INTRODUCTION:
Muscle-derived stem cells (MDSCs) have been shown to heal critical size bone defects when retrovirally transduced to produce bone morphogenetic protein 4 (BMP4). The model used in this study is the critical size calvarial defect, and its size is defined by the inability to heal without intervention during the lifetime of the animal. Murine MDSCs are known to show sexual dimorphism in osteogenic differentiation, with MDSCs isolated from males being more osteogenic than those from females. Additionally, previous work by our group has shown distinct host sex differences in MDSC-mediated bone formation in an ectopic bone model, where male hosts demonstrate more bone formation at a faster rate than female hosts; however, ovariectomy and castration appeared to have no effect on the ability of the host animal to form bone. The object of this study is to examine the role of host sex hormones in an MDSC-mediated bone healing model. Results from this study could provide clinical insight into aging and bone healing as castration and ovariectomy are often used as models of hormonal changes with age.

METHODS:
MDSCs were isolated from three week old male C57BL/10J mice using a modified preplate technique and cultured in proliferation medium (DMEM, 10% fetal bovine serum, 10% horse serum, 5% chick embryo extract, and 1% penicillin streptomycin). Cells were then transduced with a CLB2/4G retroviral vector to express human bone morphogenetic protein 4. All experiments were carried out on mature C57BL/10J mice, with n=8-10 animals per group. 4 groups were used: unaltered female, unaltered male, ovariectomized female (ovx) and castrated male (cast). Castration and ovariectomy were performed prior to acquisition from animal vendor.

To create the critical size cranial defect, the scalp was dissected and 5 mm trephine was used to create the circular bone defect. 100,000 transduced MDSCs were then applied in ~40uL of fibrin. The wound was then closed, and all animals were allowed to recover. At 1, 14 and 28 days post surgery, all groups were evaluated with a microCT scanner (VivaCT40, Scanco, Switzerland). All animal experiments were conducted with the approval of the IACUC of University of Pittsburgh.

Area of defect was computed with Northern Eclipse by analyzing 3D reconstructions computed by the Scanco software, and efficiency was calculated using volumetric measurements (total and within defect) from Scanco software (efficiency = within defect volume/ total new bone volume). All statistical analysis (ANOVA or repeated measures ANOVA) was performed with SPSS, and differences were considered significant if p<.05.

RESULTS:
Figure 1: Representative microCT scans of defect at day 1 and different host groups at 14 days. Less bone is observed forming in the female and ovx groups. At 28 days, all defects were healed and therefore all groups appeared visually similar.

Figure 2: Area of defect at 14d (mm$^3$). Ovx is significantly larger than cast (*) indicating less healing in this group. Female and male appear to show a trend of more healing (smaller area) than ovx also.

Figure 3: Total volume of new bone formed (mm$^3$) at 14 and 28d. Symbols indicate significant differences: * from same group 14d, § from cast 14d, # from ovx 28d, § from male 28d. The total amount of bone formed in ovx group at 28 days is significantly less than both male and cast groups with a trend of less total bone than female group.

Figure 4: New bone within defect site (mm$^3$) at 14 and 28d. Symbols indicate significant differences: * from same group 14d, § from cast 28d. Cast mice show the most bone formed within defect.

Figure 5: Efficiency of bone formation at 14 and 28d. Symbols indicate significant differences: * from same group 14d, § from cast 14d, # from ovx 28d. Efficiency of 1 denotes that all bone formed was within the defect area, and a smaller number represents more bone was formed outside the defect than within it. Efficiency of bone formed was significantly higher in female and ovx groups than cast mice at 14 days. An overall trend of female and ovx having higher efficiency than male and cast groups is also observed.

DISCUSSION:
The purpose of this study was to elucidate the effects of host animal sex and sex hormones on MDSC-mediated bone defect healing. In a critical size cranial defect model, differences were in fact found in healing between females, males, ovx and cast. As in previous ectopic bone formation studies, a general trend of more bone in male mice (unaltered or cast) than in female mice (unaltered or ovx) was observed. However, there were also differences in groups of the same sex (male/cast and female/ovx), indicating a divergence from ectopic formation studies and also that MDSCs behave differently in a bone formation versus a healing model. Conclusions of less bone in ovx mice could be extended to clinical models of bone healing and defect treatment with MDSCs. Older, female patients may need a different intervention compared to a young, male patient with the same bone defect. Future studies will investigate methods of overcoming these differences.