

**THE CLEVELAND CLINIC FOUNDATION  
RESEARCH INSTITUTE  
RESEARCH PROGRAMS COMMITTEES (RPC)  
INTERNAL FUNDING APPLICATION**

**ASSIGNED RPC #** \_\_\_\_\_ **DATE:** February 5<sup>th</sup>, 2004

**Submit the original and 18 copies to the RPC Office, Mail Code NB21, Room NB2-52, Ext. 42295, by the 5th of the month for review that month.**

1. a. Principal Investigator (PI): Kiran P. Nallella, M.D Empl.# 683760  
 Non-Staff P.I.'s must list Staff Sponsor: Asohk Agarwal, Ph.D  
 b. Department: Urology Location: A19.1 Phone: 4-4402

2. Title of Project: "Characterization Of Intracellular Reactive Oxygen Species In Human Spermatozoa"

3. Project Period: From: March 1, 2004 To: March 1, 2005

4. RPC Funds Requested: \$ 11,829.75 Total Project Cost: \$ 23,659.5  
 Matching Department: Glickman Urological Institute

5. **RPC approval is contingent upon approval of applicable committee(s) listed below.** Check each applicable component and submit these forms to the Committee for approval. **Please provide approval letter to the RPC Office as soon as available.**

- Human Subjects (contact IRB x42924; copy of IRB approval is required at time of RPC approval)  
 Animals (contact IACUC - Ext. 42295)  
 Recombinant DNA (contact Biosafety Committee - Ext. 46705 or 41830)  
 Product Development/Technology Protection (contact CCF Innovations - Ext. 45757)  
 Investigational Drugs (contact Hospital Pharmacy - Ext. 41128)  
 Radioactive Materials (contact Radiation Safety Director - Ext. 46645)

6. Identify the appropriate Study Section to review your project:

Biomedical Engr. & Transplantation \_\_\_\_\_ General Medical   
 Cancer \_\_\_\_\_ Cardiovascular \_\_\_\_\_ Neurosciences \_\_\_\_\_

7. List Collaborating Investigators contributing scientific efforts to this study:

Co-Investigators	Department	Mail Code	Signature
<u>Tamer M Said, M.D</u>	<u>Urology</u>	<u>A19.1</u>	_____
<u>Shyam Allamaneni, M.D</u>	<u>Urology</u>	<u>A19.1</u>	_____
<u>Suresh C. Sikka, Ph.D</u>	<u>Urology, Tulane University</u>	_____	_____
<u>Rakesh K. Sharma</u>	<u>Urology</u>	<u>A19.1</u>	_____
<u>Anthony J. Thomas, Jr., MD</u>	<u>Urology</u>	<u>A100</u>	_____
<u>Ashok Agarwal, Ph.D</u>	<u>Urology</u>	<u>A19.1</u>	_____

8. Signatures/Assurances: This research project is consistent with departmental policies and objectives. Financial commitments of departmental matching are authorized. The PI accepts scientific and financial responsibility and agrees to provide annual and final reports of progress.

\_\_\_\_\_  
Principal Investigator

\_\_\_\_\_  
Department Chairman

**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**

Physiological levels of reactive oxygen species (ROS) generated in spermatozoa play a significant role in sperm hyperactivation, capacitation, and acrosome reaction. However, high ROS levels induce peroxidative damage to the sperm cell membrane and DNA damage leading to male infertility. The primary ROS generated in human spermatozoa appears to be the superoxide anion ( $O_2^{\cdot-}$ ) that secondarily reacts with itself to generate hydrogen peroxide ( $H_2O_2$ ).

Although chemiluminescence has been the standard method for measuring ROS in a given sample, the assay entails many limitations: e.g., 1) does not specifically target intracellular ROS, 2) is not specific for any individual free radical species, and 3) requires a large number of cells to perform (usually > 2 million/mL). In contrast, assessment of ROS levels using flow cytometry offers the ability to identify specific radicals generated intracellularly in relatively low cell number. The main objectives of our proposed study are to (a) establish single flow cytometric assay for measurement of intracellular ROS using the minimal sperm concentration (b) to characterize the reactive oxygen species that are involved in the pathogenesis of male infertility, (c) to identify specific oxidants that induce sperm DNA damage, and (d) to evaluate specific antioxidants that may protect spermatozoa against such ROS induced cell damage. The clinical importance of our study is that it shall allow us to accurately identify sperm intracellular radicals and diagnose patients with high ROS levels. In addition, it may provide new therapeutic regimen for the management of male infertility.

Please provide five Key Words that best describe your project:

- (1) Spermatozoa                      (2) Leukocytes                      (3) Reactive oxygen species  
(4) Flow cytometry                      (5) Antioxidants



**MEMORANDUM**

TO: Research Programs Council  
Lerner Research Institute / NB21  
Tel: 42295; Fax: 43279

FROM: \_\_\_\_\_

DATE: \_\_\_\_\_

SUBJECT: TRANSFER OF DEPARTMENT FUNDS FOR RPC MATCHING SUPPORT

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The Department of \_\_\_\_\_

authorizes the transfer of \$ \_\_\_\_\_ from the

● \_\_\_\_\_ Fund (Fund # \_\_\_\_\_)

to support RPC # \_\_\_\_\_ for Dr. \_\_\_\_\_, Principal Investigator.

- **The information requested above must be provided at the time of protocol submission to document the departmental source of matching funds. Failure to provide department/endowment fund name and number delays the processing of approved applications.**

**If the protocol is approved for funding, this memo will be returned to the investigator to attach as documentation to a CCF Check Request form signed by the investigator's Department and Division Chairmen. This memo and the Check Request should be returned to the Research Programs Council Office/Mail Code NB21.**

TO: Research Programs Council  
Lerner Research Institute / NB21  
Tel: 45955; Fax: 43279

FROM: Ashok Agarwal, PhD, HCLD

DATE: February 5<sup>th</sup>, 2004

SUBJECT: Agreement to serve as Staff Sponsor for a fellow, resident or non-doctoral allied health professional who is applying as Principal Investigator to the RPC for research funding

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I agree to serve as Staff Sponsor for Kiran P. Nallella, M.D  
who is a \_\_\_\_\_ (Principal Investigator)

- Fellow;  
 Resident  
 Non-doctoral allied health professional

In the Glickman Urological Institute Department/Center.

If the Principal Investigator leaves CCF or becomes unable to continue to serve as Principal Investigator as prior to completion of RPC # \_\_\_\_\_, I will be responsible for either recruiting another investigator to serve as Principal Investigator, completing the project myself as Principal Investigator, or terminating this project.

Signed: \_\_\_\_\_, Staff Sponsor.  
(Staff Sponsor)