Response to Reviewers comments

SUMMARY OF PROPOSED RESEARCH PROJECT

Please provide five words that best describe your project:

(1) Male infertility (2) ROS (3) RhoGTPase

(4) Sperm immotility (5) Capacitation/acrosome reaction

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

PROJECT SUMMARY

Infertility affects approximately 10-15% of all reproductive age couples. Male infertility is present in 40-50% of these couples. Asthenozoospermia (<30% motility) accounts for about 38% of male infertility. Natural pregnancy rates are poor in asthenozoospermic patients. Therefore identifying the cause of poor sperm quality in these men and identifying strategies to overcome or minimize these defects is critical.

Before sperm can acquire the ability to fertilize and transfer genetic material essential modifications in the cell physiology must occur by capacitation, hyperactivation, and acrosome reaction. Sperm motility is governed by motor and cytoskeleton proteins. These are also important for sperm hyperactivation and sperm DNA delivery for fertilization. These steps are regulated by a group of proteins called RhoGTPase (Rho = Ras homologue; RhoA-B & Rac). Defects in these motor/cytoskeleton proteins and their regulators result in morphological abnormalities (teratozoospermia), poor motility (asthenozoospermia) and fertilization failure.

Reactive oxygen species (ROS) are necessary in physiological amounts for hyperactivation, capacitation and acrosome reaction. However uncontrolled production of ROS results in oxidative stress and affects sperm motility, DNA damage and subsequent fertilization / pregnancy. Reactive oxygen species is derived through the action of RhoGTPase. Therefore an abnormal expression of RhoGTPase may be associated with uncontrolled ROS generation especially in poor quality sperm. The goals of our study are: 1) evaluate RhoGTPase (RhoA-B, Cdc42 and Rac1) expression in spermatozoa from infertile asthenozoospermic men and normal healthy volunteers (controls), 2) study the distribution of RhoGTPase in asthenozoospermic men and controls after capacitation/ acrosome reaction, and 3) examine the binding affinity of RhoGTPase (Rac1) from asthenozoospermic men with gp91phox / p67phox from controls for assessing the defects in sperm NADPH oxidase (Nox) signaling mechanism to elucidate the involvement of ROS in these men.

Studying the RhoGTPase proteins in these men will therefore help to assess their association with poor sperm motility. Furthermore by separating spermatozoa into head and tail region and subsequently profiling RhoGTPase expression in these regions may also help identify the region of the spermatozoa that is associated with poor motility and failure in the subsequent step(s) of capacitation. Thus identification of a defective RhoGTPase and/or their regulator protein evaluation will help elucidate some of the steps necessary to alleviate this type of sperm abnormality. The results from our proposed study will further help us unravel the molecular/ genetic causes of poor sperm motility. This may allow us to seek strategies which might circumvent this defect.