FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

FERTILITY SCIENCE

The Effect of Astaxanthin (AXA) on Oxidative Stress in Sperm Post-Cryopreservation

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Introduction:

In order to understand drivers of fertility and assisted reproduction success, our lab investigates how environmental nutrients affect sperm function and metabolism. Reactive oxygen species (ROS) is a harmful molecule that can lead to oxidative stress in sperm, which could result in DNA damage and a loss of motility[1]. One way to combat these effects is to introduce low-molecular-weight antioxidants such as vitamin C. One such molecule, Astaxanthin (AXA), is a known antioxidant that has anti-cancer and anti-inflammatory properties[3]. However, there has been little research done on the effects AXA might have on bull sperm[2]. It is known to have greater antioxidant properties than canthaxanthin and beta- carotene including the ability to reduce oxidative stress in the body[4]. We hypothesize that sperm metabolism and motility will be directly related to AXA exposure.

Research Question:

What is the effect of different concentrations of Astaxanthin (Ambeed, CAT# 76974-654) on bull sperm and is it able to reverse the effects of ROS?

Hypothesis:

A concentration of 50 µM of Astaxanthin will demonstrate improved sperm health post-cryopreservation.

Materials & Methods:

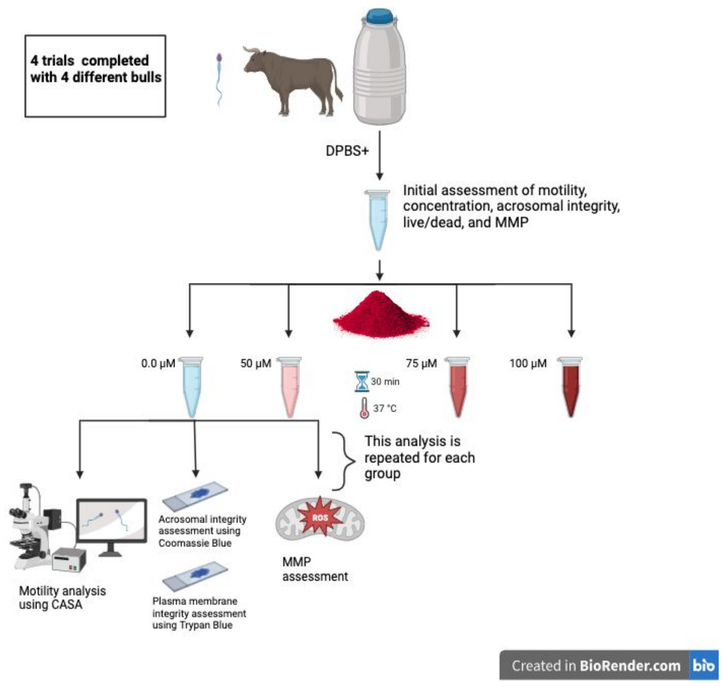

Figure 1. Experimental design and procedure
AXA was diluted in DMSO. Sperm were incubated in 0 µM, 50 µM, 75 µM, and 100 µM AXA with MT-1 dye. Motility was analyzed using the CASA system. Coomassie Blue (G-250) stain was used for acrosomal integrity analysis, and 0.4% Trypan Blue was used for plasma membrane integrity analysis. Analysis was performed after 30 minutes of incubation at 37°C.

Results:

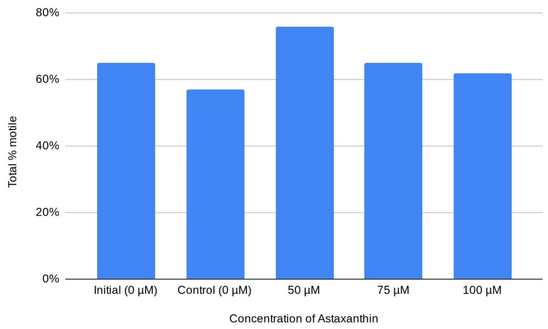

Figure 2. AXA maintains sperm motility
Sperm determined to be rapid, medium, and slow by CASA (Hamilton Thorne, Inc.) were combined as the total motility for each group. The general trend shows a negative association between concentration of AXA and motility with the 50 uM being the exception.

