The effects of ethanol on trophoblast cell differentiation in culture

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Alcohol consumption is widespread among pregnant women in Australia, particularly prior to pregnancy recognition. We have recently shown in an *in vivo* rodent model of periconceptional ethanol [EtOH] exposure, that early exposure results in sex-specific changes to placental morphology in late gestation. This included increased placenta:body weight ratio, decreased labyrinth:placenta and increased junctional zone (JZ):placenta ratios in both sexes (Gårdebjer *et al.*, 2014). This is important as the placenta is vital for mediating the growth of the developing foetus through the exchange of nutrients between mother and foetus. Alterations to placental structure and function have been implicated in intrauterine growth restriction, which in itself is an indicator of an increased predisposition to adult onset diseases.

Since the EtOH exposure occurs prior to implantation, we hypothesized that EtOH may directly alter the differentiation of trophoblast stem (TS) cells, derived from the trophectoderm, and the allocation of placental lineages, important for the formation of the definitive placenta.

In the current study, *in vitro* male mouse TS cells (RS26) were differentiated for 6 days in the presence of 0% (control), 0.2%, or 1% EtOH (n=3/treatment), and assayed for the expression of lineage-restricted trophoblast subtype markers. RNA was extracted for q-PCR and the expression of genes specific to the labyrinth (*Ctsq*, *Syna*, *Slc16a1*, *Slc16a3*) and junctional zones (*Tpbpa*, *Prl7a2*, *Prl7b1*, *Prl2c*1, *Prl3d1*) were analysed.

EtOH treatment caused reductions in the expression of *Syna* (syncytiotrophoblast layer 1 [SynT-I], $P<0.05$), *Prl7b1* (spiral-artery associated trophoblast giant cells [SpA-TGCs] and glycogen cells [GlyT], $P<0.05$), and *Prl7a2* (spongiotrophoblast [SpT] cells, $P<0.001$). No alterations were found for the remaining markers.

The observed reductions in gene expression suggest EtOH exposure can either delay TS cell differentiation or alter cell allocation to specific lineages. A reduction in the differentiation of labyrinthine cells (SynT-I) *in vivo* would lead to a reduced surface area for nutrient transport, while a reduction in the differentiation of junctional zone-derived subtypes (SpA-TGC, SpT, GlyT) would decrease trophoblast invasion and/or endocrine activity. Perturbations in all of these trophoblast subtypes have previously been implicated in foetal growth restriction. Interestingly, EtOH seems to show a biphasic response to the differing doses, with the 0.2% EtOH treatment causing a mild increase in expression in all genes.

We have shown that direct EtOH exposure results in alterations to placental cell types present within differentiating male TS cell cultures. Future analysis of female TS cells will determine if these changes are sexually dimorphic.

Gårdebjer EM, Cuffe JS, Pantaleon M, Wlodek ME, Moritz KM. (2014) *Placenta* **35**: 50-7.