AIM: Mesenchymal stem cells (MSCs) are pluripotent cells present in marrow, which have the ability to differentiate into osteoblasts, chondrocytes and adipocytes. Potential skeletal tissue engineering uses include healing bone defects, spinal fusion and revision arthroplasty surgery. A means of storing viable Mesenchymal stem cells is necessary in order for these cells to be readily available for clinical use. The aim of this study was to determine whether cryopreservation has any effect on the osteogenic potential of human bone marrow derived MSCs.

METHOD: Ten normal iliac crest bone marrow aspirates were obtained following informed consent from patients. Each aspirate was divided into two equal samples. Ficoll-separation was used to isolate the MSCs. The fresh MSCs from one sample were cryopreserved (cryopreserved populations), while the other was cultured as a control population. To assess the osteogenic potential of the MSCs after cryopreservation $2 \times 10^5$ MSCs from each aspirates’ population was cultured with osteogenic supplements and the increased level of alkaline phosphatase (ALP) protein production when the MSCs were cultured with osteogenic supplements was measured after 7 & 14 days. Osteopontin is another protein known to be produced by osteoblasts, so the amount of this protein was also measured after 14 days for both MSCs cultured in standard media and compared with the control. The difference between MSCs that had been cryopreserved compared with the control was compared between the samples that had been cultured in standard or osteogenic medium.

The effect of cryopreservation on the proliferation rate of MSCs in culture was compared with the control, after days 1, 5 & 15 days, after passage 1, 2 & 4. Proliferation rate was measured using alamar blue assay on 8 samples of cells in culture from each of eight aspirates.

RESULTS and DISCUSSION: Cryopreservation was not observed to affect the primary cultures of MSCs, which became confluent after a similar period in culture (12-14 days), forming colonies with recognized MSCs morphology. The expression of ALP and osteocalcin, after stimulating the MSCs to differentiate with osteogenic supplements, was not significantly reduced by the cryopreservation process ($P<0.2$), see figure 1 & 2.

The proliferation rate was significantly different after day 1 for MSCs that had been cryopreserved compared with the control, see figure 3.

CONCLUSION: MSCs obtained from fresh human bone marrow aspirates can be cryopreserved without compromise to their proliferation rate or osteogenic potential, confirming that this is a useful means of storing viable cells for future clinical use.