Surgical model for Genome-editing via Oviductal Nucleic Acid Delivery (GONAD) in rats

Animal Modeling Core
University of Missouri

Allison M. Tomasino[1], Daniel J. Davis[2], Elizabeth C. Bryda[2,3]

[1]University of Illinois College of Veterinary Medicine, Urbana, IL, [2]MU Animal Modeling Core, University of Missouri and [3]Rat Resource and Research Center, College of Veterinary Medicine, University of Missouri, Columbia, MO

RRRC Rat Resource & Research Center www.rrrc.us

INTRODUCTION

- *Ex vivo* handling of embryos is required to make genetically modified rats.
- Entails sacrificing the pregnant female to retrieve the embryos, making the genetic modification in culture, and then transferring them back into a surrogate "pseudo pregnant" female rat to give birth to the genetically modified animals.
- Genome-editing via Oviductal Nucleic Acid Delivery (GONAD) can produce genetically engineered rats *in vivo* and eliminate the need for surrogate dams and sacrifice of the embryo donor.
- This procedure has the potential to be translated for use in other species for which embryo collection, culture and transfer conditions are not well-established.

GOAL

- Determine the optimal surgical and reagent delivery parameters to perform GONAD successfully in Sprague Dawley (outbred stock) rats.
- Hypothesis - *In vivo* genetic modification of rat embryos via GONAD can be used to reduce the number of female animals needed to create genetically modified rats.

METHODS

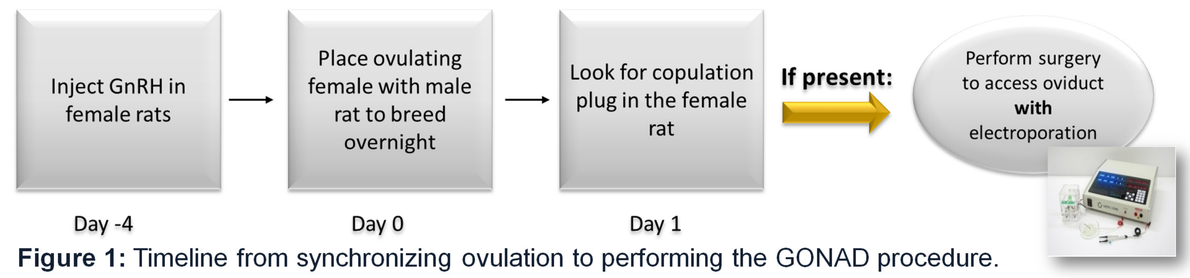

Figure 1: Timeline from synchronizing ovulation to performing the GONAD procedure.

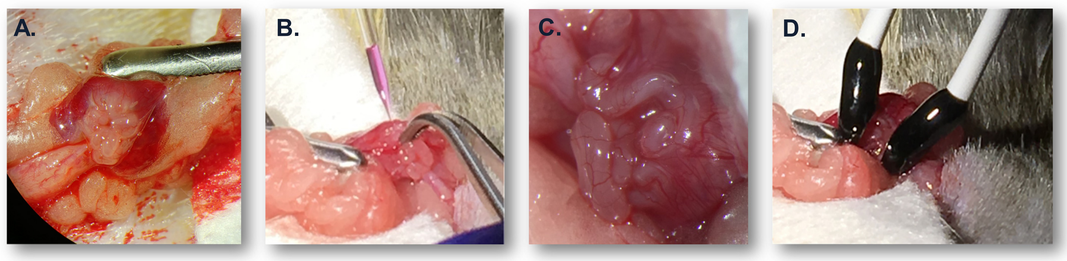

Figure 2: (A) The oviduct was surgically accessed. **(B)** 5µl (2.5µg) of EGFP mRNA was injected directly into the oviductal lumen using a glass micropipette. **(C)** The oviduct filled with EGFP mRNA. **(D)** The Nepa21 Super Electroporator (Japan) was used to electroporate the embryos at the 1-cell stage. The machine was set to 50V and the oviducts were electroporated for 6 pulses.

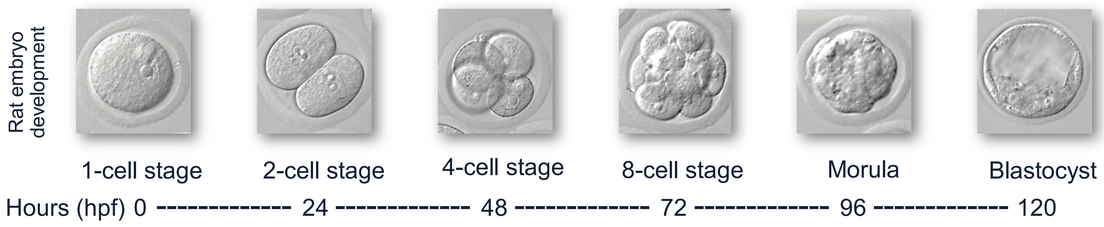

Figure 3: The GONAD procedure took place *in situ* while the embryos were at the 1-cell stage. The female was allowed to recover from surgery. Prior to implantation at ~48h-post-fertilization (hpf), the embryos were flushed out of the oviducts and uterus with R2CH and cultured *in vitro* in KSOM-R media to the blastocyst stage.

