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Supplementary Material

Haemoglobin expression in *in vivo* murine preimplantation embryos suggests a role in oxygen-regulated gene expression

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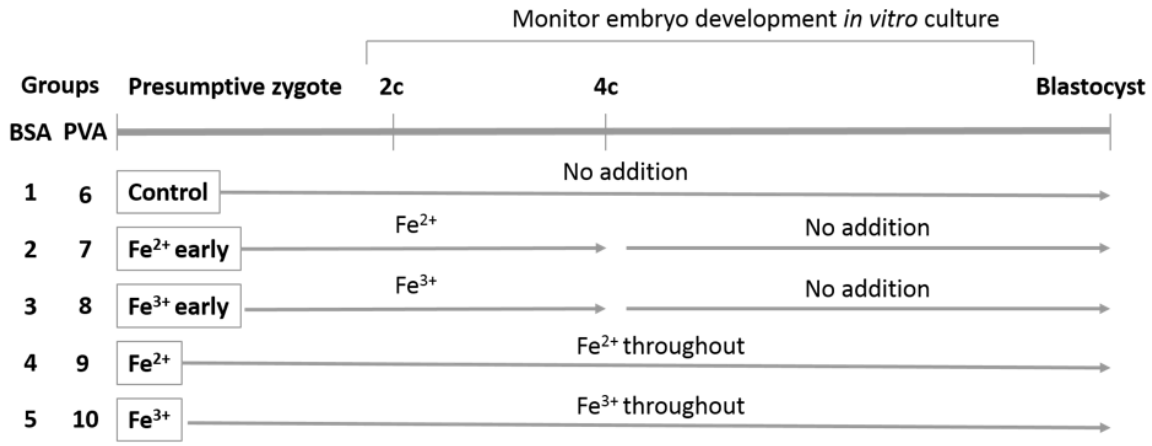

Fig. S1. Experimental design for ferrous and ferric haemoglobin addition in MEA media. Presumptive murine zygotes were placed into separate culture medium (BSA, PVA), making a total of ten treatment groups: Control, ferrous Hb (Fe^{2+}) to the 4-cell stage, Fe^{2+} to blastocyst stage, ferric Hb (Fe^{3+}) to the 4-cell stage and Fe^{3+} to blastocyst stage in BSA/PVA.

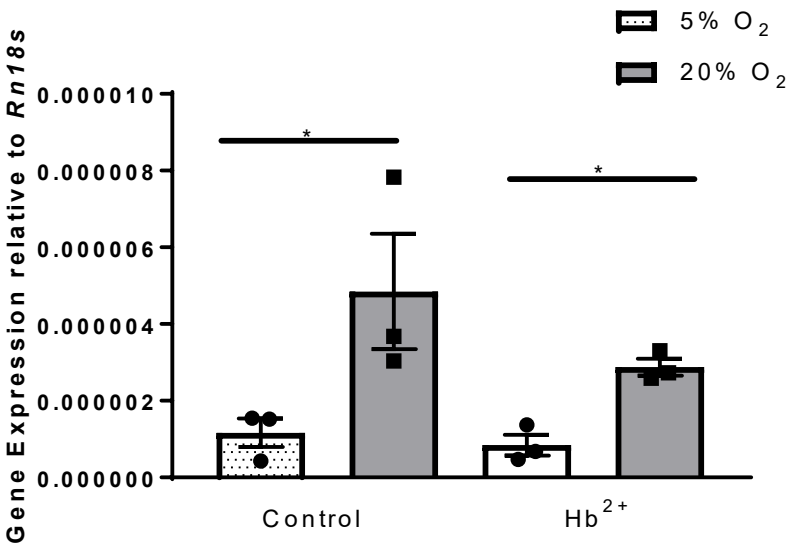

Fig. S2. *Hba-a1* expression is higher in blastocysts cultured at 20% oxygen compared to 5% oxygen, in the presence and absence of Hb. Presumptive murine zygotes were placed into MEA culture with BSA, and ferrous Hb added from Day 1 of culture. Three experimental replicates of 20 blastocysts per replicate per treatment were used for RT-qPCR analysis. Error bars are mean ± SEM. A Student's t-test was carried out on log-transformed data. **P*<0.05

