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Exposure of Mouse Embryos to Ethanol During Preimplantation Development: Effect on DNA-Methylation in the H19 Imprinting Control Region

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ABSTRACT

Ethanol is a classic teratogen capable of inducing a wide range of developmental abnormalities that vary in severity, from the barely perceptible to spontaneous abortion. These defects are collectively referred to as foetal alcohol spectrum disorders (FASD). Foetal alcohol syndrome (FAS) lies at the extreme end of this spectrum and is associated with three broad domains: prenatal and/or postnatal growth retardation, distinctive facial features and brain damage. Epidemiological and animal studies clearly indicate that the clinical variability of FASD is related to four distinct window periods: preconception, preimplantation, gastrulation and postorganogenesis. These developmental windows are correlated with peak periods of epigenetic reprogramming, suggesting a common mechanism of ethanol teratogenesis. Together with experimental evidence that ethanol inhibits DNA-methyltransferase, as well as folate metabolism, this suggests an 'epigenetic model of FASD'.

The aim of the present study was to explore the validity of this model by investigating the relationship between ethanol-induced growth retardation and imprinting, following ethanol exposure during the preimplantation period. Employing an

experimental study design, together with a hybrid mouse model, embryos and placentae were harvested at 10.5 days post coitus (dpc). The weights of embryos and placentae, as well as methylation profiles at the *H19* imprinting control region (ICR) – an important regulator of growth - were measured.

It was found that ethanol-treated embryos and placentae were severely growth retarded in comparison to controls: r=-0.760 (p<0.01, one-tailed) and r=-0.816 (p<0.05, two-tailed), respectively. Bisulphite genomic sequencing revealed that the methylation profile at the *H19* ICR was unaffected in ethanol-treated embryos, in comparison to saline-treated controls. Conversely, methylation at the paternal and maternal alleles in placentae was found to be reduced and increased, respectively, in comparison to embryos. These results imply that mechanisms for the maintenance of imprinting in the embryo are more robust than in the placenta. This is consistent with the relatively long-lived nature of the embryo, which must maintain imprinting for a considerably longer period of time than the placenta.

Bisulphite sequencing also revealed that the paternal allele of the *H19* ICR had significantly decreased levels of methylation, while the maternal allele had increased levels of methylation, in ethanol treated-placentae, in comparison to saline controls. The changes observed at the paternal allele were localized to the CTCF1 DNA-binding site, while a trend for increased methylation at the maternal allele was observed at the CTCF2 site. A partial correlation further revealed that demethylation at the paternal allele in placentae partly mediated the effect of ethanol on placental weight. An ‘epigenetic switch model’, whereby paternal *Igf2* is downregulated by the epigenetic switching of the paternal allele to the maternal epigenotype, is proposed to explain this relationship.

However, partial correlations also indicated that demethylation at the paternal allele of the *H19* ICR, as well as placental growth retardation, did not mediate the effect of ethanol on embryo growth.

Collectively, these data suggest that imprinting at the *H19* ICR is not a mechanism of embryo growth retardation prior to 10.5 dpc. In explaining these results, it is proposed that the growth retarded placenta was able to meet the nutritional demands of the similarly growth retarded embryo up until 10.5 dpc. However, an important question for future research would be to examine the relationship between ethanol-induced growth retardation and imprinting during late gestation. During the final growth spurt (>14.5 dpc) the growth retarded placenta may become unable to meet the increased demands for nutrition, which would exacerbate foetal growth restriction.

In sum, the present study revealed a novel mechanism of ethanol-induced growth retardation in the placenta but indicated that imprinting at the *H19* ICR does not mediate the effect of ethanol on the early embryo. Further research is required to resolve the relationship between imprinting and ethanol-induced growth retardation.