INTRODUCTION:
The ovariectomised (OVX) rat is currently the most popular animal model used for studies on estrogen deficiency-induced or postmenopausal osteoporosis. However, an issue with the OVX rat model is that there is no standard model. Throughout the literature, investigators have used animals that were ovariectomised at different ages, started their observations, treatments, etc. at various time points post-OVX, as well as examining different skeletal sites. Such factors are important to consider as they may affect the temporal pattern of bone loss, and consequently the interpretation of results for a given study. The purpose of this study was to examine the temporal pattern of bone loss in the OVX rat with respect to the age of the animal at OVX and the skeletal site being observed, in order to facilitate better study design (e.g. when to administer treatment), interpretation of data, and comparison of results between investigators, as well to further understand the long-term effects on estrogen-deficiency on bone.

METHODS:
With ethical approval, a total of 64 virgin female Wistar rats were used in this study. There were two age groups, 12 weeks and 24 weeks (n=32). Half of the rats in each age group (n=16) received bilateral ovariectomy (OVX) and the other half received sham operation (Sham). Two rats from each group were sacrificed at 2, 5, 10, 15, 20, 25, 30, and 35 weeks post-surgery, and their femurs, tibias, and spines harvested. End points for this study were plain X-rays, DEXA, microCT, and histology. To date only X-ray and microCT data are up to date. Bones were fixed in formalin for at least 48hrs prior to being scanned and their bone microarchitecture analysed using the Skyscan 1072 machine and CTAn software (SkyScan, Belgium). The bone parameters measured included the percent bone volume (BV/TV, %), bone surface to bone volume ratio (BS/BV, mm$^{-1}$), trabecular pattern factor ( Tb.Pf, mm$^{-1}$), trabecular thickness ( Tb.Th, mm), trabecular number (Tb.N, mm$^{-1}$), trabecular separation (Tb.Sp, mm), and structure model index (SMI). The sites examined in this study were the proximal femur (femoral head and neck), distal femur (below the growth plate), proximal tibia metaphysis (distally from 1mm below the growth plate, and above the growth plate), and the spine (L3 and L4 vertebrae). To date only the data for the proximal tibia metaphysis and spine are available up to 15 weeks post-surgery for the rats that underwent surgery at 24 weeks old, and up to 25 weeks post-surgery for the rats that underwent surgery at 12 weeks old.

Preliminary examination of microCT data showed BV/TV, Tb.N, and Tb.Sp, to be of most interest and was focused on for statistical analyses using GraphPad Prism version 5.00 for Windows (GraphPad Software, California USA). One-way ANOVA (p < 0.05) with Tukey post-test were performed to determine the effect of time post-surgery for each group (sham vs. OVX), at each age (surgery at 12 weeks vs. 24 weeks old), for each skeletal site (spine vs. tibia). Two-way ANOVA (p < 0.05) with Bonferroni adjustment were also performed to determine: 1) the effect of OVX over time at each skeletal site, for each age group, and 2) the effect of age at OVX on bone loss due to estrogen-deficiency.

RESULTS:
Body weights of OVX rats were higher than sham rats at both ages from about 3 weeks post-surgery confirming successful OVX. At about 20-25 weeks post-surgery a reversal of this trend was observed in the rats that underwent surgery at 12 weeks old.

MicroCT analyses of the proximal tibia metaphysis showed decreases in Tb.N and increases in Tb.Sp over time in rats OVX at 24 weeks old (OVX24), which were significantly different to their sham counterparts (Sham24) (Figure 1a). Rats OVX at 12 weeks old (OVX12) also showed similar decreases in Tb.N and increases in Tb.Sp over time, as well as significant differences to their sham counterparts (Sham12), but with larger individual variations (Figure 1b). At this site, BV/TV of both OVX groups were significantly lower than their sham counterparts (Figure 2) at each time point from 5 weeks post-surgery.

At the spine, decreases in Tb.N and increases in Tb.Sp were also found in OVX12 and OVX24 groups. However, the differences between them and their sham counterparts only became significant at later time points compared to the tibia. At the spine, BV/TV of both OVX groups only became significantly lower than their sham counterparts from 10 weeks post-surgery. The decrease in BV/TV found in spines and tibias of OVX12 and OVX24 rats over time only became significant at later time points.

DISCUSSION:
Estrogen-deficiency due to menopause is a long-term process in which its onset and duration influences the temporal pattern of bone loss. The OVX rat is the most commonly used animal model of postmenopausal osteoporosis as it has been shown to represent the most important clinical features of estrogen deficiency-induced bone loss in humans whilst offering certain advantages compared to other animal models. Even with a relatively small sample size the results from this study showed that the age at OVX (onset of estrogen-deficiency), length of time post-OVX (duration of estrogen deficiency), and skeletal site being observed plays a significant role in the resulting pattern of bone loss. Based on our current results it appears that: 1) Young rats may only be used up to about 25 weeks post-OVX as this when their differences to the sham group become reduced, making the effect of treatments more difficult to detect. 2) Compared to the spine, the proximal tibia metaphysis is more sensitive to the effects of estrogen-deficiency making it a more suitable site to observe the effects of treatments. 3) Regardless of age at OVX, treatments should not be administered until at least around 5 weeks post-OVX as this is when the differences between the OVX and sham groups first become significant. 4) The interaction between the age at OVX, time post-OVX, and skeletal site must be carefully considered when designing and consequently interpreting, and comparing studies using the OVX rat.