

SUMMARY OF PROPOSED RESEARCH

Please provide five Key Words that best describe your project:

(1). knockout mouse (2). GST5mu (3). testis
(4). oxidative stress (5). mating

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

Our long term goal is to understand how the lack of a major antioxidant gene (glutathione-S-transferase, GST5mu) in the testis predisposes a subpopulation of male infertility patients to damaging effects of oxidative stress. We will identify the genetic and the environment factors that may be increased over a period of time due to increased production of reactive oxygen species (ROS) and may be associated at least in part, with secondary male infertility.

Our immediate aims are to use a knockout mouse deficient for one of the isozymes glutathione-S-transferase mu (GST5mu), which is reported to be similar in human, rats and mouse. Lack of the major antioxidant system in the testis may result, in increased susceptibility to oxidative stress, which increases over time compared to the wild type. This may be analogous to men who may be more susceptible to oxidative stress and present with secondary infertility. We will validate GST5mu knockout model that may duplicate the testicular alterations seen after exogenous (environmental) or endogenous (genetic changes) exposure to oxidative stress, and obtain preliminary data to support a related NIH RO1 grant application. The aims of our proposed study are:

Aim 1: To confirm that GST5mu expression in the testis of the wild-type is limited to post-meiotic germ cells and is absent in these cells in the knockout.

Aim 2: To determine whether the time-dependent loss of fertility in GST5mu knockout male animals is the result of increased ROS and/or decreased antioxidant capacity and establish whether the testicular cell types, which normally express GST5mu, are preferentially sensitive to oxidative stress in the knockout or whether sensitivity to oxidative stress is a more general phenomenon.

Seventy male mouse (70) from the GST5mu knockout mouse and 70 from the wild type will be split and grouped according to their ages. We will localize GST5mu in the wild type mouse testis and study how alterations in oxidative stress markers (histological, biochemical, immunohistochemical, DNA fragmentation, lipid peroxidation, apoptosis and antioxidant status) are different in the knockout mouse compared to the wild type. We will identify the cell types (post-meiotic germ cells, spermatids, spermatozoa with excessive residual cytoplasm) in the testis where alterations in oxidative stress biomarkers occur. The presence of these markers will be correlated with the specific cell types that are positive for GST5mu.

The study should demonstrate that we have a useful knockout mouse model for study of GST5mu gene that may be critical in preventing oxidative stress and conferring protection to the developing germ cells, and postmeiotic cells (spermatids and spermatozoa). This data should greatly increase our chances for NIH funding for a more extensive study that will further examine the pregnancy outcome in the absence of this gene following prolonged exposure to external stressors, construct and perform gene array analysis, elucidate the mechanism of action and study the testicular biopsies from men with secondary infertility to validate our global hypothesis.