Veterinary Research Scholars Program
University of Missouri

Improving Cumulus-Oocyte-Complex Yield in a Superovulation Protocol for *Peromyscus* mice

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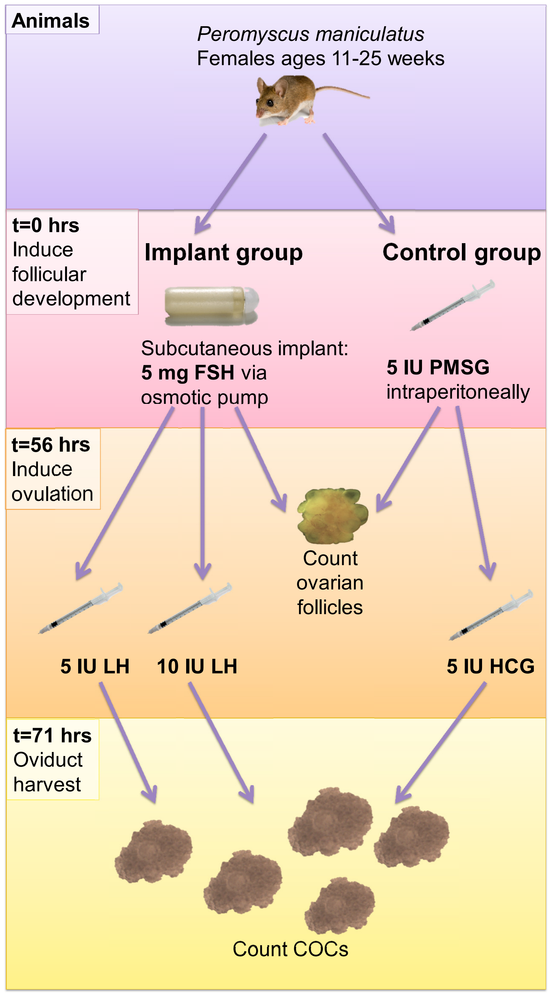
Introduction

- The *Peromyscus* genus of mice, or deer mice, are used in research on evolution, gene imprinting, monogamy, and environmental toxicology.
- Preserving and resurrecting strain variants or generating new transgenic lines is impossible with current protocols, and superovulation protocols for *Mus* mice are ineffective.
- Previous superovulation studies in *Peromyscus* have yielded similar numbers of cumulus-oocyte-complexes (COCs) to that which a female would naturally ovulate, or require cumbersome *in vitro* maturation techniques.[1]
- The purpose of the present study was to optimize an efficient, scalable superovulation protocol for deer mice.
- Since the timing of *Peromyscus* follicular waves is unknown, we hypothesized that a sustained release of FSH via osmotic pump would increase COC yield compared to previously published injection methods.

Methods



Statistical analysis: Data are plotted as mean +/- SD. Histograms were plotted (not shown) to determine that data was non-parametric. Dichotomous data was analyzed by Mann-Whitney test (Fig. 1A, Fi. 3B) with Bonferroni correction for multiple tests (Fig. 3B). Polychotomous data was analyzed by Kruskal-Wallis test (Fig. 3A). * $p<0.05$.

Results

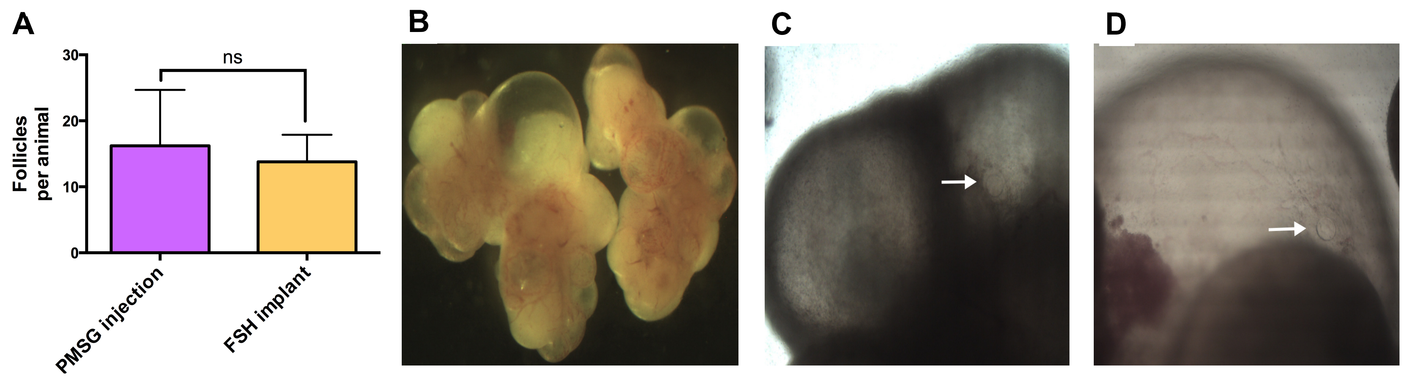


Figure 1. Pump effect on ovarian folliculogenesis. Ovaries were harvested at 56 hours from *Peromyscus* aged 11-15 weeks that had received 5 IU PMSG I.P. (n=10) or an osmotic pump containing 5 mg FSH (n=9). **(A)**The number of follicles per ovary are similar between groups (p=0.92). **(B)** Two ovaries from *Peromyscus* that received FSH via osmotic pump showing mature follicles, which were larger and more uniform than the control PMSG group (data not shown). **(C)** and **(D)** Oocyte and zona pellucida (arrow) are visible within follicles of control PMSG group **(C)** or FSH pump treated **(D)** mice.

