# Whole genome Nanopore DNA analysis shows that chronic corticosterone supplementation results in altered sperm DNA methylation.

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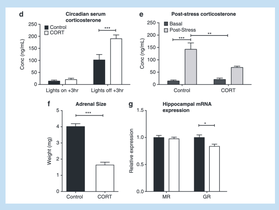
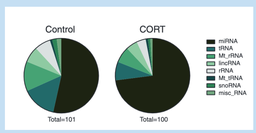
## Introduction

- Corticosterone (CORT) is the main stress hormone in mice.
- CORT supplementation models Hypothalamic-Pituitary-Adrenal (HPA) axis activation, the neuroendocrine system that regulates stress but also plays a role in digestion, emotions, immune system and metabolism.
- 4 weeks of CORT supplementation in male mice lead to altered sperm small non coding RNA (sncRNA) changes in F0 [1].
- Paternal CORT leads to behavioral changes in their offspring (F1 and F2). Including alterations in memory, anxiety and depression phenotypes [1,2].
- Paternal CORT leads to expression changes in the paternal imprinted gene Igf2 in hippocampus of F1 and F2 male offspring and F1 female offspring [1].

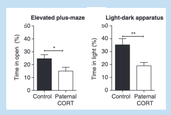



F0



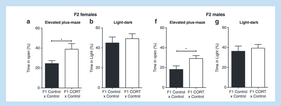

Altered sncRNA profile in sperm

**Behavioral changes shifts in offspring**




F1

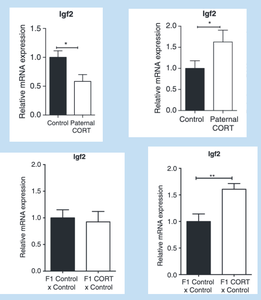
Increased anxiety in F1 male offspring



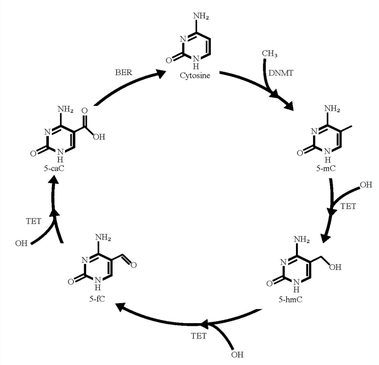

F2

Decreased anxiety in F2

**Igf2 mRNA expression in hippocampus at 8 weeks**



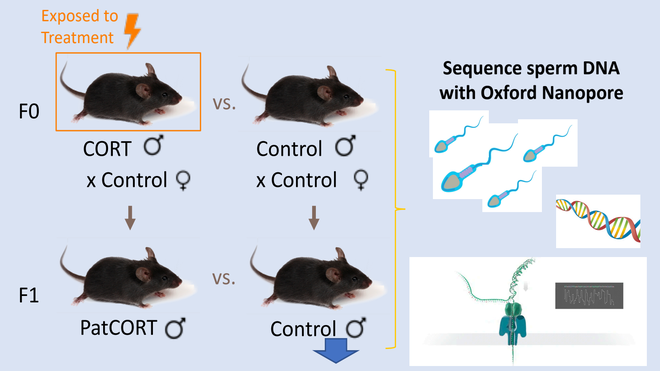

## Cytosine methylation cycle




- 5mC is transformed into 5hmC.
- 5mC stabilizes chromosomes.
- 5hmC destabilizes chromosomes
- 5mc and 5hmC cannot coexist in the same cytosine.

5mC: 5-methylcytosine.
5hmC :5-hydroxymethylcytosine.

