

ANKRD49 is Required for Peri-implantation Embryogenesis

Julie Park¹; Deqiang Miao²; Michela Ciccarelli¹; Tessa Lord³; Jon M. Oatley¹

1. School of Molecular Biosciences, Center for Reproductive Biology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 USA.

2. Rodent Genetics Core, Cedars-Sinai Medical Center, D5007, Davis Building, 8700 Beverly Boulevard, Los Angeles, CA 90048, USA.

3. Priority Research Centre for Reproductive Science, Discipline of Biological Sciences, the University of Newcastle, Callaghan, NSW, Australia.

Studies of early pregnancy loss in humans indicate that less than 30% of conceptions progress to live birth and approximately 80% of all cases of pregnancy loss occur within the first trimester. Some cases of early pregnancy loss can be attributed to environmental factors while others may originate from deficiencies in preimplantation or peri-implantation embryo health. At present, the genetic factors required to support embryogenesis have not been fully defined. We previously identified ankyrin repeat domain 49 (*Ankrd49*) as a transcription factor that is expressed throughout preimplantation embryogenesis. To examine the functional importance of *Ankrd49*, CRISPR/Cas9 methodology was used to engineer mouse embryos with inactivated alleles. Founders with monoallelic deletions in *Ankrd49* were generated, but no biallelic edited offspring were born. In addition, the building of lines from founders of either deletion allele yielded heterozygous *Ankrd49*^{+/-} offspring at expected Mendelian ratios, but homozygous *Ankrd49*^{-/-} offspring were never produced. Both heterozygous and wild-type embryos could be detected at E7.5, E9.5, and E12.5 from timed mating of *Ankrd49*^{+/-} mice, but knockout embryos were absent, suggesting that loss of ANKRD49 function leads to peri- or post-implantation death. In corroboration, an expected Mendelian ratio of 22.5% of blastocyst stage embryos at E3.5 were found to be *Ankrd49*^{-/-} in heterozygous intercrosses by genotyping analysis. Interestingly, an abnormally high percentage (12.5–37.5%) of implantation sites at E12.5 of *Ankrd49*^{+/-} intercrosses were found to be empty of the embryo proper but have persistent placental tissue. Additionally, outcomes of outgrowth assays revealed that *Ankrd49*^{-/-} blastocysts can hatch and form typical inner cell mass and trophectodermal outgrowth colonies *in vitro*, suggesting that *Ankrd49* null blastocysts are competent to initiate implantation. Collectively, these findings demonstrate that ANKRD49 is required for development of the embryo proper or gastrulation, with loss of function in mice leading to embryonic death between E4.5 and E7.5. Ongoing studies focusing on this developmental window will determine which aspects of peri-implantation embryo development are impacted by *Ankrd49* ablation to better understand the genetic factors underlying embryogenesis and early pregnancy loss.