The absence of the copulatory plug disrupts pregnancy in mice

Michael Lough-Stevens[1], Caleb Ghione[1], Adelaide Hobbs[1], Matthew Urness[1], Colleen Sweeney[1], Matthew D. Dean[1]

Molecular and Computational Biology, University of Southern California, 1050 Childs Way, Los Angeles, CA 90089

Introduction

- Understanding the biological reasons for implantation failure is important because pregnancies may fail even when there are no known clinical abnormalities.
- A significant proportion of pregnancy failures may be prevented with a deeper understanding of the molecular pathways required to initiate and maintain pregnancy.
- We used a mouse model to study pregnancy failure caused by a reproductive defect in the ejaculate of male mice: the copulatory plug, a solid mass formed from male seminal fluid in the female mouse's reproductive tract (see Fig. 1).
- We investigated 1) whether implantation rates go down when females do not receive a plug and 2) whether progesterone, which is essential for the implantation of fertilizing eggs in mammals and is upregulated at the time of implantation, is affected by the presence or absence of a plug.

Methods

- We mated female mice to males that could form a plug, or to genetically engineered males that lacked Transglutaminase IV and could not form a plug.
- We developed a technique to study implantation using microscopic beads that mimic the size and activity of fertilized eggs. The beads were transported into the female's uterus using a Non-Surgical Embryo Transfer (NSET) device (see Fig. 2) and then scored. Female Progesterone was collected via cardiac puncture followed by Enzyme-Linked Immunosorbent Assay (ELISA).