## Methods



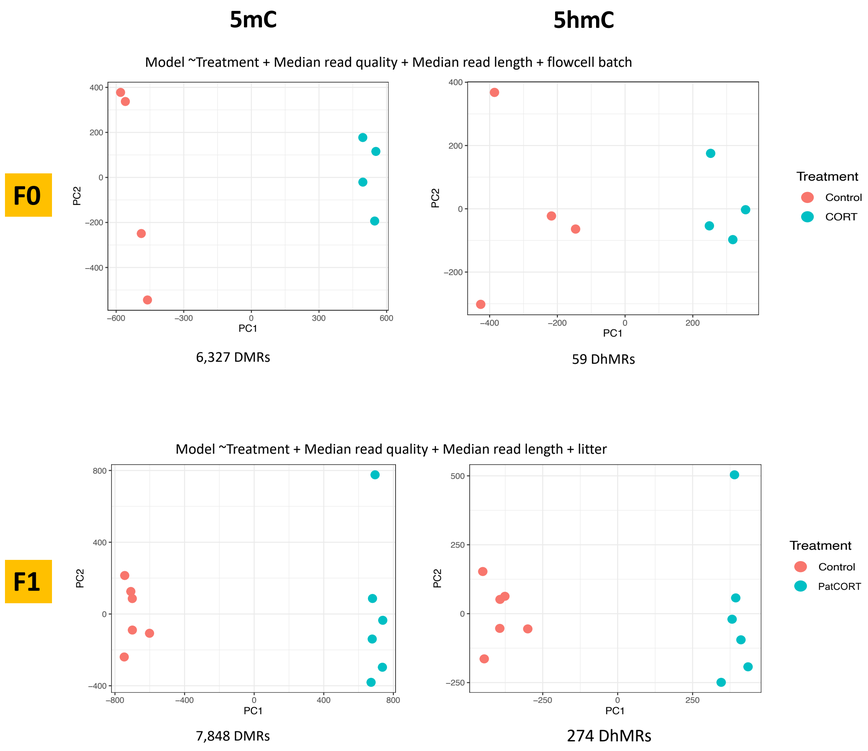

**Direct DNA Cytosine methylation detection**

Directly from the pore current read. No bisulfite conversion

- Imprinting Control Regions (ICRs), are regions of the genome with parent-of-origin specific methylation.
- DNA methylation at ICRs survives the waves of demethylation that occur during embryogenesis, so can be inherited across generations.
- Methylation of sperm- ICRs guarantees paternal expression of the paternally expressed genes (Igf2, Dlk1 and Rasgfrf1)

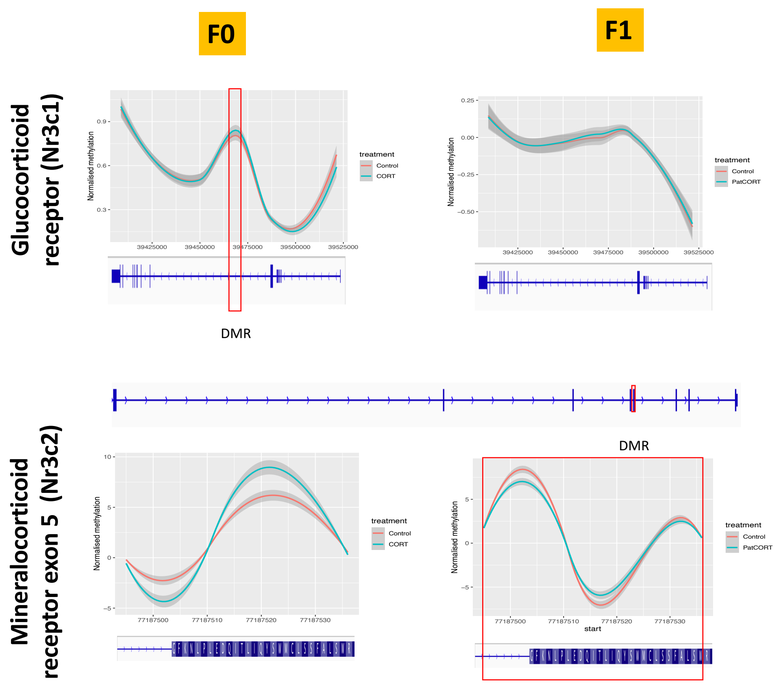
**Hypothesis:** sperm from CORT-treated mice (F0) and their male offspring (F1) have DNA cytosine methylation/hydroxymethylation changes that contribute to the epigenetic inheritance of offspring phenotype.

