Investigating Global Changes to Histone Lysine Methylation in *Kdm6a* Conditional Knockout Sperm

David Kelly, Ben Walters PhD, Bluma Lesch MD, PhD, *Department of Genetics, Yale School of Medicine*

The Research Experience for Veteran Undergraduates
Where enlisted U.S. veteran undergraduates in STEM develop world-class research skills.

Yale SCHOOL OF MEDICINE

Abstract

Epigenetic changes regulate gene expression without altering the DNA sequence and can be associated with diseases such as cancer.[1] A gene implicated in cancer development is *Kdm6a*, a histone demethylase that helps control gene activity in sperm.[2] Our lab previously found that deleting Kdm6a in developing sperm increases the risk of cancer in offspring. The epigenetic mechanisms by which this risk is conferred are unknown. *Kdm6a* is known to regulate H3K4 methylation. Alterations to this modification may contribute to the cancer risk observed in offspring. Therefore, we assessed global changes to H3K4 methylation in sperm from wild-type and *Kdm6a* knockout male mice using Western blotting.

Introduction

Histones- Package DNA and regulate gene expression.
Nucleosome- Chromatin's basic unit, consists of DNA base pairs wrapped around a group of histone proteins.[3]
Histone Demethylation- Removes methyl groups on histones, altering chromatin structure and gene activity.[4]
Kdm6a- A lysine demethylase that usually alters H3K27me3, previously shown to be a subunit in the COMPASS complex, which regulates H3K4 methylation.[2]
Conditional Knockout (cKO)- Gene deletion in specific tissues using enzymes like Cre Recombinase.[5]

Figures 1–5. *adapted from BioRender.*[6]

Figure 1. DNA

Figure 2. Histone

Figure 3. H3K4me1/3

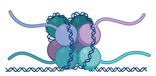


Figure 4. Nucleosome

Figure 5. Conditional Knockout (cKO)

Research Question

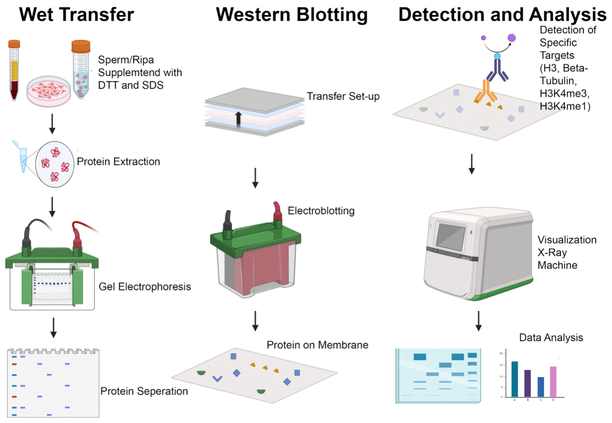
How does deletion of *Kdm6a* in the male germline alter global H3K4 methylation in sperm?

Methods

Sample Preparation: Testes from male mice were dissected and sperm was collected via swim-out from adult control and *Kdm6a* cKO mice. Sperm lysates were then prepared using RIPA buffer.

Quantification: After developing western blot images using an X-ray imager, band intensities were quantified using Fiji/ImageJ. Intensities were normalized to Beta Tubulin or H3. The Data were then analyzed using Excel and graphed using GraphPad Prism.

Figure 6. Western blotting workflow. Adapted from Clinisciences Experimental Protocol for Western Blotting 2025.[7]



Results

Figure 7. Western blots showing validation of histone extraction from sperm, the dark band in each lane represents the presence of protein in these samples.

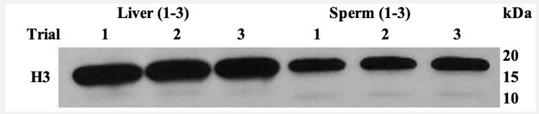


Figure 8. Western blot showing RIPA+DTT+SDS successfully solubilizes protamine.

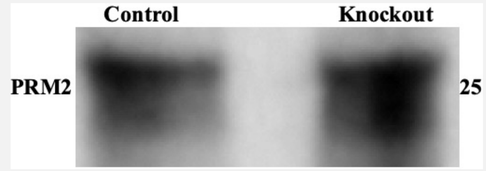
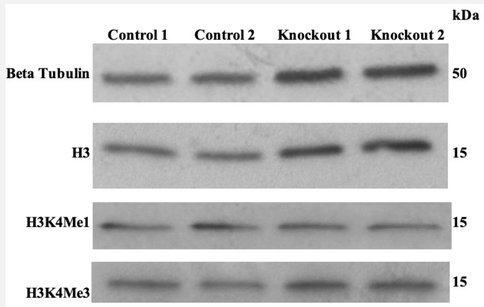
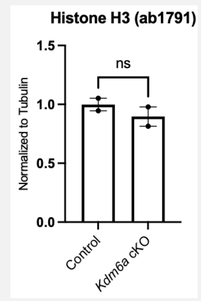
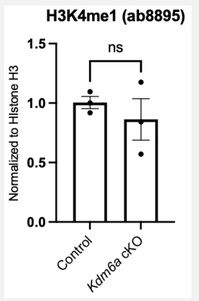
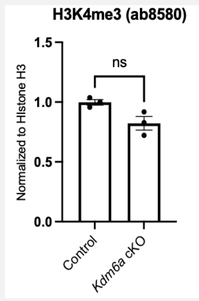


Figure 9. Western blots of H3K4me1, H3K4me3, and total histone H3 in control vs *Kdm6a* cKO sperm. Beta Tubulin was used as a loading control. Graphs show densitometric quantification normalized to histone H3 or Beta Tubulin.









Limitations

1. Looking at global levels, of all the histones, there could be localized ones which could require a different technique to analyze.
2. We are assuming that the sperm is homogenous in epigenetic state, but recent evidence suggests this may be heterogenous.
3. The technique of Western blot may not be precise enough to detect slight variances in global levels of histone modifications.

Conclusion

1. RIPA+DTT+1%SDS successfully extracts histone H3.
2. PRM2 is detectable in sperm lysates.
3. Beta Tubulin is a good housekeeping gene for sperm lysates.
4. *Kdm6a* deletion does not significantly alter global levels of histone H3, H3K4me1, or H3K4me3 in sperm. This suggests that the increased cancer risk associated with *Kdm6a* loss is unlikely to result from global changes in histone modifications, but may instead involve more specific, localized epigenetic changes.