Rat age at ovariectomy and duration of estrogen deficiency influence knee cartilage histology
Yu, Y; Francisco, J I; Oliver, R A; +Walsh, W R
Surgical and Orthopaedic Research Laboratories, University of New South Wales, Sydney. Australia
w.walsh@unsw.edu.au

INTRODUCTION
An increased prevalence of postmenopausal osteoarthritis (OA) has stimulated a great interest in the relationship between osteoporosis and osteoarthritis. Animal models of ovariectomy have been used to assess the estrogen deficiency on joint tissues and the effects of estrogen treatment on cartilage. A relationship between osteoarthritis and loss of ovarian function has been reported [1-3]. The effect of estrogen treatment on OA in humans and animal models remains inconclusive.

This study investigated the effect of age at time of ovariectomy and duration of estrogen deficiency on knee cartilage histology in rats. We hypothesized that age as well duration of OVX rats would influence the articular cartilage of the knee.

METHODS
Eighty-four virgin female Wistar rats in three age groups: 12, 24 and 44 weeks (n=28) were used. Half of the rats in each group received bilateral ovariectomy (OVX) or sham operation (Sham). Two rats from each test group were sacrificed at 2, 5, 10, 15, 20, 25, 30 weeks post-surgery.

Animals were weighed weekly and the ovariectomy confirmed at necropsy by uterine atrophy. Bone mineral density (BMD) of the skeleton was measured using Dual-Energy X-ray Absorptiometry (DEXA). The micro-computed tomography was performed using a Skyscan 1072 micro-computed tomography (micro-CT) system and CTAn software.

The knee joints were processed for routine paraffin histology and haematoxylin and eosin (H&E) and Safranin O staining. The sections were analyzed under microscope with normal and polarized light. The articular cartilage at the distal femur and the proximal tibia were evaluated using the OARSI cartilage OA histopathology grading system [4] in a blinded fashion.

RESULTS
No cartilage changes consistent with OA were found out to 30 weeks following ovariectomy in the animals that were 12 weeks at the time of the procedure (Figure 1a). In contrast, cartilage in the animals that were 24 weeks old at the time ovariectomy began to degrade at 20 weeks post-OVX (Figure 1b) and continued to progress at 30 weeks (Figure 1c). The cartilage in the Sham operated rats in the 12 and 24 week age groups remained normal throughout the study (Figure 1d). In contrast, the Sham and OVX animals in the 44 group demonstrated severe cartilage degradation as early as 2 weeks following surgery (Figure 2a and 2b). Changes in both the cartilage as well as bone were noted in this group.

The severity of cartilage degradation in different aged OVX rats demonstrated in this study correlated well with the osteoporotic bone structures detected by DEXA and micro-CT [5].

DISCUSSION
Our studies demonstrated a strong correlation of osteoporosis and osteoarthritis induced by OVX in rats. Rat age at OVX plays an important role for model standardization. Twelve week old rats were too young for OVX model to study both osteoporosis and osteoarthritis. Forty-four week old rats were too old due to the postmenopausal osteoarthritis features presented in the Sham group. Twenty-four week old rats were suitable as OVX induced osteoarthritis and osteoporosis models however a length of 20 weeks post-OVX is required to show the cartilage degradation as well the osteoporotic bone micro-architecture. This model is useful to investigate the molecular pathways of postmenopausal osteoarthritis and to select specific targeted therapies.

REFERENCES