## CORT supplementation in male mice leads to sperm DNA changes in 5mC and 5hmC across two generations (F0 and F1)




**DMR:** Differentially methylated region with coef=TreatmentCORT, adj.pval<0.05.
**DhMR:** Differentially hydroxymethylated region with coef=TreatmentCORT, adj.pval<0.05

## CORT supplementation in male mice leads to sperm DNA methylation changes in the glucocorticoid and mineralocorticoid receptors




- F0 CORT sperm has increased methylation at Nr3c1 intron 8. Nr3c1, or glucocorticoid receptor, is the main receptor of corticosterone.
- F1 PatCORT offspring have altered methylation at the intron exon boundary at the beginning of Nr3c2 exon 5. N3c2 or mineralocorticoid receptor is the main receptor of aldosterone.

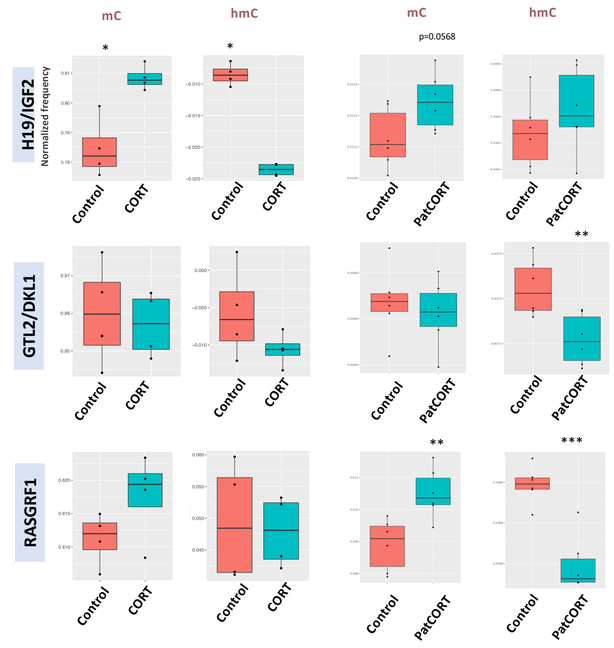
## CORT treatment leads to increased mC and decreased hmC at sperm ICR in both F0 and F1

**F0**

Model ~Treatment + Median read quality + Median read length + flow cell batch

**F1**

Model ~Treatment + Median read quality + Median read length + litter




- Increased mC at sperm IH19/gf2 ICR explains increased Igf2 expression in Paternal CORT male offspring (F1). The mechanism for decreased expression of Igf2 in female offspring needs further analysis.
- Increased methylation and decreased hydroxymethylation due to CORT suggests increased expression of paternally expressed genes and likely propensity to bigger, stronger pups.

## Summary

- We were able to identify changes both 5mC and 5hmC in mouse sperm using Oxford Nanopore sequencing and find differentially methylated and hydroxy-methylated regions (DMRs, DhMRs).
- Four weeks CORT supplementation in mouse produces methylation and hydromethylation changes in sperm of exposed mice (F0) and their unexposed male offspring (F1), being the effect on 5mC larger than in 5hmC.
- Corticoid receptor methylation is affected by corticosterone supplementation.
- CORT treatment leads to changes in sperm Imprinting Control Regions (ICRs)in F0 and F1, marked by increased methylation and decreased hydroxymethylation.
- Changes in sperm ICR methylation translate to changes in offspring gene expression.

## Acknowledgements

This research was undertaken using the LIEF HPC-GPGPU Facility hosted at the University of Melbourne. This Facility was established with the assistance of LIEF Grant LE170100200.
Shian Su
Chloe Goldsmith

## References

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2. Yeshurun, S., Rogers, J., Short, A. K., Renoir, T., Pang, T. Y., & Hannan, A. J. (2017). Elevated paternal glucocorticoid exposure modifies memory retention in female offspring. *Psychoneuroendocrinology*, *83*, 9–18. https://doi.org/10.1016/j.psyneuen.2017.05.014

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**github** (incomplete): https://github.com/Coracollar/DNA_mC_hmC_ONT/