# Cellular mechanisms linking paternal stress with reproductive function and embryo development

LABORATORY OF TRANSLATIONAL PSYCHIATRY

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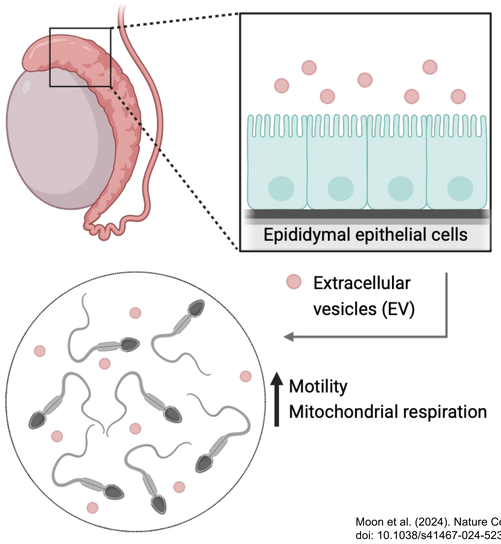
## Background

Stress is an important determinant of human behavior and physiology and can lead to long-term health issues. In males, studies have identified prolonged effects of stress on reproductive somatic cells that can further influence offspring development. Within the epididymis, sperm undergo a critical maturation process facilitated by factors secreted into the caput lumen by epididymal epithelial cells (EECs), including extracellular vesicles (EVs).



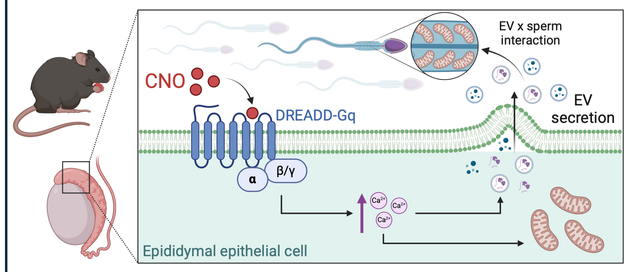

Moon et al. (2024). Nature Comm. doi: 10.1038/s41467-024-52319-0.

## Hypothesis

We hypothesize that chronic activation of epididymal epithelial cells will increase intracellular calcium signaling, thereby mimicking cellular stress effects, increasing sperm mitochondrial activation and extracellular vesicle secretion, and ultimately influencing offspring developmental outcomes.

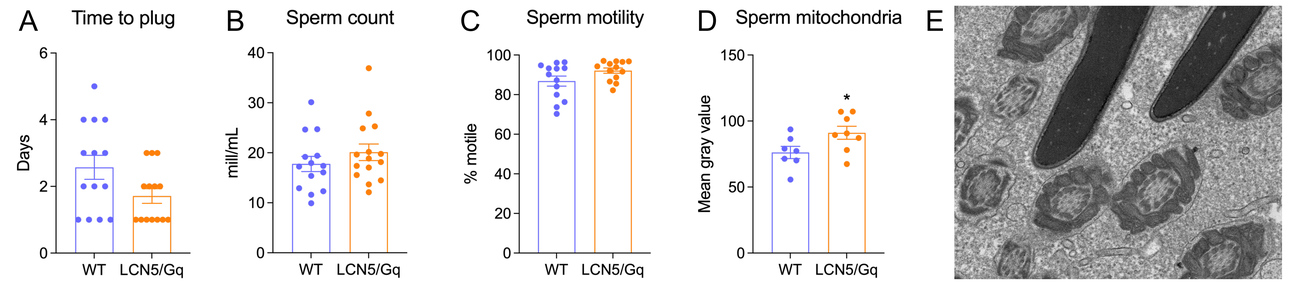
## Experimental timeline


## Transgenic mouse model



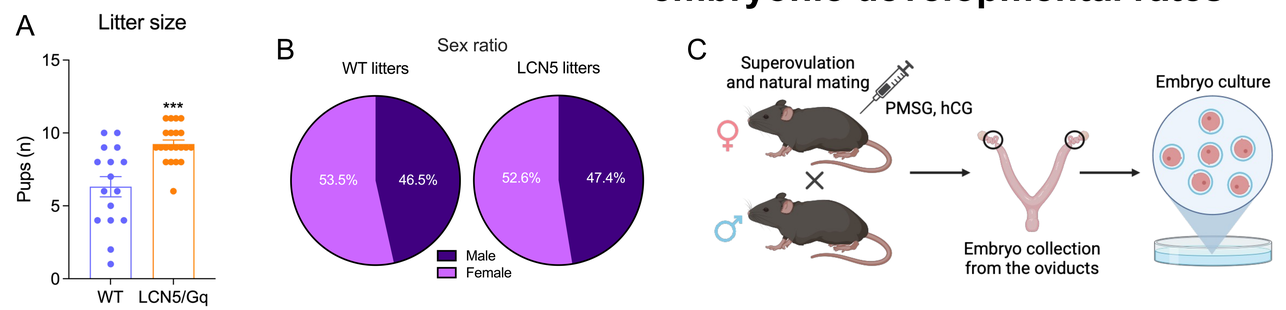

Schematic of the transgenic mouse model targeting caput EECs (LCN5-expressing cells) that express the Gq-coupled Designer Receptor Exclusively Activated by Designer Drugs (DREADD). System activation done through chronic treatment with the DREADD ligand clozapine-N-oxide (CNO). Intracellular calcium modulates mitochondrial activity and EV secretion, which in turn influences sperm function.

