Induction of Pseudopregnancy in Rats

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ABSTRACT

Rat embryo transfer is a commonly used technique when generating genetically engineered rat models. Once genetically manipulated ex vivo, embryos are transferred into a pseudopregnant surrogate dam. The current standard practice of inducing pseudopregnancy is to pair females with vasectomized males and allow them to mate. This requires research facilities to house males for this sole purpose and perform an abdominal surgery to vasectomize the male rats. The objective of our project was to compare the efficacy of induction of pseudopregnancy using 1) vasectomized males, 2) genetically sterile *Nanos2* knockout males, and 3) a novel cervical manipulation device. We hypothesize that the cervical manipulation device and *Nanos2* knockout males will have the same success rate of inducing pseudopregnancy as vasectomized males. To perform this experiment, we synchronized mature (> 8 weeks of age) female rats using luteinizing hormone releasing hormone (LHRH) and then split them up randomly into equal groups of three to four. One group was paired with vasectomized males, one group was paired with *Nanos2* knockout males, and one group was not mated but instead was cervically stimulated the next day. Three days after the mating pairs were separated and cervical manipulation was performed, we collected samples for vaginal cytology for three consecutive days to check stage of estrous in the females. Pseudopregnant females are expected to show persistent diestrus. The outcome of this study has implications for reducing the need for surgical vasectomy and/or the use of males altogether to achieve pseudopregnancy in rats.

INTRODUCTION

- Embryo transfer is used when creating genetically engineered models
- The surrogate dam must be pseudopregnant to accept the embryos
- Vasectomized males are used to induce pseudopregnancy
- Males undergo abdominal surgery to be vasectomized
- *Nanos2* KO genetically sterile males and cervical manipulation are potential alternatives to vasectomy

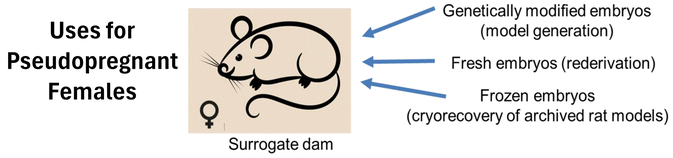


Figure 1. Schematic listing techniques and procedures that involve embryo transfer and require the use of pseudopregnant females.

RESULTS

Table 1. Trial 1: Vasectomized Group

Trial 1 Mating and Cytology Results: Vasectomized Group					
Female ID	Plug Present?	Cytology Day 8	Cytology Day 9	Cytology Day 10	Pseudo-pregnant?
830DJ	No	Diestrus	Diestrus	Diestrus	Yes
829DJ	No	Diestrus	Diestrus	Diestrus	Yes
831DJ	Yes	Diestrus	Diestrus	Diestrus	Yes

Table 2. Trial 1: *Nanos2* Group

Trial 1 Mating and Cytology Results: *Nanos2* Group					
Female ID	Plug Present?	Cytology Day 8	Cytology Day 9	Cytology Day 10	Pseudo-pregnant?
867DJ	Yes	Diestrus	Diestrus	Diestrus	Yes
866DJ	Yes	Estrus	Diestrus	Diestrus	No
865DJ	Yes	Diestrus	Diestrus	Diestrus	Yes

Table 3. Trial 1: Cervical Manipulation Group

Trial 1 Cytology Results: Cervical Manipulation Group				
Female ID	Cytology Day 8	Cytology Day 9	Cytology Day 10	Pseudo-pregnant?
841DJ	Estrus	Estrus	Diestrus	No
842DJ	Proestrus	Estrus	Diestrus	No
840DJ	Diestrus	Diestrus	Estrus	No
839DJ	Diestrus	Proestrus	Estrus	No

Table 4. Trial 2: Vasectomized Group

Trial 2 Mating and Cytology Results: Vasectomized Group					
Female ID	Plug Present?	Cytology Day 8	Cytology Day 9	Cytology Day 10	Pseudo-pregnant?
Right	Yes	Diestrus	Diestrus	Diestrus	Yes
Both	Yes	Diestrus	Diestrus	Diestrus	Yes
Left	Yes	Estrus	Diestrus	Diestrus	No
None	Yes	Diestrus	Diestrus	Diestrus	Yes

Table 5. Trial 2: *Nanos2* Group

Trial 2 Mating and Cytology Results: *Nanos2* Group					
Female ID	Plug Present?	Cytology Day 8	Cytology Day 9	Cytology Day 10	Pseudo-pregnant?
None	No	Diestrus	Estrus	Estrus	No
Right	Yes	Diestrus	Diestrus	Diestrus	Yes
Both	Yes	Diestrus	Diestrus	Diestrus	Yes
Left	Yes	Diestrus	Diestrus	Diestrus	Yes

