

Hemoglobin A1 and B mRNA are expressed in the pre-implantation stage mouse embryo

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Abstract: An increasing number of tissues have been found to express intracellular hemoglobin, with functions ranging from O₂ and NO sequestering, and as an antioxidant. We have previously published that granulosa and cumulus cells from the murine ovarian antral follicle contains hemoglobin mRNA and protein, with levels under endocrine control over the peri-ovulatory period, peaking at 12 h post hCG in eCG/hCG treated female mice [1]. Here we determined if the early embryo also expressed mRNA of *Hba-a1* and *Hbb* over the course of *in vivo* development from the 2-cell stage to the blastocyst stage.

Pre-pubertal CBAB6 F1 female mice were treated with 5IU eCG/5IU hCG to stimulate follicular development, and placed with males immediately after hCG. Copulation was confirmed by presence of a plug and females sacrificed at 40, 52, 72 and 86 post-hCG treatment, corresponding 2-cell, 4 cell, morula and blastocyst stage embryos. RNA extraction, primer design, cDNA production and RT-PCR followed the protocol described in [1].

RT-PCR revealed, for the first time, hemoglobin A1 and B transcripts were present from the 2-cell stage, declining throughout subsequent development to almost undetectable levels by the morula and blastocyst stage (Figure 1), demonstrating these transcripts are most likely maternal in origin. Haptoglobin and 2,3-bisphosphoglycerate mutase fell even more sharply to be very low at the 4-cell stage. The function of hemoglobin within embryos remains to be ascertained, but we have proposed that sequestering gases, in particular O₂, is an attractive hypothesis. Further work is continuing to characterize protein levels and to elucidate functionality.

Brown, H.M. *et al.* (2015) Hemoglobin: A gas-transport molecule that is hormonally regulated in the ovarian follicle. *Biology of Reproduction* BIOLREPROD/2014/124594.

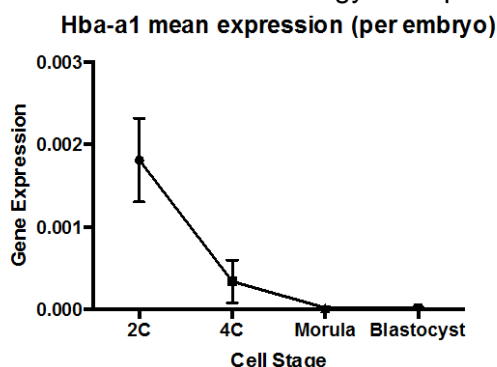


Figure 1 Hba-a1 expression during early embryo development.