REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1.	Name and designation of ICMR- IF	Dr. Dipty Singh
2.	Address	Neuroendocrinology, ICMR-NIRRH, Parel, Mumbai-12
3.	Frontline area of research in which training/research was carried out	Male Infertility
4.	Name & address of Professor and host institute	Dr. Ashok Agarwal American Center for Reproductive Medicine Cleveland Clinic | Mail Code X-11 | 10681 Carnegie Avenue |Cleveland | OH 44195 | United States |
5.	Duration of fellowship	6 Months (8th January 2019 to 5th July 2019)
6.	Highlights of work conducted **i.Technique/expertise acquired** **Training Obtained:** Undergone andrology training for semen analysis and advance sperm function tests as per 2010 World Health Organization Criteria at Andrology Center, Cleveland Clinic **Protocol/ Assay Developed:** 1. Evaluation of sperm mitochondrial membrane potential (MMP) by flow cytometry 2.Evaluation of sperm intracellular reactive oxygen species (iROS) by flow cytometry **Techniques Learnt:** 1. Flow cytometry; 2. Sperm selection techniques; 3. Sperm cryopreservation; 4. ORP test – MiOXSYS™ system; 5. TUNEL assay;	

ii. Research results, including any papers, prepared/submitted for publication

Summary of work done:

Title: Association of Sperm Mitochondrial Dysfunction and mtDNA Methylation Changes in Infertile Men with Clinical Varicocele - A Pilot Study

Varicocele is a leading cause of male infertility. Current evidence indicates that mitochondrial dysfunction is a key contributing factor to the mechanism by which varicocele impairs sperm function. Mitochondrial membrane potential (MMP) and intracellular reactive oxygen species (iROS) are prime indicators of mitochondrial dysfunction. Recent studies also indicate the perturbed gene expression in spermatozoa of men with varicocele. Epigenetics plays an important role in the regulation of gene expression through modulation of DNA activity without altering the basic nucleotide structure. Very few studies have reported epigenetic changes in infertile men with clinical varicocele. Therefore, the objective of this study was to evaluate mitochondrial dysfunction and mtDNA methylation changes in spermatozoa of men with clinical varicocele.

This case-controlled study included 9 infertile men with unilateral and bilateral varicocele (grade II–III), and 9 healthy fertile men of reproductive age. Routine semen parameters (2010 World Health Organization Criteria) were analyzed following liquefaction. MMP (JC-1 dye) and iROS (2',7'-dichlorofluorescin diacetate, DCFDA) assays were performed using an Accuri C6 flow cytometer (Becton and Dickinson, San Jose, CA) after sperm separation with a 65% density gradient. Oxidation-reduction potential (ORP) was measured by MiOXSYS (Male Infertility Oxidative System). Furthermore, the sperm DNA from healthy fertile men and infertile varicocele men were isolated using QIAamp DNA mini kit (Qiagen, Hinden,Germany) for methylation studies. Sperm DNA fragmentation was evaluated using a terminal deoxynucleotidyl transferase–mediated fluorescein-TUNEL assay with an Apo-Direct kit (Pharmingen, San Diego, CA). Statistical analysis was carried out with an independent samples t-test using MedCalc Statistical Software (version 17.8; Ostend, Belgium).

Our results indicated that infertile men with varicocele had significantly lower sperm count and motility compared to healthy fertile donors. Patients with varicocele had a significantly lower MMP than healthy fertile donors. iROS produced by live spermatozoa