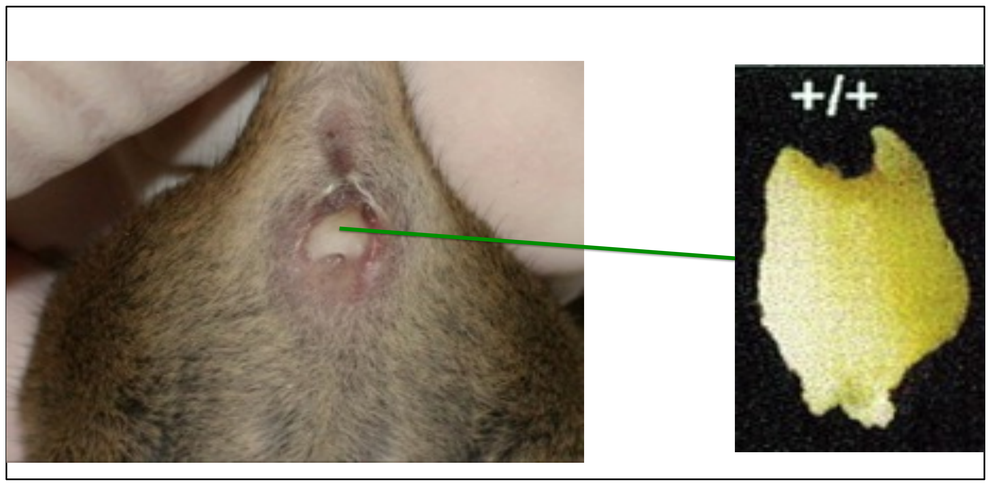

Figure 1: The location of the copulatory plug

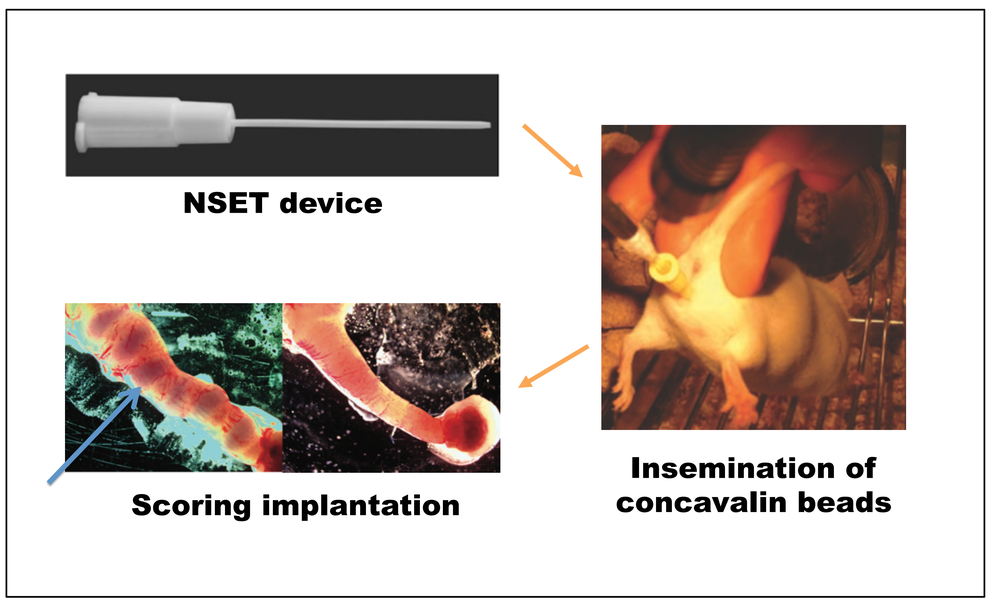

Figure 2: Workflow from inserting beads into the uteruses of mice to scoring implantation sites.

Results

- 0% of females housed alone implanted, demonstrating that ejaculation is minimally required for pregnancy.
- 75% of females that received a plug implanted versus 50% of females that did not, a mildly non-significant result (X^2=3.07, df=1, p=0.079)(Table 1).
- Mean progesterone between treatments showed no difference between plugged and non-plugged females, but a significant difference between the control and paired females (Kruskal-Wallis X^2=33.8, df=4, p<<<0)(Fig. 3).

	IMPLANTATION	NO IMPLANTATION
Females, ejaculation+plug	**30**	**10**
Females, ejaculation+no plug	**15**	**15**
Female_Alone	**0**	**42**

Table 1: Number of implantations and no implantations for females in three treatment groups.

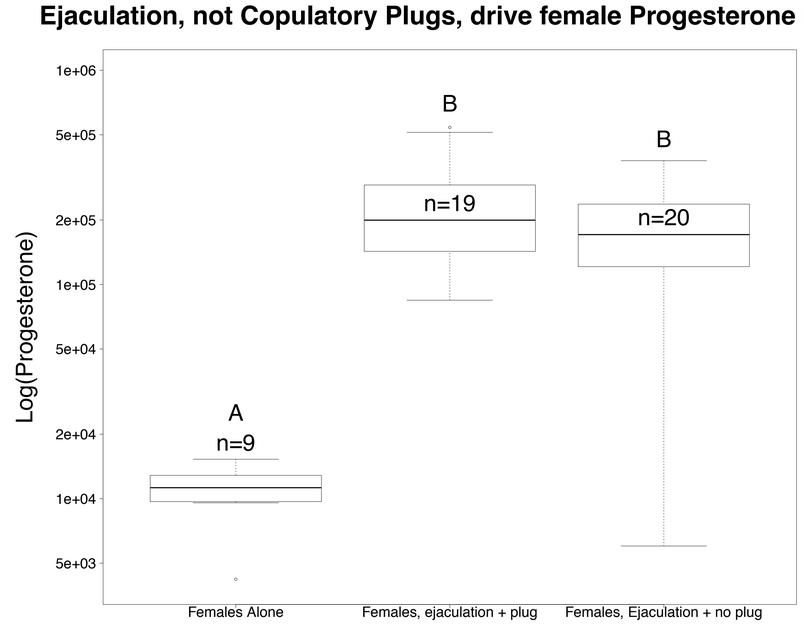

Figure 3: Mean progesterone (pg/mL) per treatment group, where groups A and B are statistically significant based on Kruskal-Wallis Rank Sums Test and a post-hoc Dunn's Test.

Discussion

- Implantation success is achieved partially by the presence of a copulatory plug, but is not sufficient.
- Progesterone is not differentially expressed based on plug status, suggesting other compounds must drive implantation success.
- We have identified a powerful mouse model to test the early steps in initiation and maintenance of pregnancy.

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