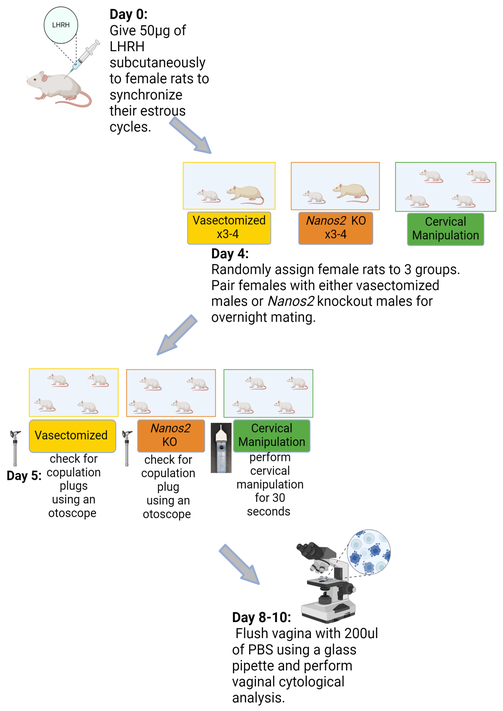
Table 6. Trial 2: Cervical Manipulation Group

Trial 2 Cytology Results: Cervical Manipulation Group				
Female ID	Cytology Day 8	Cytology Day 9	Cytology Day 10	Pseudo-pregnant?
None	Diestrus	Diestrus	Estrus	No
Both	Diestrus	Diestrus	Estrus	No
Right	Diestrus	Estrus	Diestrus	No
Left	Diestrus	Diestrus	Diestrus	Yes

METHODOLOGY

Figure 2. Three groups of 3-4 mature female Hsd:SD and HsdHlr:ZUCKER rats (>8 weeks) were used. Females were paired with surgically vasectomized males (Group 1), genetically sterile males that were homozygous for a knockout (KO) mutation in the *Nanos2* gene (Group 2), or were not mated and instead were cervically manipulated using a novel cervical manipulation device (Group 3).

Figure 3. Experimental design. We gave 50µg of luteinizing hormone releasing hormone (LHRH) subcutaneously to every female in each group between 9am and 10am to synchronize their estrous cycles (Day 0). On Day 4 at 5pm, we paired females with vasectomized males (Group 1) or *Nanos2* KO males (Group 2) in individual cages for each pair for overnight mating. Group 3 females were group housed without males overnight. On Day 5 at 7am, we checked for successful mating of the Group 1 and 2 females using an otoscope to check for copulation plugs. Group 3 females were subjected to cervical manipulation for 30 seconds. On Day 8-10, vaginal cytological analysis was performed by flushing the vagina with 200µg of PBS using a glass pipette, placing one drop on a glass side, staining with Kwik Diff (American MasterTech Scientific St. Lodi, CA) following standard procedures and analyzing the slides to assess the stage of estrous. Pseudopregnancy appears as persistent diestrus.



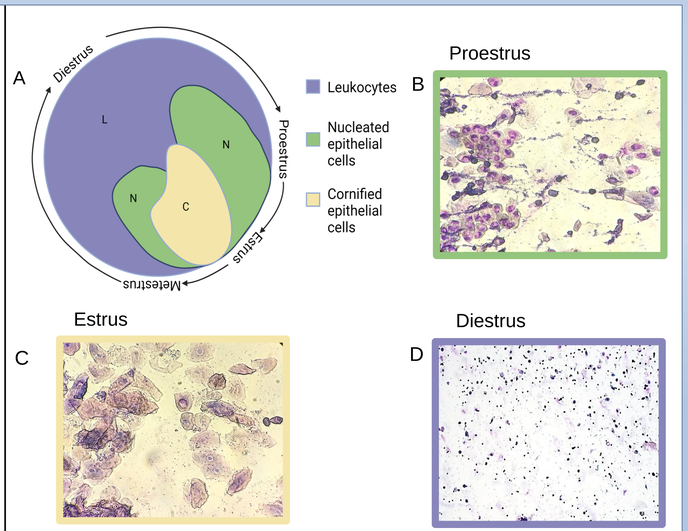


Figure 4. Rat estrous cycle and vaginal cytology. **A)** Schematic of rat estrous cycle, indicating which cell types predominate during each stage. Purple indicates predominant leukocytes during diestrus, green indicates predominant nucleated epithelial cells during proestrus, and yellow indicates predominant cornified epithelial cells during estrus. **B-D)** Representative examples of vaginal cytology during proestrus **(B)**, estrus **(C)**, and diestrus **(D)**.

CONCLUSIONS/ FUTURE DIRECTIONS

Table 7. Summary of Results from Trial 1 and 2

Groups	Number of animals	Copulation plug positive	Number pseudopregnant	% Pseudopregnant
Vasectomized	7	5	6	86
Nanos2	7	6	5	71
Cervical manipulation	8	Not mated	1	13

- *Nanos2* males are potentially good alternatives to vasectomized males
- Cervical manipulation was not effective
- Adjustments to the cervical manipulation device might improve results

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