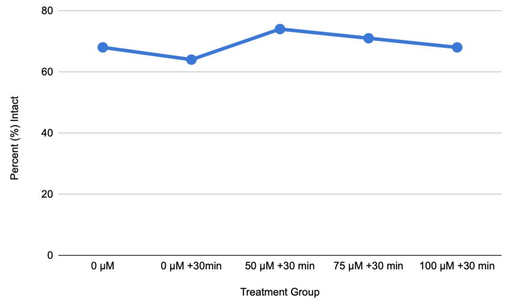

Figure 3. Acrosomal integrity is not impacted by AXA incubation
There was no effect on the percent of intact acrosomes per sample following AXA incubation, as assessed using Coomassie Blue G-250 dye for acrosomal integrity.

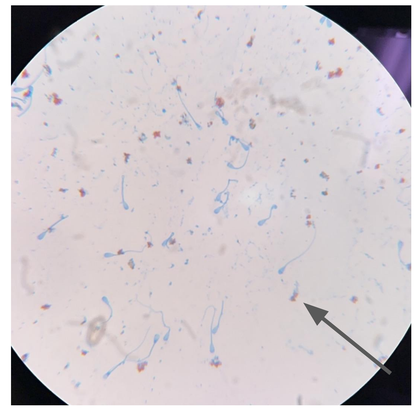

Figure 4. Acrosomal integrity after incubation in AXA (50 µM)
A representative image of sperm acrosomal staining using Coomassie Blue G-250 (Amresco, CAT# M140) and Astaxanthin particles (red). Intact acrosomes are identified by the dark-blue sperm heads. Arrow depicts astaxanthin circulating about a sperm head.

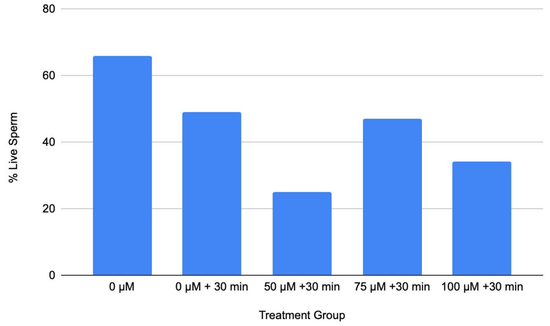

Figure 6: Sperm Viability using Trypan Blue Staining
Sperm were assessed with Trypan Blue stain (HyClone, CAT# SV30084.01) to determine the percentage of sperm that were alive before and after incubation. There is a negative association with concentrations of AXA and percent of live sperm with a notable drop in % of live sperm in the 50 uM group.

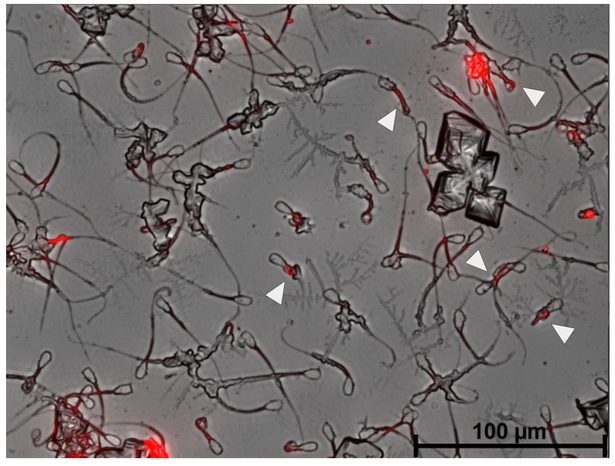

Figure 5 (left). Mitochondrial membrane potential (MMP) of sperm following AXA incubation
These images (Leica DM 16000) show representative staining patterns for MMP (Dojindo, CAT#MT13-10) in sperm following incubation in varying concentrations of AXA (A. 0µM + 0 minutes, B. 0µM + 30 minutes, C. 50µM + 30 minutes, D. 75µM + 30 minutes, E. 100µM + 30 minutes). Red fluorescence is concentrated in the sperm midpiece of many sperm cells (arrow head). Quantification yielded inconclusive results for mitochondrial membrane potential, and further assessment is needed.

Discussion / Future Directions:

Despite an overall decrease in health after 30 minutes of incubation, sperm incubated with 50 µM AXA demonstrated an improvement in sperm health compared to the control. Further investigation is necessary to understand the molecular mechanisms of how astaxanthin is beneficial to sperm, and additional experimentation using more controlled conditions and ROS quantification (Promega,CAT# G8820) will help to elucidate this effect as well. This research can be applied to the continued efforts by medical, agricultural, and conservation teams to improve human and animal fertility across a variety of disciplines.

References:

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