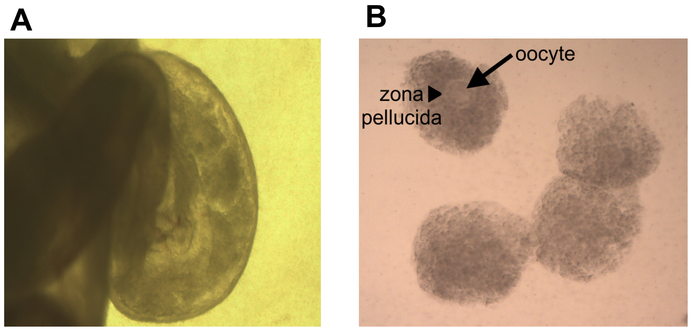


Figure 2. Morphology of COCs. COCs within the oviduct **(A)** or after dissection into media **(B)**. COCs form an elongated structure in the oviduct that is removed by an action similar to "squeezing a tube of toothpaste", unlike the clustered clutch produced by *Mus* mice. This suggests that superovulation is not a simultaneous event in *Peromyscus*.

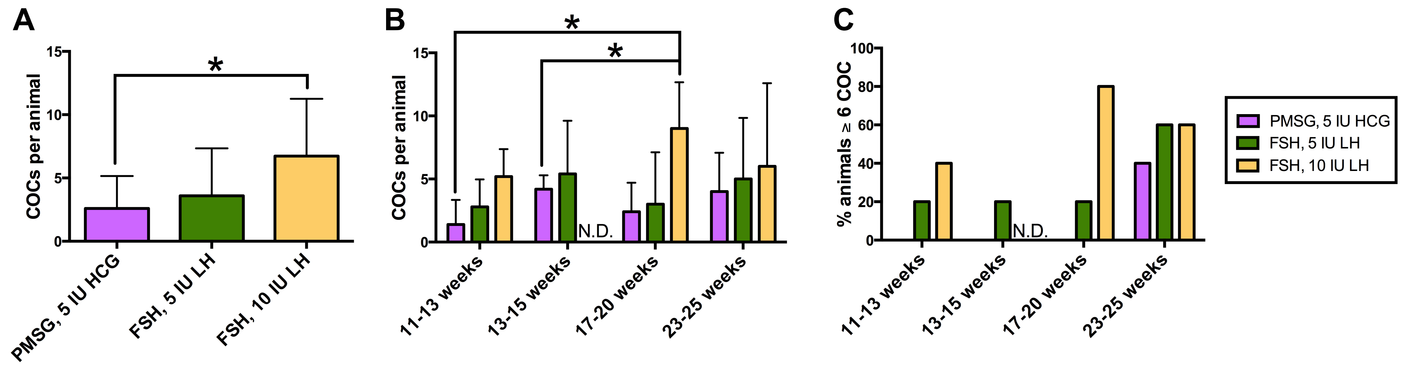


Figure 3. Optimization of COC yield. FSH implant followed by 10 IU LH yields more COCs. **(A)** COC yield by treatment group (n=15). **(B)** COC yield stratified by age group (n=5). *Peromyscus* 17-20 weeks old treated with FSH osmotic pump and 10 IU LH yield the most oocytes, significantly higher than the conditions previously published, 12-14 week old mice treated with 5 IU PMSG and 5 IU HCG. **(C)** Superovulation success rate, stratified by age group (n=5). Success was defined as the percent of mice that produced ≥6 COCs and therefore more than one standard deviation above our colony's average litter size of 4.2+/-1.5 pups.

Conclusions

New efficient conditions for superovulation of *Peromyscus* mice

	New protocol	Previously published conditions
Animal age	17-20 weeks	12-14 weeks
First hormone	5 mg FSH via subcutaneous osmotic pump implant	5 IU PMSG
Second hormone	10 IU LH i.p., t=56 hours	5 IU HCG, t=56 hours
COC collection	t=71 hours (needs optimization)	t=71 hours
Average COC yield	9 (range 5-15)	4.2 (range 0-5)
Superovulation success rate	80%	0%

- Many unovulated follicles remained and ovulation did not appear synchronous; higher yields may be achieved by waiting longer before COC collection.
- We did not test the 13-15 week time point, which may be the most effective age.
- This efficient, practical method of superovulation is the first step toward eventual embryo transfer protocols or transgenic manipulation of *Peromyscus*.

References

1. Choi JK, He X (2013) *In Vitro* Maturation of Cumulus-Oocyte Complexes for Efficient Isolation of Oocytes from Outbred Deer Mice. PLoS ONE 8(2): e56158. doi:10.1371/journal.pone.0056158

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