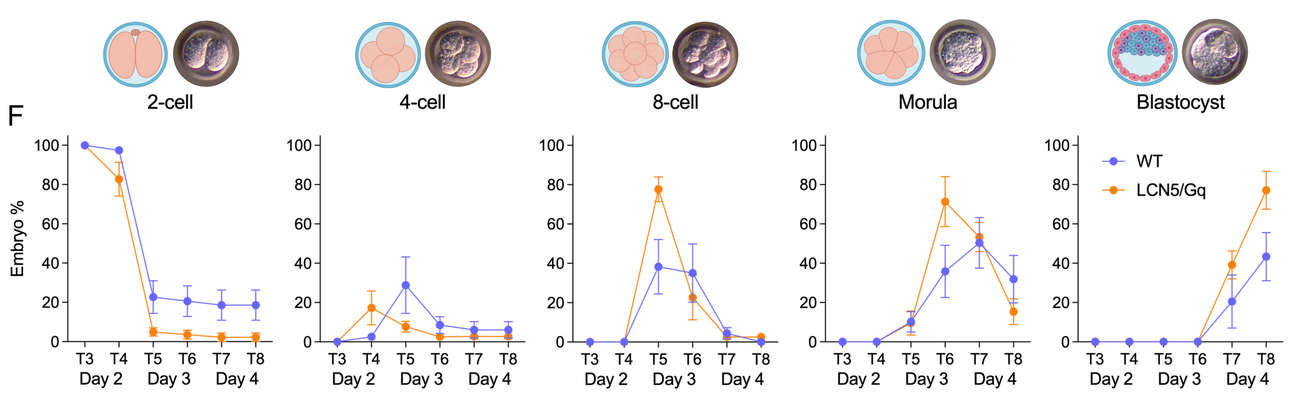
## Sperm mitochondria respond to environmental cues




**A** Average time to plug per male. Unpaired *t*-test, $p = 0.052$, $n = 14$ males/group. **B** Sperm count performed with the Computer Assisted Sperm Analysis (CASA) system. Unpaired *t*-test, $p = 0.306$, $n = 14$-15 males/group. **C** Percentage of motile sperm from CASA analysis. Unpaired *t*-test, $p = 0.07$, $n = 13$ males/group. **D** Mean gray value of sperm mitochondria. Unpaired *t*-test, $^{*}p = 0.049$, $n = 7$-8 males/group, 45-70 mitochondria per male. **E** Transmission electron microscopy (TEM) image of sperm mitochondria. For all panels, data are presented as mean ± SEM.

## Chronic activation of epididymal epithelial cells enhances reproductive outcomes and accelerates embryonic developmental rates




**A** Litter size at gestational day 12.5. Unpaired *t*-test, $^{***}p < 0.001$, $n =$ 16-21 litters/group. **B** Sex ratio of litters. 2-way ANOVA, sex effect $p =$ 0.273, genotype effect $^{***}p < 0.001$, interaction effect $p = 0.962$, $n =$ 16-21 litters/group. **C** Schematic illustrating the superovulation protocol, embryo collection and culture. 10UI intraperitoneal injection for both Pregnant Mare Serum Gonadotropin (PMSG) and Human chorionic gonadotropin (hCG) hormones. **D** Total number of retrieved oocytes from oviducts. Unpaired *t*-test, $p = 0.776$, $n =$ 5-6 males/group, 1 female for each male. **E** Percentage of fertilized embryos per male. Unpaired *t*-test, $^{*}p = 0.019$, $n =$ 5-6 males/group. **F** Representative images of each cell stage during embryonic development. Percentage of embryos at each developmental stage over the course of 3 days. T3, T5, and T7 were acquired in the morning (~9AM) and T4, T6, and T8 were acquired in the afternoon (~5PM). $n =$ 5-7 males/group, one embryo culture per male. For all panels, data are presented as mean ± SEM.

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## Summary & Conclusions

Here we indicate that epididymal epithelial cells respond to environmental cues and regulate sperm function, mediating the effects of paternal stress on offspring. We also suggest that embryonic accelerated development may influence the offspring brain maturation, potentially increasing the risk for neuropsychiatric disorders later in life. Understanding this pathway provides key insights into how paternal experiences may shape reproductive outcomes and ultimately offspring neurodevelopment, linking epididymal signaling to intergenerational stress transmission.