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## Effects of culture conditions on cytosine methylation and MeCP2 binding in preimplantation mouse embryos

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Imprinting is an epigenetic modification in which one gene of a pair of alleles is silenced in a parent-specific way. Imprinting is commonly controlled by methylation of the C5 position of cytosines which are located next to guanines in DNA sequences. CpG methylation usually confers a silencing signal to genes. Methyl-CpG Binding proteins, (e.g. MeCP2), have also been shown to play a role in silencing genes when bound to methylated DNA regions. Oxidative stress has been shown to form 8-hydroxyguanine lesions on guanine bases which may hinder MeCP2 binding activity, and therefore hinder gene silencing. It has been shown that culture of preimplantation mouse embryos can cause gene misexpression, including loss of imprinting. The purpose of this study was to use immunofluorescence and confocal microscopy to examine whether culturing preimplantation mouse embryos in different culture conditions causes alterations in DNA methylation, binding of the MeCP2 methyl-CpG binding proteins, and the formation of 8-oxoguanine lesions on DNA when compared to in-vivo produced mouse embryos. Preimplantation mouse embryos were subjected to four different culture conditions in a 2X2 factorial design using 2 levels of oxygen tension (5% and 20%) and 2 different media (KSOM + amino acids, and Whitten's medium). Cultured embryos will be compared to two different in-vivo produced control groups; a) super-ovulated and b) not super-ovulated. The super-ovulated in-vivo group embryos were collected from females 96h post hCG. The culture groups embryos were collected at the two-cell stage approximately 44 hours post hCG. Two-cell embryos were then divided in four groups and cultured until 108-120h post hCG. At the completion of each treatment, embryos were fixed or frozen for immunofluorescence or real time RT-PCR analyses, respectively. Results will be discussed.