

SUMMARY OF PROPOSED RESEARCH

(Do not exceed the space provided)

Describe clearly and concisely, in language readily understandable to a biomedical scientist who may not be a specialist in the research project's field, the broad objectives, specific aims, general procedures, and the potential significance of the research.

Project Summary

Freezing and thawing results in alterations in cellular structure. In addition, human reproduction and early development *in vivo* is influenced by oxygen concentration. It is well known that under *in vitro* culture conditions, oxygen concentration is higher than *in vivo* and this increases the susceptibility of both the oocytes and embryos to oxidative stress. Oxidative stress could tend to induce alterations in the cytoskeleton (microtubule and microfilament) of the oocytes. Damage of cytoskeleton especially the spindle comprised of microtubules and chromosomes in mature oocyte will result in failure of the final meiotic reduction division. This has the potential of forming an aneuploid embryo, a condition that is fatal in nearly all instances in human development. Therefore, morphology of the microtubule and chromosome alignment especially in mature metaphase II oocyte is critical for further embryo development. Alteration in the cytoskeleton in metaphase II oocyte can therefore be used as an indicator of the ability of oocyte to form a chromosomally balanced embryo.

The present study will examine the alterations in the cytoskeleton and chromosome alignment of the metaphase II oocyte in fresh and frozen conditions, as well as under conditions of oxidative stress using a mouse model. We also will examine how the damage to the cytoskeleton may be reduced by antioxidant supplementation. This may help improve both fertilization rate and development rate in assisted reproductive technology (ART) programs.

Specific aims:

1. To examine the effect of freezing and thawing on the spindle structure and compare with fresh oocytes.
2. To examine the effect of oxidative stress on cytoskeleton damage.
 - a. To examine the effect of oxidative stress on the microtubule and microfilament
 - b. To examine the effect of oxidative stress on the chromosomal alignment.
3. To examine the use of potential antioxidants (vitamins C and E) in reducing oxidative stress-induced changes in the cytoskeleton.

General procedure:

- Step 1: Oocyte retrieval from controlled ovarian hyperstimulated mouse.
- Step 2: Cryopreservation and thawing of oocytes.
- Step 3: Induction of oxidative stress.
- Step 4: Immunohistochemical staining the microtubules.
- Step 5: Staining the microfilament using fluorescent-labeled dye.
- Step 6: Staining the chromosome.
- Step 7: Evaluation by Fluorescence and Confocal microscopy.

Potential significance: This is the first study, which will examine the effect of cryopreservation and oxidative stress on the cytoskeleton in mature metaphase II oocytes and investigate the beneficial effect of antioxidants in reducing the susceptibility of cytoskeleton to oxidative stress such as during *in vitro* handling of oocytes in ART.

Please provide five Key Words that best describe your project

(1) Cryopreservation

(2) Oxidative stress

(3) Cytoskeleton

(4) Chromosome

(5) Confocal microscope