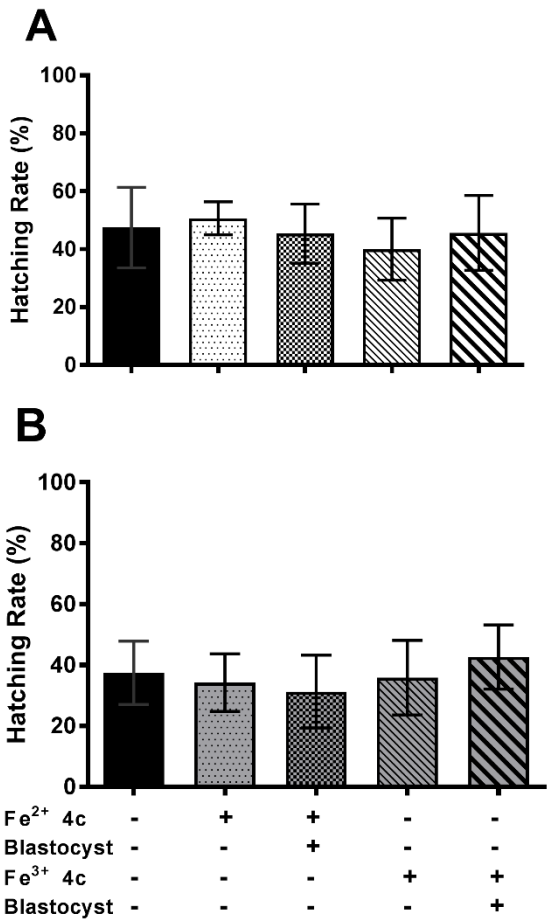

Fig. S3. Development to hatching blastocyst from cleaved 2-cell is not affected by haemoglobin addition. Presumptive murine zygotes were placed into separate culture medium (**A**: BSA, **B**: PVA) with five treatment groups: Control, ferrous Hb (Fe^{2+}) to the 4-cell stage, Fe^{2+} to blastocyst stage, ferric Hb (Fe^{3+}) to the 4-cell stage and Fe^{3+} to blastocyst stage. Five experimental replicates of 10-30 embryos per replicate per group were used. Error bars are mean ± SEM. A one-way ANOVA with Tukey's multiple comparisons test was carried out on arcsine-transformed data.

Table S1. Primers were designed from *mus musculus* mRNA sequences on the National Center for Biotechnology Information PubMed database using Primer 3 software (Whitehead Institute for Biomedical Research, CA, USA (Untergasser *et al.* 2012)) and synthesised by Geneworks (Geneworks, SA, Australia; Invitrogen, Australia Pty. Ltd.)

Primers were diluted to 25 μM

Gene	**Forward**	**Reverse**
Hba-a1	GTGTGGATCCCGTCAACTTC	AGAGGCAAGGAATTTGTCCA
Hbb	GCTGGTTGTCTACCCTTGGA	ACGATCATATTGCCCAGGAG
Bpgm	ACCGGAGGTACAAAGTGTGC	CTCCAGCAGAATCGGAACTC
Rn18s	AGAAACGGCTACCACATCCAA	CCTGTATTGTTATTTTTCGTCACTACCT
Bnip3	ACCCGCCTAGGTCCCACTT	GGGAGGGCGGCTGTTT
Glut1	CCAGCTGGGAATCGTCGTT	CAAGTCTGCATTGCCCATGAT
Elovl6	AGCAGTTCAACGAGAACGAAGC	CCGACCACCAAAGATAAAGGC
Ndrg1	ACCCGCCTAGGTCCCACTT	GGGAGGGCGGCTGTTT
Hp	GGGAGCTGTTGTCACTCTCC	TCACATTCGGGGAGTTTCTC
Ndufa4l2	CCTGCGCAGTCCTGATGTCT	GGTTGAAACGGCAAGGAACTT

Reference

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., and Rozen, S. G. (2012). Primer3 – new capabilities and interfaces. *Nucleic Acids Res.* **40**, e115. doi:10.1093/nar/gks596