–"means no specimen was selected. There are 16 lumbar vertebra, 16 femoral neck, 16 mandibular angle and 16 rib specimens in EG and CG respectively.