Detecting DNA oxidation in Sperm

Alexie Zwerdling, Miles Wandersee, Sierra Lopez, Mirella L. Meyer-Ficca, Ralph G. Meyer
Department of Animal, Dairy, & Veterinary Sciences, Utah State University, Logan, UT 84321

Introduction

- Decreasing levels of nicotinamide adenine dinucleotide (NAD) are an integral part of aging. However, low levels of NAD have been linked to DNA damage in sperm causing a decrease in quality and integrity due to increased levels of oxidative stress in the DNA.
- A transgenic mouse model, ANDY mouse, is used to simulate aging males with low NAD levels.
- The DNA oxidation of guanine bases due to oxidative stress leads to the formation of 8-oxoguanine that can be used as a biomarker for detecting DNA single strand breaks in sperm cells.

Hypothesis

Low levels of NAD increase oxidative stress causing DNA damage in sperm cells of aging men. Therefore, oxidized DNA can be detected

To test this hypothesis, direct and indirect methods of 8-oxoguanine detection using antibodies were performed on HeLa cells and sperm samples, respectively.

Methods

Two methods of 8-oxoguanine detection were used. For each method, a fluorescent microscope was used to detect 8-oxoguanine.

Direct Method

- Cultured HeLa cells, treated with hydrogen peroxide to induce oxidative stress and stained with fluorescent- labeled antibodies to detect 8-oxoguanine, were used to establish the method (data not shown). This method was not sensitive enough.

Indirect Method

- Sperm samples were treated with FPG which creates single strand breaks in the DNA. Fluorescent-labels nucleotides are used to elongate the single strand break allowing for detection of the oxidized bases in the sperm samples.

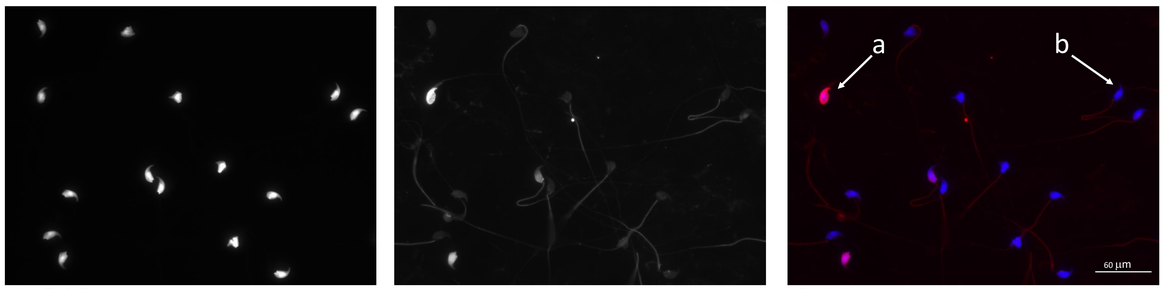
Results

Direct Method

- The direct method with hydrogen peroxide induced oxidative stress in HeLa cells was unsuccessful for detecting 8-oxoguanine with fluorescent labeled antibodies. Subsequently, the indirect method was employed.

Indirect Method

- Compared to the positive and negative controls upon analysis with the fluorescent microscope, the sperm samples showed positivity with DNA strand breaks within the nuclei of individual sperm cells. The fluorescent labeled nucleotides used to elongate the site of the oxidized guanine are utilized to quantify the amount of single stranded breaks in the DNA are present in the sperm samples.
- Analysis of the sperm samples was conducted using image pro software.



A. DAPI B. CY3-dUTP C. Merge

Figure 1: Sperm of NAD-deficient transgenic ANDY mice have oxidative damage and are labeled by terminal deoxynucleotidyl transferase (TdT), using CY3 labeled dUTP nucleotide incorporation (middle). DAPI: sperm DNA, merge: Overlay of the DAPI and CY3-dUTP, arrow a corresponds to a positive sperm cell with DNA strand breaks ,while arrow b is negative with no DNA strand breaks detected.

Conclusion

- NAD levels play an important role in aging for sperm quality and integrity in males.
- Using an indirect method to detect DNA strand breaks will enable quantification of oxidized DNA within the sperm cells.
- The next step for the indirect method will be to use it with sperm samples from the ANDY mouse model that simulate aging males with increased oxidative stress due to low levels of NAD.

Acknowledgements

This work was funding by the R15 and R56 grants.
I would also like to thank the Meyer Lab for allowing me to work on this project.