

**EVALUATION OF THE TECHNOLOGY DEVELOPED FOR THE THREE
LABORATORY TEST KITS FOR ASSESSMENT OF HUMAN SPERM FUNCTION:
HYPOOSMOTIC SWELLING, ACROSOME STATUS AND FUNCTION AND
NUCLEAR DECONDENSATION**

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Date of completion (as approved by PAC):	6/2010
Expected date of completion:	9/2010

General Objective:

To test the efficacy of the modified laboratory kits for sperm function, namely hypoosmotic swelling, acrosome status and function and nuclear chromatin decondensation using semen from proven fertile and different groups of infertile subjects.

Specific Objectives:

1. To test the efficacy, sensitivity and reproducibility of these kits among proven fertile and different groups of infertile subjects attending the infertility clinic at NIHFW in comparison with the existing established methods in order to match the negative and positive predictive values for each of these tests.
2. To correlate the data on sperm function to other sperm parameters like count, motility, viability and morphology.

Methodology:

Semen samples from proven fertile subjects and from those attending the infertility clinic at NIHFW will be utilized for testing the efficacy of the new technologies on sperm function tests developed in the laboratory. All the subjects will be categorized in specific groups depending on their sperm count, motility and presence or absence of leucocytospermia. There are 8 groups and in each group at least 50 subjects are to be recruited for the study. The protocols of the sperm function tests will be tested in comparison with the old method (WHO) available for the purpose.

Progress:

With the support from the part-time faculty and the Senior Laboratory Assistant engaged in the project work, there has been substantial progress in the research study as per the laid

down objectives. A sum total of 270 subjects were included in the study and the semen samples were evaluated as per the study protocols. Based on the study findings, a manuscript has been published in the international Journal "Fertility and Sterility" and another has been submitted for publication.

Summary of the Work so far:

Sperm function test scores (HOS, AS, NCD) were observed below normal in all the infertile groups excepting the group with normal count and motility. In contrast, ROS was observed significantly elevated irrespective of sperm count and motility with teratospermia depicting the maximum. Seminal plasma catalase was estimated lower in asthenozoospermia but elevated in leukocytospermia with good sperm motility. Barring few categories, a positive and significant correlation of sperm function with TAC and SOD was observed in all the infertile subjects.

Besides ROS, the effect of nitric oxide as promoter or inhibitor of sperm function has been debated for a very long time. The role of specific free nitrogen radicals and their association with altered sperm functions among different groups of infertile subjects was investigated further. Specified tests of sperm function like hypo-osmotic swelling test (HOS), nuclear chromatin decondensation (NCD), and acrosomal status (AS) along with assays for nitric oxide synthase (NOS) in sperms and total nitric oxide (NO) in seminal plasma were carried out in infertile subjects categorized into subgroups based on sperm count, motility, morphology and presence of leucocytes. All infertile subjects with the exception of normospermia demonstrated subnormal sperm function scores. Sperm viability in most of the infertile subjects was found directly proportional to sperm function. However, in leukocytospermia (L), subnormal sperm function was seen in spite of normal viability. Exceptionally high nitric oxide levels were estimated in the seminal plasma of subjects with asthenospermia, irrespective of the presence or absence of leucocytes. In other groups, no significant trend was apparent. Levels of NO in the seminal plasma, however, corresponded well with the activities of nitric oxide synthase present in the sperms of fertile as well as different groups of infertile subjects. NO demonstrated a significant inverse relation with sperm function among asthenospermia (A), leukocytospermia (L) and leucocytoasthenospermia (LA). The decrease in sperm function in leukocytospermia (L) was independent of sperm motility. Attenuated activities of enzymes either superoxide dismutase or catalase or both were observed in all infertile subjects excepting normospermia. The above findings indicate the fact that infertile subjects in which sperm motility is not affected, subnormal sperm function may have contribution from the imbalance in the production and decomposition of reactive nitrogen species present in the semen.

Any Other Comments:

The extra time (three months) is needed to complete the remaining project work and correlation of sperm function parameters on sperm motility variables as determined by computer assisted sperm analysis which is now in progress and to submit the final report.

Tentative Budget

Sl.No.	Details	Period	Amount (Rs)
1	<u>Personnel</u> Part-Time Faculty (1 No.)	January to September	1,80,000.00
2.	Laboratory Assistant	January to March	30,000.00
3.	Chemical and Labware	January to September	2,00,000.00
	Total		Rs. 4,10,000.00/-