RESULTS

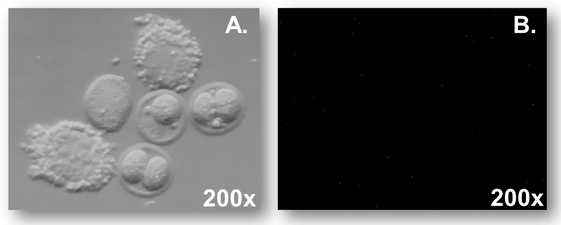

Figure 4: Imaging of embryos ~48hpf following delivery of EGFP mRNA using GONAD. **(A)** Bright field image. Embryos at the 1-2 cell stage as well as damaged embryos were observed. **(B)** Fluorescent microscopy (488nm excitation laser and 507nm emission filter) was used to detect EGFP expression. No fluorescence was observed in any of these embryos..

TROUBLESHOOTING

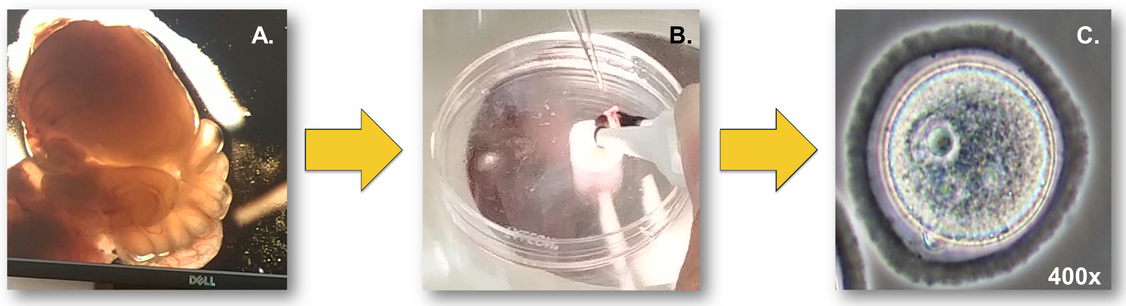

Figure 5: (A) Oviducts were collected and placed in a petri dish with R2CH. Either 5µl (2.5µg) or 10µl (5µg) of EGFP mRNA was injected into the oviducts. **(B)** The oviducts were electroporated at 50V for 6 pulses. **(C)** Embryos were flushed out and placed in KSOM-R to culture overnight.

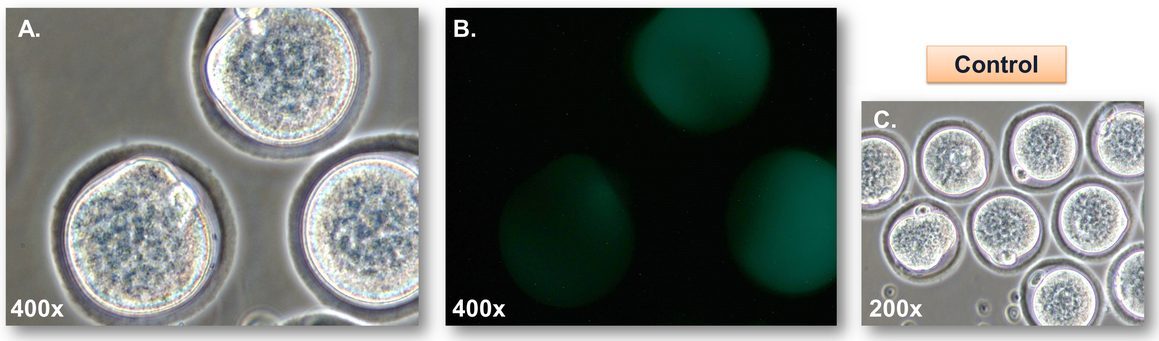

Figure 6: (A) Representative bright field image of 1-cell embryos cultured overnight following *ex vivo* electroporation of oviducts with EGFP mRNA. **(B)** 1-cell embryos did not develop further following overnight culture; however, they did exhibit EGFP fluorescence. **(C)** Bright field image of un-manipulated control 1-cell embryos cultured overnight did not develop further.

CONCLUSIONS

- ***In vivo* trial**:
  - ❖ The embryos did not express EGFP. This is potentially due to insufficient amounts of EGFP mRNA solution being injected and retained in the oviductal lumen.
- **Troubleshooting**:
  - ❖ 50V for 6 pulses using 5µl EGFP mRNA was sufficient to yield 1-cell embyros with EGFP expression when oviducts were electroporated *ex vivo*.
  - ❖ Based on the developmental arrest of the un-manipulated control embryos, failure of the *ex vivo* GONAD embryos to develop was most likely due to sub-optimal culturing conditions rather than failure of the GONAD technique.
- **Future Directions:**
  - ❖ Optimize injection techniques and solution volumes to maximize amount of EGFP mRNA retained in the oviductal lumen *in vivo*.
  - ❖ Once optimized, use GONAD to deliver genome-editing reagents to make genetically modified embryos *in vivo*.

ACKNOWLEDGEMENTS

This work was supported by NIH grant P40 OD011062 (ECB) and a grant from the American Society of Laboratory Animal Practitioners Foundation (funding from ASLAP Foundation) and an endowment established by IDEXX-BioResearch. We thank Corinne E. Piotter and members of the RRRC /MU MMRRC Cryobiology/Reproductive Lab for technical assistance.

Veterinary Research Scholars Program
University of Missouri

ASLAP The American Society of Laboratory Animal Practitioners

ILLINOIS College of Veterinary Medicine